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Notes:

1) Segmented by Journal which publish articles on Basic Research and Human Disease

2) Journals are listed alphabetically from A to I

Acta Cytol (1)

OBJECTIVE: To compare the performance of human papillomavirus (HPV) DNA detection by polymerase chain reaction (PCR) and Hybrid Capture II (HCII) test (Digene, Gaithersburg, Maryland, U.S.A.) in residual cells left in the collection vials of the DNACitoliq system (Digene Brasil, São Paulo, Brazil). STUDY DESIGN: A series of 263 cervical samples collected for liquid-based cytology with the DNACitoliq system was tested for oncogenic HPV types first with HCII and subsequently with PCR. After DNA purification with GFX Genomic Blood DNA Purification Kit (Amersham, Piscataway, New Jersey, U.S.A.), PCR was performed using AmpliTaq Gold DNA polymerase (Applied Biosystems). PGMY09/11 L1 consensus primers and GH20/PCO4 primers for human beta-globin target were coamplified. RESULTS: Altogether, 260 samples were positive for beta-globin, and 3 negative ones were excluded from the analysis. PCR and HCII yielded concordant results in 199 cases (76.5%) (102 positive and 97 negative), with Cohen's kappa of.577 (95% CI.477-.677) and weighted kappa of.733 (95% CI.659-.791). HPV prevalence in different categories of cytologic abnormalities was practically identical with HCII and PCR assays (P=.989). Among the 61 (23.5%) discrepant cases, 28 samples were HCII+/PCR- cases. Of these, 27 of 28 samples showed a low viral load, and 1 had an intermediate viral load. CONCLUSION: The data suggest that residual material from the DNACitoliq system adequately preserves HPV DNA for detection by HCII and PCR, with performance similar to that of specimen transport medium.

Alcohol (4)

http://www.sciencedirect.com/science/article/B6T40-3XR8CH-H/2/de85fc7ddb426d8e9e618cf61533a3ad

Deficiency of mitochondrial aldehyde dehydrogenase (ALDH2) has been previously reported in South American Indians. We therefore assayed five individuals from each of five South American India populations (Quechua, Karitiana, Ticuna, Surui, Guahiba), and two North American
populations (Maya and Moskoke) for the presence of the Oriental ALDH22 variant. These samples were also surveyed for other alleles altering ALDH2 function. Allele-specific amplification assay (ASA) did not detect the ALDH22 allele in any of the New World populations studied. The entire coding sequence of the ALDH2 cDNA was enzymatically amplified in partially overlapping fragments. Each fragment was digested using restriction endonucleases and subfragments 148-285 b.p. in length were analyzed by the single-stranded conformation polymorphism (SSCP) technique. No variants were detected within the coding region of the ALDH2 gene in any of the seven American Indian populations. Three potentially correct explanations for these results are suggested. First, an ALDH2 polymorphism is present but undetectable by SSCP; second, none of the studied individuals were ALDH2 negative; third, the polymorphism occurs beyond the coding region of ALDH2 gene.


http://www.sciencedirect.com/science/article/B6T40-485N5G8-5/2/9f6effb70fe9add3d6f38d2c89ba0f1e

Results of recent studies have indicated an association between voluntary alcohol intake and activities of [kappa]-opioid receptor systems in animal models. We assessed the possibility that genetic differences observed in alcohol preference among mouse strains are related to possible polymorphisms of the [kappa]-opioid receptor gene (Oprk1). We compared DNA sequences of the coding region and the promoter/regulatory region of Oprk1 among C57BL/6ByJ (B6, alcohol-preferring), BALB/cJ (alcohol-avoiding), CXBI (alcohol-avoiding), and six B6.C and B6.1 Recombinant QTL Introgression (RQI) strains, which carry ~3% of the donor BALB/cJ genome in the background B6 genome and showed various alcohol preferences. Although there were no sequence differences in the coding region, BALB/cJ had a single nucleotide polymorphism (SNP) in the promoter region, which was not detected in other strains. The results indicate that the difference in alcohol preference between B6 and BALB/cJ is not correlated with polymorphisms of Oprk1. However, results of further studies comparing Oprk1 mRNA expression between B6 and BALB/cJ showed that Oprk1 expression is regulated differently in these strains. Also, DBA/2J mice (alcohol-avoiding) showed expression of Oprk1 mRNA subtypes (alternatively spliced) different from B6 and BALB/cJ mice. Search of the Celera Genomics database indicated that DBA/2J had several SNP sites in the promoter/regulatory regions, which might explain the different expression of Oprk1 mRNA subtypes in this strain. The strain-dependent variation in the expression of alternatively spliced genes can be a significant source of phenotypic variation of complex traits such as alcohol preference.


http://www.sciencedirect.com/science/article/B6T40-3YN91YK-8/2/26ce7eaca3872edef2f10b5b99d6b58cf

Ethanol preference, a component of alcoholism, has been known for four decades to differ greatly between C57BL/6 and BALB/c inbred mouse strains. For mapping quantitative trait loci (QTLs) that affect ethanol preference, we used a set of B6.C Recombinant QTL Introgression (RQI) strains, which carry about 5% of the donor BALB/cJ (C) genome on a C57BL/6ByJ (B6) background. After characterizing males of the progenitor and RQI strains for variations in ethanol preference, we scanned their genome for polymorphisms at 244 dinucleotide-repeat marker loci known to differ between B6 and C. Because of the introgression of BALB/c-type QTLs onto the B6 background, some strains showed ethanol preference significantly lower or higher than that of
the background strain, suggesting that genetic interaction between ethanol preference QTLs and the background can be operative. The genomic region showing the strongest influence on ethanol preference was on mouse chromosome 15, and corresponds to human chr.12 q11-q13.


http://www.sciencedirect.com/science/article/B6T40-41TMS9K-4/2/0848f5d5be495e310c3ecc37ce56a2df

In our present genetic study to map Quantitative Trait Loci (QTLs) for alcohol-related behaviors, we used 44 B6.C and 36 B6.I inbred congenic Recombinant QTL Introgression (RQI) mouse strains of the b5i7 series carrying genes of BALB/cJ (C) or CXBI (I) origin on C57BL/6ByJ (B6) genetic background. Ethyl alcohol consumption (EAC) was measured in adult males, and chromosomes 1, 2, 3, 9, and 15 were scanned with polymorphic microsatellite markers. In the B6.C set of strains, multiple regression analysis yielded a model with three microsatellite markers, which explained 32% of the genetic variance (p=0.0006). The two markers with the highest significance levels in the model, D1Mit167 and D2Mit74, have been mapped to chromosome regions close to the gene opioid receptor kappa 1 (chr. 1) and opioid receptor kappa 3 (chr. 2), respectively. The results of this gene-mapping study suggest that genetic polymorphisms in kappa opioid receptors may contribute to genetic predisposition to voluntary alcohol-drinking behavior.

Alcohol Alcohol. (1)


http://alcalc.oupjournals.org/cgi/content/abstract/38/5/407

Aims: Alcohol dehydrogenase \{beta\} subunit (ADH2) Arg47His and aldehyde dehydrogenase 2 (ALDH2) Glu487Lys were genotyped by a duplex polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP), which allows DNA amplification with one-tube PCR including eight primers, and subsequent electrophoresis. Methods: Several PCR conditions were tested to establish the optimal conditions for distinguishing the allele-specific bands for the two polymorphisms. Under the optimal PCR conditions, 454 Japanese health check-up examinees were genotyped. Results: The allele-specific bands were successfully amplified under the optimal conditions of the duplex PCR-CTPP. The genotype distributions were within the Hardy-Weinberg equilibrium. The bands produced by the duplex PCR-CTPP genotyping were clearer than those produced by PCR-CTPP, conducted solely for ADH2. Conclusions: ADH2 Arg47His and ALDH2 Glu487Lys were successfully genotyped by this newly developed duplex PCR-CTPP, an inexpensive and time-saving genotyping tool, which will be useful in epidemiological studies on alcoholism, as well as risk estimation of alcohol-related diseases.
The cardiac L-type calcium current (ICa) can be modified by activation of protein kinase C (PKC). However, the effect of PKC activation on ICa is still controversial. Some studies have shown a decrease in current, whereas other studies have reported a biphasic effect (an increase followed by a decrease in current or vice versa). A possible explanation for the conflicting results is that several isoforms of PKC with opposing effects on ICa were activated simultaneously. Here, we examined the influence of a single PKC isoform (PKC-[beta]II) on L-type calcium channels in isolation from other cardiac isoforms, using a transgenic mouse that conditionally expresses PKC-[beta]II. Ventricular cardiac myocytes were isolated from newborn mice and examined for expression of the transgene using single cell RT-PCR after ICa recording. Cells expressing PKC-[beta]II showed a twofold increase in nifedipine-sensitive ICa. The PKC-[beta]II antagonist LY-379196 returned ICa amplitude to levels found in non-PKC-[beta]II-expressing myocytes. The increase in ICa was independent of Cav1.2-subunit mRNA levels as determined by quantitative RT-PCR. Thus these data demonstrate that PKC-[beta] is a potent modulator of cardiac L-type calcium channels and that this specific isoform increases ICa in neonatal ventricular myocytes.


Angiotensin II (ANG II) has been etiologically linked to vascular disease; however, its role in the alterations of endothelial function that occur in vascular disorders is not completely understood. Matrix metalloproteinases (MMPs) and proinflammatory cytokines are involved in the pathological remodeling of blood vessels that occurs in vascular disease. In this study we evaluated the effects of ANG II on tumor necrosis factor (TNF)-(alpha) and MMP-2 production in endothelial cells. Human umbilical vein endothelial cells (HUVECs) were stimulated with ANG II (0.1-10 \text{ \mu}M) for 24 h, in the presence or absence of antagonists of ANG II type 1 (AT1R) and type 2 (AT2R) receptors, and the production and release of TNF-(alpha) and MMP-2 were assessed. ANG II increased TNF-(alpha) mRNA and protein expression and the release of bioactive TNF-(alpha). Moreover, ANG II induced MMP-2 release and reduced the secretion of tissue inhibitor of MMP (TIMP)-2 from endothelial cells. To elucidate whether endogenous TNF-(alpha) could mediate the effects of ANG II on MMP-2 release, cells were pretreated with anti-TNF-(alpha) neutralizing antibodies or pentoxifylline (an inhibitor of TNF-(alpha) synthesis). TNF-(alpha) inhibition prevented the secretion of MMP-2 induced by ANG II. Furthermore, AT1R antagonism with candesartan prevented the formation of MMP-2 and TNF-(alpha) and the reduction of TIMP-2 induced by ANG II. These results indicate that ANG II, via AT1R, modulates the secretion of TNF-(alpha) and MMP-2 from endothelial cells and that TNF-(alpha) mediates the effects of ANG II on MMP-2 release.
Purinergic inhibition of Na-K-Cl cotransport has been noted in various renal epithelial cells derived from the collecting tubule, including Madin-Darby canine kidney (MDCK) cells. In recent studies, we have observed purinergic inhibition of Na-K-Cl cotransport in C11-MDCK subclones (alpha-intercalated-like cells). Interestingly, Na-K-Cl cotransport activity was also detected in C7-MDCK subclones (principal-like cells) but was not affected by ATP. In this investigation, we have transfected the human Na-K-Cl cotransporter (huNKCC1) in both C11 and C7 cells to determine whether these differences in NKCC regulation by ATP were due to cell-specific purinoceptor signaling pathways or to cell-specific isoforms/splice variants of the transporter. In both cell lines, we found that endogenous as well as huNKCC1-derived cotransport activity was restricted to the basolateral side. In addition, we were able to show that extracellular application of 100 \( \mu \text{M} \) ATP or 100 \( \mu \text{M} \) UTP abolished NKCC activity in both mock- and huNKCC1-transfected C11 cells but not in mock- and huNKCC1-transfected C7 cells; in C11 cells, intriguingly, this inhibition was not affected by inhibitors of RNA and protein synthesis and occurred even though expression levels of UTP-sensitive P2Y2-, P2Y4-, and P2Y6-purinoceptors were not different from those observed in C7 cells. These results suggest that C11 cells express an undetermined type of UTP-sensitive P2-purinoceptors or a unique P2Y-purinoceptor-triggered signaling cascade that leads to inhibition of NKCC1.

The aim of the present study was to determine the distribution of monocarboxylate transporter (MCT) subtypes 1-4 in the various structures of the rat eye by using a combination of conventional and real-time RT-PCR, immunoblotting, and immunohistochemistry. Retinal samples expressed mRNAs encoding all four MCTs. MCT1 immunoreactivity was observed in photoreceptor inner segments, Muller cells, retinal capillaries, and the two plexiform layers. MCT2 labeling was concentrated in the inner and outer plexiform layers. MCT4 immunolabeling was present only in the inner retina, particularly in putative Muller cells, and the plexiform layers. No MCT3 labeling could be observed. The retinal pigment epithelium (RPE)/choroid expressed high levels of MCT1 and MCT3 mRNAs but lower levels of MCT2 and MCT4 mRNAs. MCT1 was localized to the apical and MCT3 to the basal membrane of the RPE, whereas MCT2 staining was faint. Although MCT1-MCT4 mRNAs were all detectable in iris and ciliary body samples, only MCT1 and MCT2 proteins were expressed. These were present in the iris epithelium and the nonpigmented epithelium of the ciliary processes. MCT4 was localized to the smooth muscle lining of large vessels in the iris-ciliary body and choroid. In the cornea, MCT1 and MCT2 mRNAs and proteins were detectable in the epithelium and endothelium, whereas evidence was found for the presence of MCT4 and, to a lesser extent, MCT1 in the lens epithelium. The unique distribution of MCT subtypes in the eye is indicative of the pivotal role that these transporters play in the maintenance of ocular function.
Primary cultures of granule cells (GC) from rat cerebellar cortex were used to determine whether bioelectric activity, via a Ca2+/calmodulin-dependent kinase (CaMK) signaling cascade, modulates expression and exon selection in the inositol trisphosphate receptor type 1 (IP3R1). IP3R1 contains or lacks three exons (S1, S2, and S3) that are regulated in a regionally and temporally specific manner. The neuronal, or long, form of IP3R1 is distinguished from peripheral tissues by inclusion of the S2 exon. Although previous studies indicated that IP3R1 are undetectable in the cerebellar granular layer in vivo, receptor protein and mRNA are induced in cultured GC grown in medium supplemented with 25 mM KCl or NMDA, two trophic agents that promote long-term survival, compared with GC grown in 5 mM KCl. IP3R1 induction in response to 25 mM KCl or NMDA is attenuated by coaddition of voltage-sensitive calcium channel or NMDA receptor antagonists, respectively. Actinomycin D, CaMK, and calcineurin antagonists likewise suppress induction. Unlike the major variants of IP3R1 in Purkinje neurons, which lack S1 and S3, GC grown with trophic agents express mRNA containing these exons. Both neuronal types contain S2. Evidence obtained using mutant mice with Purkinje cell lesions, laser-microdissected GC neurons from slices, and explant cultures indicates that GC predominantly express the S1-containing variant of IP3R1 in vivo.


ATP-sensitive K+ (KATP) channels are composed of pore-forming Kir6.x subunits and regulatory sulfonylurea receptor (SUR) subunits. SURs are ATP-binding cassette proteins with two nucleotide-binding folds (NBFs) and binding sites for sulfonylureas, like glibenclamide, and for channel openers. Here we report the identification and functional characterization of four novel splice forms of guinea pig SUR1. Three splice forms originate from alternative splicing of the region coding for NBF1 and lack exons 17 (SUR1[Delta]17), 19 (SUR1[Delta]19), or both (SUR1[Delta]17[Delta]19). The fourth (SUR1C) is a COOH-terminal SUR1-fragment formed by exons 31-39 containing the last two transmembrane segments and the COOH terminus of SUR1. RT-PCR analysis showed that these splice forms are expressed in several tissues with strong expression of SUR1C in cardiomyocytes. Confocal microscopy using enhanced green fluorescent protein-tagged SUR or Kir6.x did not provide any evidence for involvement of these splice forms in the mitochondrial KATP channel. Only SUR1 and SUR1[Delta]17 showed high-affinity binding of glibenclamide (Kd[approx] 2 nM in the presence of 1 mM ATP) and formed functional KATP channels upon coexpression with Kir6.2.


Epithelial cells of the epididymis and vas deferens establish an optimum luminal environment in which spermatozoa mature and are stored. This is achieved by active transepithelial transport of various ions including Cl[-] and H+. We investigated the localization of three closely related members of the ClC family, ClC-3, ClC-4, and ClC-5, in the epididymis and vas deferens. RT-PCR using mRNA isolated by laser capture microdissection (LCM)-detected ClC-3 and ClC-5 transcripts but did not detect any ClC-4-specific transcript. Western blot and immunofluorescence analysis demonstrated that ClC-3 and ClC-5 proteins are present in all regions of the epididymis.
and in the vas deferens. CIC-5 is expressed exclusively in H+-ATPase-rich cells (narrow and clear cells). Confocal microscopy showed that CIC-5 partially colocalizes with the H+-ATPase in the subapical pole of clear cells. CIC-3 is strongly expressed in the apical membrane of principal cells of the caput epididymidis and the vas deferens and is less abundant in principal cells of the body and cauda epididymidis. These findings are consistent with a potential role for CIC-3 in transepithelial chloride transport by principal cells and for CIC-5 in the acidification of H+-ATPase-containing vesicles in narrow and clear cells. CIC-5 might facilitate endosome trafficking in the epididymis, as has been proposed in the kidney.


http://ajpcell.physiology.org/cgi/content/abstract/283/4/C1009

Fish oils (FOs) have been noted to reduce growth and proliferation of certain tumor cells, effects usually attributed to the content of polyunsaturated fatty acids of the n-3 family, which are thought to modulate cellular signaling pathways. We investigated the influence of FO on cell cycle kinetics of cultured Chinese hamster ovary cells. Exponentially growing cells were labeled with 5-bromo-2'-deoxyuridine (BrdU) and analyzed by flow cytometry after 5-day treatment with exogenous fat. Bivariate BrdU-DNA analysis indicated slower progression through S phase and thus longer S phase duration time in FO- but not corn oil-treated or control cells. We hypothesize that FO treatment might interfere with spatial/temporal organization of replication origins. Therefore, we mapped the well-characterized replication origin ori-[beta] downstream of the dihydrofolate reductase gene with the nascent strand length assay. Three DNA marker segments with known positions relative to this origin were amplified by PCR. By quantitatively assessing DNA length of the fragments in all fractions containing these markers, the location of ori-[beta] was established. In control or corn oil-treated cells, the location of ori-[beta] was consistent with previous studies. However, in FO-treated cells, DNA replication appears to start from a new site located farther upstream from ori-[beta], suggesting a different replication initiation pattern. This study suggests novel mechanism(s) by which fats affect cell proliferation and DNA replication in mammalian cells.


http://ajpcell.physiology.org/cgi/content/abstract/284/2/C547

Synaptotagmin I (Syt I), a low-affinity Ca2+-binding protein, is thought to serve as the Ca2+ sensor in the release of neurotransmitter. However, functional studies on the calyx of Held synapse revealed that the rapid release of neurotransmitter requires only approximately micromolar [Ca2+], suggesting that Syt I may play a more complex role in determining the high-affinity Ca2+ dependence of exocytosis. Here we tested this hypothesis by studying pituitary cells, which possess high- and low-affinity Ca2+-dependent exocytic pathways and express Syt I. Using patch-clamp capacitance measurements to monitor secretion and the acute antisense deletion of Syt I from differentiated cells, we have shown that the rapid and the most Ca2+-sensitive pathway of exocytosis in rat melanotrophs requires Syt I. Furthermore, stimulation of the Ca2+-dependent exocytosis by cytosol dialysis with solutions containing 1 {micro}M [Ca2+] was completely abolished in the absence of Syt I. Similar results were obtained by the preinjection of antibodies against the CAPS (Ca2+-dependent activator protein for secretion) protein. These results indicate that synaptotagmin I and CAPS proteins increase the probability of vesicle fusion at low cytosolic [Ca2+].

http://ajpcell.physiology.org/cgi/content/abstract/282/1/C172

First published September 5, 2001; 10.1152/ajpcell.00048.2001.---Intestinal strictures are frequent in Crohn's disease but not ulcerative colitis. We investigated the expression of transforming growth factor (TGF)-[beta] isoforms by isolated and cultured primary human intestinal myofibroblasts and the responsiveness of these cells and intestinal epithelial cells to TGF-[beta] isoforms. Normal intestinal myofibroblasts released predominantly TGF-[beta]3 and ulcerative colitis myofibroblasts expressed both TGF-[beta]1 and TGF-[beta]3, whereas in myofibroblast cultures from fibrotic Crohn's disease tissue, there was significantly lower expression of TGF-[beta]3 but enhanced release of TGF-[beta]2. These distinctive patterns of TGF-[beta] isoform release were sustained through several myofibroblast passages. Proliferation of Crohn's disease intestinal myofibroblasts was significantly lower than that of myofibroblasts derived from normal and ulcerative colitis tissue. In contrast to cells from normal and ulcerative colitis tissue, neutralization of the three TGF-[beta] isoforms did not affect the proliferation of Crohn's disease intestinal myofibroblasts. Studies on the effect of recombinant TGF-[beta] isoforms on epithelial restitution and proliferation suggest that TGF-[beta]2 may be the least effective of the three isoforms in intestinal wound repair. In conclusion, the enhanced release of TGF-[beta]2 but reduced expression of TGF-[beta]3 by Crohn's disease intestinal myofibroblasts, together with their enhanced proliferative capacity, may lead to the development of intestinal strictures.


http://ajpcell.physiology.org/cgi/content/abstract/287/1/C22

Neonatal sciatic nerve injury is known to result in an extensive loss of lumbar motor neurons as well as the disappearance of their respective muscle fibers in the hindlimb musculature. The loss of motor neurons and muscle fibers can be prevented by immediate administration of target-derived neurotrophic factors to the site of injury. In the present study, we investigated the role of ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) in the survival and maturation of a subset of motor neurons innervating the extensor digitorum longus (EDL) and tibialis anterior (TA) muscles. We have shown that combined administration of CNTF and BDNF prevented the loss of motor units after neonatal nerve injury and contributed to the maintenance of muscle mass. Importantly, this combined neurotrophin regimen also prevented the disappearance of muscle fibers that express myosin heavy chain IIB (MyHC IIB) in both EDL and TA muscles 3 mo after neonatal sciatic nerve crush. In parallel studies, we observed a higher level of BDNF in EDL muscle during the critical period of development when motor neurons are highly susceptible to target removal. Given our previous findings that combined administration of CNTF with neurotrophin-3 (NT-3) or neurotrophin-4/5 (NT-4/5) did not result in the rescue of MyHC IIB fibers in EDL, the present results show the importance of muscle-derived BDNF in the survival and maturation of a subpopulation of motor neurons and of MyHC IIB muscle fibers during neonatal development of the neuromuscular system.

We have previously reported that the hEAG K+ channels are responsible for the potential membrane hyperpolarization that induces human breast cancer cell progression into the G1 phase of the cell cycle. In the present study, we evaluate the role and functional expression of the intermediate-conductance Ca2+-activated K+ channel, hIK1-like, in controlling cell cycle progression. Our results demonstrate that hIK1 current density increased in cells synchronized at the end of the G1 or S phase compared with those in the early G1 phase. This increased current density paralleled the enhancement in hIK1 mRNA levels and the highly negative membrane potential. Furthermore, in cells synchronized at the end of G1 or S phases, basal cytosolic Ca2+ concentration ([Ca2+]i) was also higher than in cells arrested in early G1. Blocking hIK1 channels with a specific blocker, clotrimazole, induced both membrane potential depolarization and a decrease in the [Ca2+]i in cells arrested at the end of G1 and S phases but not in cells arrested early in the G1 phase. Blocking hIK1 with clotrimazole also induced cell proliferation inhibition but to a lesser degree than blocking hEAG with astemizole. The two drugs were essentially additive, inhibiting MCF-7 cell proliferation by 82% and arresting >90% of cells in the G1 phase. Thus, although the progression of MCF-7 cells through the early G1 phase is dependent on the activation of hEAG K+ channels, when it comes to G1 and checkpoint G1/S transition, the membrane potential appears to be primarily dependent on the hIK1-activity level.

High mobility group box 1 (HMGB1) protein, a DNA binding protein that stabilizes nucleosomes and facilitates transcription, was recently identified as a late mediator of endotoxin lethality. High serum HMGB1 levels in patients with sepsis are associated with increased mortality, and administration of HMGB1 produces acute inflammation in animal models of lung injury and endotoxemia. Neutrophils occupy a critical role in mediating the development of endotoxemia-associated acute lung injury, but previously it was not known whether HMGB1 could influence neutrophil activation. In the present experiments, we demonstrate that HMGB1 increases the nuclear translocation of NF-κB and enhances the expression of proinflammatory cytokines in human neutrophils. These proinflammatory effects of HMGB1 in neutrophils appear to involve the p38 MAPK, phosphatidylinositol 3-kinase/Akt, and ERK1/2 pathways. The mechanisms of HMGB1-induced neutrophil activation are distinct from endotoxin-induced signals, because HMGB1 leads to a different profile of gene expression, pattern of cytokine expression, and kinetics of p38 activation compared with LPS. These findings indicate that HMGB1 is an effective stimulus of neutrophil activation that can contribute to development of a proinflammatory phenotype in diseases characterized by excessively high levels of HMGB1.

Stimulation of ATP or adenosine receptors causes important physiologic changes in retinal pigment epithelial (RPE) cells that may influence their relationship to the adjacent photoreceptors. While RPE cells have been shown to release ATP, the regulation of extracellular ATP levels and
the production of dephosphorylated purines is not clear. This study examined the degradation of ATP by RPE cells and the physiologic effects of the adenosine diphosphate (ADP) that results. ATP was readily broken down by both cultured human ARPE-19 cells and the apical membrane of fresh bovine RPE cells. The compounds ARL67156 and (βγm)ATP inhibited this degradation in both cell types. RT-PCR analysis of ARPE-19 cells found mRNA message for multiple extracellular degradative enzymes; ectonucleotide pyrophosphatase/phosphodiesterase (eNPP)1, eNPP2 and eNPP3, the ectoATPase ecto-nucleoside triphosphate diphosphohydrolase (NTPDase)2, NTPDase3, and some message for NTPDase1. Considerable levels of ADP bathed RPE cells, consistent with a role for NTPDase2. ADP and ATP increased levels of intracellular Ca2+. Both responses were inhibited by thapsigargin and P2Y1 receptor inhibitor MRS 2179. Message for both P2Y1 and P2Y12 receptors was detected in ARPE-19 cells. These results suggest that extracellular degradation of ATP in subretinal space can result in production of ADP. This ADP can stimulate P2Y receptors and augment Ca2+ signaling in the RPE.


http://ajpcell.physiology.org/cgi/content/abstract/283/1/C347

In this study, we examined the role of the nuclear factor-[kappa]B (NF-[kappa]B)-inducing kinase (NIK) in distinct signaling pathways leading to NF-[kappa]B activation. We show that a dominant-negative form of NIK (dnNIK) delivered by adenoviral (Ad5dnNIK) vector inhibits Fas-induced [kappa]B phosphorylation and NF-[kappa]B-dependent gene expression in HT-29 and HeLa cells. Interleukin (IL)-1[beta]- and tumor necrosis factor-[alpha] (TNF-[alpha])-induced NF-[kappa]B activation and [kappa]B-dependent gene expression are inhibited in HeLa cells but not in Ad5dnNIK-infected HT-29 cells. Moreover, Ad5dnNIK failed to sensitize HT-29 cells to TNF-[alpha]-induced apoptosis at an early time point. However, cytokine- and Fas-induced signals to NF-[kappa]B are finally integrated by the [kappa]B kinase (IKK) complex, since [kappa]B[alpha] phosphorylation, NF-[kappa]B DNA binding activity, and IL-8 gene expression were strongly inhibited in HT-29 and HeLa cells overexpressing dominant-negative IKK[beta] (Ad5dnIKK[beta]). Our findings support the concept that cytokine signaling to NF-[kappa]B is redundant at the level of NIK. In addition, this study demonstrates for the first time the critical role of NIK and IKK[beta] in Fas-induced NF-[kappa]B signaling cascade.


http://ajpcell.physiology.org/cgi/content/abstract/00610.2004v1

Lysophosphatidic acid (LPA) is a mediator of multiple cellular responses. LPA mediates its effects predominantly through the G protein-coupled receptors, LPA1, LPA2, and LPA3. In the present work, we studied LPA2-mediated signaling using human colon cancer cell lines, which predominantly express LPA2. LPA2 activated Akt and Erk1/2 in response to LPA. LPA mediated Akt activation was inhibited by pertussis toxin (PTX), whereas Erk1/2 activation was completely inhibited by a blocker of phospholipase C[β], U73122. LPA also induced interleukin-8 (IL-8) synthesis in the colon cancer cells by primarily activating LPA2 receptor. We also found that LPA2 interacts with Na+/H+ exchanger regulatory factor 2 (NHERF2). Activation of Akt and Erk1/2 was significantly attenuated by silencing of NHERF2 expression by RNA interference, suggesting a pivotal role of NHERF2 in LPA2-mediated signaling. We also found that expression of LPA2 was elevated, whereas expression of LPA1 down-regulated in several types of cancers, including ovarian and colon cancer. We conclude that LPA2 is the major LPA receptor in colon...
cancer cells and cellular signals by LPA2 are largely mediated through its ability to interact with NHERF2.

Am J Physiol Endocrinol Metab (5)


http://ajpendo.physiology.org/cgi/content/abstract/286/6/E896

Micro- and macroangiopathy are major causes of morbidity and mortality in patients with diabetes. Our aim was to characterize IGF-I receptor (IGF-IR) and insulin receptor (IR) in human micro- and macrovascular endothelial cells. Cultured human dermal microvascular endothelial cells (HMVEC) and human aortic endothelial cells (HAEC) were used. Gene expression was measured by quantitative real-time RT-PCR and receptor protein by ligand-binding assay. Phosphorylation of IGF-IR (beta)-subunit was analyzed by immunoprecipitation and Western blot. Glucose metabolism and DNA synthesis was assessed using [3H]glucose and [3H]thymidine incorporation, respectively. We detected gene expression of IGF-IR and IR in HAEC and HMVEC. IGF-IR gene expression was severalfold higher than that of IR. The specific binding of 125I-IGF-I was higher than that of 125I-insulin in HAEC and HMVEC. Insulin and the new, long-acting insulin analog glargine interacted with the IGF-IR with thousand- and hundred-fold less potency than IGF-I itself. Phosphorylation of the IGF-IR (beta)-subunit was shown in HAEC for IGF-I (10-8 M) and insulin (10-6 M) and in HMVEC for IGF-I and glargine (10-8 M, 10-6 M). IGF-I 10-7 M stimulated incorporation of [3H]thymidine into DNA, and 10-9-10-7 M also the incorporation of [3H]glucose in HMVEC, whereas glargine and insulin had no significant effects at 10-9-10-7 M. Human micro- and macrovascular endothelial cells express more IGF-IR than IR. IGF-I and high concentrations of glargine and insulin activates the IGF-IR. Glargine has a higher affinity than insulin for the IGF-IR but probably has no effect on DNA synthesis at concentrations reached in vivo.


http://ajpendo.physiology.org/cgi/content/abstract/282/3/E608

Using in vitro and in vivo methods, we have demonstrated increased sensitivity of adrenocortical steroidogenesis to ACTH in Milan hypertensive (MHS) compared with normotensive (MNS) rats and have investigated whether this is caused by mutations of steroidogenic enzymes. Genes encoding aldosterone synthase (CYP11B2) and 11[beta]-hydroxylase (CYP11B1) in MHS and MNS have been cloned and sequenced. Nucleotide 752 (G) in exon 4 of MHS CYP11B2 differs from that of MNS (A); CYP11B1 sequences were identical. The nucleotide 752 mutation caused a Q251R substitution in the amino acid sequence of MHS CYP11B2. The phenotype of MHS CYP11B2 alleles, when expressed in COS-1 cells, differed from that of MNS alleles. The relative activities of the three reactions catalyzed by COS-1 cells were identical to that of MNS alleles. The relative activities of the three reactions catalyzed by COS-1 cells were the same as that of MHS alleles. The relative activities of the three reactions catalyzed by COS-1 cells were the same as that of MNS alleles. The relative activities of the three reactions catalyzed by COS-1 cells were the same as that of MHS alleles. The relative activities of the three reactions catalyzed by COS-1 cells were the same as that of MNS alleles.
[14C]deoxycorticosterone and analysis of radioactivity associated with deoxycorticosterone, corticosterone, 18 hydroxycorticosterone, and aldosterone. Both 11- and 18-hydroxylase activities were lower (19 and 12%, respectively; P < 0.01 and P < 0.05) in cells transfected with MHS compared with MNS alleles, whereas 18-oxidase activity was 42% higher (P < 0.01). To assess the significance of the CYP11B2 mutation in vivo, DNA from F2 hybrid MHS x MNS rats was genotyped. MHS alleles were associated with lower urine volumes in both sexes, lower ventricle weights in male rats, but no difference in systolic or diastolic blood pressures between the sexes. We conclude that a mutation in CYP11B2 may affect aldosterone secretion in MHS; however, under normal environmental circumstances, we were unable to demonstrate any influence of this mutation on blood pressure.


http://ajpendo.physiology.org/cgi/content/abstract/282/5/E1119

Type II 5'-iodothyronine deiodinase (D2), produces triiodothyronine (T3) and is stimulated by cold exposure via norepinephrine (NE) release in brown adipose tissue. Cultured rat brown adipocytes require T3 for the adrenergic stimulation of D2 activity. D2 mRNA expression in cultured brown adipocytes is undetectable with the use of basal conditions or NE without T3. Full D2 expression is achieved using NE + T3, especially after prolonged T3 exposure. [beta]3-Adrenergic agonists mimic the NE action, whereas cAMP analogs do not. Prolonged exposure to T3 alone increases D2 mRNA. High T3 doses (500 nM) inhibit the adrenergic stimulation of D2 activity while increasing D2 mRNA. The effects obtained with NE + T3 or T3 alone are suppressed by actinomycin, but not by cycloheximide, which leads to accumulation of short D2 mRNA transcripts. Prolonged or short exposure to T3 did not change D2 mRNA half-life, but T3 seemed to elongate it. In conclusion, T3 is an absolute requirement for the adrenergic stimulation of D2 mRNA in brown adipocytes. T3 upregulates D2 mRNA, an effect that might involve stimulation of factors required for transcription or for stabilization of D2 mRNA.


http://ajpendo.physiology.org/cgi/content/abstract/283/5/E899

The transport mechanism mediating brain uptake of tumor necrosis factor (TNF)-[alpha] has been studied. When 125I-labeled rat TNF-[alpha] was used in internal carotid artery perfusions in rats, the cytokine showed transcytosis through the blood-brain barrier in intact form (permeability-surface area product 0.34 {+/-} 0.13 {micro}l {middle dot} min^{-1} {middle dot} g^{-1}). Uptake was inhibited by low nanomolar concentrations of unlabeled rat TNF-[alpha]. Human TNF-[alpha], which does not interact with the p80 TNF receptor in rodents, showed no brain uptake. mRNA expression of both p60 and p80 receptors could be demonstrated in native brain microvessel preparations. These transcripts increased to 149% (p60) and 127% (p80) of control 4 h after a systemic immune stimulation (2 mg/kg bacterial endotoxin ip). Lipopolysaccharide treatment did not alter the rate of brain uptake of TNF-[alpha] measured between 4 and 24 h later. In conclusion, a receptor-mediated mechanism is responsible for the transcytosis of TNF-[alpha]. Saturable transport, requiring the p80 receptor, occurs at concentrations encountered under pathophysiological conditions and therefore constitutes a relevant mechanism of communication between the immune system and the brain.

http://ajpendo.physiology.org/cgi/content/abstract/284/5/E931

The incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are gut hormones that act via the enteroinsular axis to potentiate insulin secretion from the pancreas in a glucose-dependent manner. Both GLP-1 receptor and GIP receptor knockout mice (GLP-1R[-]/[-] and GIPR[-]/[-], respectively) have been generated to investigate the physiological importance of this axis. Although reduced GIP action is a component of type 2 diabetes, GIPR-deficient mice exhibit only moderately impaired glucose tolerance. The present study was directed at investigating possible compensatory mechanisms that take place within the enteroinsular axis in the absence of GIP action. Although serum total GLP-1 levels in GIPR knockout mice were unaltered, insulin responses to GLP-1 from pancreas perfusions and static islet incubations were significantly greater (40-60%) in GIPR[-]/[-] than in wild-type (GIPR+/+) mice. Furthermore, GLP-1-induced cAMP production was also elevated twofold in the islets of the knockout animals. Pancreatic insulin content and gene expression were reduced in GIPR[-]/[-] mice compared with GIPR+/+ mice. Paradoxically, immunocytochemical studies showed a significant increase in [beta]-cell area in the GIPR-null mice but with less intense staining for insulin. In conclusion, GIPR[-]/[-] mice exhibit altered islet structure and topography and increased islet sensitivity to GLP-1 despite a decrease in pancreatic insulin content and gene expression.

*Am J Physiol Gastrointest Liver Physiol* (15)


http://ajpgi.physiology.org/cgi/content/abstract/282/3/G480

Regional differences in the ontogeny of mouse intestinal [alpha]-2,6-sialyltransferase activities ([alpha]-2,6-ST) and the influence of cortisone acetate (CA) on this expression were determined. High ST activity and [alpha]-2,6-ST mRNA levels were detected in immature small and large intestine, with activity increasing distally from the duodenum. As the mice matured, ST activity (predominantly [alpha]-2,6-ST) in the small intestine decreased rapidly to adult levels by the fourth postnatal week. CA precociously accelerated this region-specific ontogenic decline. A similar decline of ST mRNA levels reflected ST activity in the small, but not the large, intestine. Small intestinal sialyl [alpha]-2,6-linked glycoconjugates displayed similar developmental and CA induced-precocious declines when probed using Sambucus nigra agglutinin (SNA) lectin. SNA labeling demonstrated age-dependent diminished sialyl [alpha]2,6 glycoconjugate expression in goblet cells in the small (but not large) intestine, but no such regional specificity was apparent in microvillus membrane. This suggests differential regulation of sialyl [alpha]-2,6 glycoconjugates in absorptive vs. goblet cells. These age-dependent and region-specific differences in sialyl [alpha]-2,6 glycoconjugates may be mediated in part by altered [alpha]-2,6-ST gene expression regulated by trophic factors such as glucocorticoids.

http://ajpgi.physiology.org/cgi/content/abstract/285/5/G919

Tumor necrosis factor-{alpha} (TNF-{alpha}) is a multifunctional cytokine involved in the expression of many genes integral to the inflammatory response. In addition, it activates both apoptotic and survival pathways, the latter being mediated through the activation of the transcription factor nuclear factor-{kappa}B (NF-{kappa}B). Protein kinase CK2, a serine-threonine kinase that is universally upregulated in human malignancies, may be involved at multiple levels in this process. However, its role in mediating a survival response within colon cancer cells remains incompletely understood. Here we report that inhibition of CK2 in HCT-116 and HT-29 cells with the use of two specific CK2 inhibitors, 5,6-dichloro-ribifuranosylbenzimidazole (DRB) and apigenin, effected a synergistic reduction in cell survival when used in conjunction with TNF-{alpha}. Furthermore, there was a demonstrable synergistic reduction in colony formation in soft agar with the use of the same combinations. Western blot analysis showed that poly-ADP ribose polymerase and procaspase-3 cleavage complemented the fluorescence-activated cell sorter analysis findings of significantly increased subdiploid DNA-containing cell populations using these conditions. Remarkably, these events occurred in the absence of any reduction in the expression of the Bcl-2 family members Bcl-2, Mcl-1, and Bcl-xL or any change in the proapoptotic molecules Bad or Bax. One-hybrid NF-{kappa}B promoter assays utilizing a Gal4-p65 transactivation domain construct revealed that the TNF-induced transactivation was inhibited by both DRB and apigenin. This was associated with a concomitant reduction in the expression of a recognized anti-apoptotic NF-{kappa}B target, manganese superoxide dismutase, demonstrated by Q-PCR. Our findings indicate a potentially novel strategy for the treatment of colon cancer, one that targets CK2 simultaneous with TNF-{alpha} administration.


http://ajpgi.physiology.org/cgi/content/abstract/284/6/G883

Previous studies suggest that ether-a-go-go related gene (ERG) KCNH2 potassium channels contribute to the control of motility patterns in the gastrointestinal tract of animal models. The present study examines whether these results can be translated into a role in human gastrointestinal muscles. Messages for two different variants of the KCNH2 gene were detected: KCNH2 V1 human ERG (HERG) (28) and KCNH2 V2 (HERGUSO) (13). The amount of V2 message was greater than V1 in both human jejunum and brain. The base-pair sequence that gives rise to domains S3-S5 of the channel was identical to that previously published for human KCNH2 V1 and V2. KCNH2 protein was detected immunohistochemically in circular and longitudinal smooth muscle and enteric neurons but not in interstitial cells of Cajal. In the presence of TTX (10[-]6 M), atropine (10[-]6 M), and L-nitroarginine (10[-]4 M) human jejunal circular muscle strips contracted phasically (9 cycles/min) and generated slow waves with superimposed spikes. Low concentrations of the KCNH2 blockers E-4031 (10[-]8 M) and MK-499 (3 x 10[-]8 M) increased phasic contractile amplitude and the number of spikes per slow wave. The highest concentration of E-4031 (10[-]6 M) produced a 10-20 mV depolarization, eliminated slow waves, and replaced phasic contractions with a small tonic contracture. E-4031 (10[-]6 M) did not affect [14C]ACh release from enteric neurons. We conclude that KCNH2 channels play a fundamental role in the control of motility patterns in human jejunum through their ability to modulate the electrical behavior of smooth muscle cells.
[gamma]/[delta] T cells might play an important role in autoimmune conditions like inflammatory bowel disease (IBD). In the present study, we characterized the T cell receptor (TCR)-[delta] repertoire by complementarity determining region 3 (CDR3) spectratyping in the inflamed and noninflamed mucosa and in the peripheral blood of subjects with Crohn's disease and ulcerative colitis. In contrast to previously published data about [alpha]/[beta] T cells, we rarely found oligoclonal expansions of [gamma]/[delta] T cells specific only for the inflamed mucosa. The same dominant [gamma]/[delta] T cell expansions were also present in the noninflamed colon. Furthermore, the peripheral [gamma]/[delta] TCR repertoire was oligoclonal but clearly distinct from that in the inflamed intestine. Thus our results do not support a role for antigen-specific [gamma]/[delta] T cells in IBD, and dominant [gamma]/[delta] T cells of the peripheral blood are not likely to be derived from the inflamed gut. However, in several patients, the TCR-[delta]-repertoire was highly diversified, whereas in others we observed a loss of dominant [gamma]/[delta] T cell clones when inflamed and noninflamed mucosa were compared. In conclusion, those changes indicate that [gamma]/[delta] T cells might play an important role in a subset of patients with IBD.

Numerous genes expressed by intestinal epithelial cells are developmentally regulated, and the influence that adaptive (AI) and passive (PI) immunity have in controlling their expression has not been evaluated. In this study, we tested the hypothesis that both PI and AI influenced enterocyte gene expression by developing a breeding scheme that used T and B cell-deficient recombination-activating gene (RAG) mice. RNA was isolated from the liver and proximal/distal small intestine at various ages, and the steady-state levels of six different transcripts were evaluated by RNase protection assay. In wild-type (WT) pups, all transcripts [Fc receptor of the neonate (FcRn), polymeric IgA receptor (pIgR), GLUT5, lactase-phlorizin hydrolase (lactase), apical sodium-dependent bile acid transporter (ASBT), and Na+/glucose cotransporter (SGLT1)] studied were developmentally regulated at the time of weaning, and all transcripts except ASBT had the highest levels of expression in the proximal small intestine. In WT suckling pups reared in the absence of PI, pIgR mRNA levels were increased 100% during the early phase of development. In mice lacking AI, the expression of pIgR and lactase were significantly attenuated, whereas FcRn and GLUT5 levels were higher compared with WT mice. Finally, in the absence of both passive and active immunity, expression levels of pIgR and lactase were significantly lower than similarly aged WT mice. In summary, we report that the adaptive and passive immune status of mice influences steady-state mRNA levels of several important, developmentally regulated enterocyte genes during the suckling and weaning periods of life.
The nitric oxide (NO) synthase inhibitor N[omega]-nitro-L-arginine (L-NNA) inhibits heat stress (HS)-induced NO production and the inducible 70-kDa heat shock protein (HSP-70i) in many rodent organs. We used human intestinal epithelial T84 cells to characterize the inhibitory effect of L-NNA on HS-induced HSP-70i expression. Intracellular Ca2+ concentration ([Ca2+]i) was measured using fura-2, and protein kinase C (PKC), and PKA activities were determined. HS increased HSP-70i mRNA and protein in T84 cells exposed to 45(°)C for 10 min and allowed to recover for 6 h. L-NNA treatment for 1 h before HS inhibited the induction of HSP-70i mRNA and protein, with an IC50 of 0.0471 ± 0.0007 (micro)M. Because the HS-induced increase in HSP-70i mRNA and protein is Ca2+ dependent, we measured [Ca2+]i after treating cells with L-NNA. L-NNA at 100 (micro)M significantly decreased resting [Ca2+]i. Likewise, treatment with 1 (micro)M GF-109203X or H-89 (inhibitors of PKC and PKA, respectively) for 30 min also significantly decreased [Ca2+]i and inhibited HS-induced increase in HSP-70i. GF-109203X- or H-89-treated cells failed to respond to L-NNA by further decreasing [Ca2+]i and HSP-70i. L-NNA effectively blocked heat shock factor-1 (HSF1) translocation from the cytosol to the nucleus, a process requiring PKC phosphorylation. These results suggest that L-NNA inhibits HSP-70i by reducing [Ca2+]i and decreasing PKC and PKA activity, thereby blocking HSF1 translocation from the cytosol to the nucleus.


Dietary lipid acutely upregulates apolipoprotein (apo) A-IV expression by sevenfold at the pretranslational level in neonatal swine jejunum. To determine the mechanism of this regulation, two-day-old female swine received intraduodenal infusions of low- and high-triaclylglycerol (TG) isocaloric diets for 24 h. Nuclear runoff assay confirmed apo A-IV gene transcriptional regulation by the high-TG diet. Footprinting analysis using the swine apo A-IV proximal promoter sequence (+14 to [-]246 bp) demonstrated three regions protected by the low-TG extracts. Of these three motifs, only ACCTTC showed 100% homology to the human sequence and was further studied. EMSA was performed using probes containing wild-type (WT) and mutant (M) motifs. A shift was noted with the low-TG nuclear extracts with the WT probe but not with the M probe. Excess unlabeled free WT probe competed out the shift, whereas the M probe did not. No significant shift occurred with either probe using high-TG extracts. These results suggest that a repressor protein binds to the ACCTTC motif and becomes unbound during lipid absorption, allowing transcriptional activation of the apo A-IV gene in newborn swine small intestine.


The chemokine CCL28 is constitutively expressed by epithelial cells at several mucosal sites and is thought to function as a homeostatic chemoattractant of subpopulations of T cells and IgA B cells and to mediate antimicrobial activity. We report herein on the regulation of CCL28 in human colon epithelium by the proinflammatory cytokine IL-1, bacterial flagellin, and n-butyrate, a product of microbial metabolism. In vivo, CCL28 was markedly increased in the epithelium of pathologically inflamed compared with normal human colon. Human colon and small intestinal xenografts were used to model human intestinal epithelium in vivo. Xenografts constitutively
expressed little, if any, CCL28 mRNA or protein. After stimulation with the proinflammatory cytokine IL-1, CCL28 mRNA and protein were significantly increased in the epithelium of colon but not small intestinal xenografts, although both upregulated the expression of another prototypic chemokine, CXCL8, in response to the identical stimulus. In studies of CCL28 regulation using human colon epithelial cell lines, proinflammatory stimuli, including IL-1, bacterial flagellin, and bacterial infection, significantly upregulated CCL28 mRNA expression and protein production. In addition, CCL28 mRNA expression and protein secretion by those cells were significantly increased by the short-chain fatty acid n-butyrate, and IL-1- or flagellin-stimulated upregulation of CCL28 by colon epithelial cells was synergistically increased by pretreatment of cells with n-butyrate. Consistent with its upregulated expression by proinflammatory stimuli, CCL28 mRNA expression was attenuated by pharmacological inhibitors of NF-κB activation. These findings indicate that CCL28 functions as an "inflammatory" chemokine in human colon epithelium and suggest the notion that CCL28 may act to counterregulate colonic inflammation.


http://ajpgi.physiology.org/cgi/content/abstract/285/1/G235

Numerous therapies used for inflammatory bowel disease (IBD) target the transcription factor NF-κB, which is involved in the production of cytokines and chemokines integral for inflammation. Here we show that curcumin, a component of the spice turmeric, is able to attenuate colitis in the dinitrobenzene sulfonic acid (DNB)-induced murine model of colitis. When given before the induction of colitis it reduced macroscopic damage scores and NF-κB activation. This was accompanied by a reduction in myeloperoxidase activity, and using semiquantitative RT-PCR, an attenuation of the DNB-induced message for IL-1β was detected. Western blotting analysis revealed that there was a reproducible DNB-induced activation of p38 MAPK detected in intestinal lysates by using a phosphospecific antibody. This signal was significantly attenuated by curcumin. Furthermore, we show that the immunohistochemical signal is dramatically attenuated at the level of the mucosa by curcumin. We conclude that the widely used food additive curcumin is able to attenuate experimental colitis through a mechanism correlated with the inhibition of the activation of NF-κB and effects a reduction in the activity of p38 MAPK. We propose that this agent may have therapeutic implications for human IBD.


http://ajpgi.physiology.org/cgi/content/abstract/288/3/G514

The interleukin-2-deficient (IL-2-) mouse model of ulcerative colitis was used to test the hypothesis that colonic epithelial cells (CEC) directly respond to bacterial antigens and that alterations in Toll-like receptor (TLR)-mediated signaling may occur during the development of colitis. TLR expression and activation of TLR-mediated signaling pathways in primary CEC of healthy animals was compared with CEC in IL-2- mice during the development of colitis. In healthy animals, CEC expressed functional TLR, and in response to the TLR4 ligand LPS, proliferated and secreted the cytokines IL-6 and monocyte chemoattractant protein-1 (MCP-1). However, the TLR-responsiveness of CEC in IL-2- mice was different with decreased TLR4 responsiveness and augmented TLR2 responses that result in IL-6 and MCP-1 secretion. TLR signaling in CEC did not involve NF-(κappa)B (p65) activation with the inhibitory p50 form of NF-(κappa)B predominating in CEC in both the healthy and inflamed colon. Development of colitis
was, however, associated with the activation of MAPK family members and upregulation of MyD88-independent signaling pathways characterized by increased caspase-1 activity and IL-18 production. These findings identify changes in TLR expression and signaling during the development of colitis that may contribute to changes in the host response to bacterial antigens seen in colitis.


http://ajpgi.physiology.org/cgi/content/abstract/284/2/G328

Proliferation and carcinogenesis of the large intestinal epithelial cells (IEC) cells is significantly increased in transgenic mice that overexpress the precursor progastrin (PG) peptide. It is not known if the in vivo growth effects of PG on IEC cells are mediated directly or indirectly. Full-length recombinant human PG (rhPG1-80) was generated to examine possible direct effects of PG on IEC cells. Surprisingly, rhPG (0.1-1.0 nM) was more effective than the completely processed gastrin 17 (G17) peptide as a growth factor. Even though IEC cells did not express CCK1 and CCK2 receptors (-R), fluorescently labeled G17 and Gly-extended G17 (G-Gly) were specifically bound to the cells, suggesting the presence of binding proteins other than CCK1-R and CCK2-R on IEC cells. High-affinity (Kd = 0.5-1.0 nM) binding sites for 125I-rhPG were discovered on IEC cells that demonstrated relative binding affinity for gastrin-like peptides in the order PG [G-Gly => COOH-terminally extended G17] [G-Gly > G17 > *CCK-8 (* significant difference; P < 0.05). In conclusion, our studies demonstrate for the first time direct growth effects of the full-length precursor peptide on IEC cells in vitro that are apparently mediated by the high-affinity PG binding sites that were discovered on these cells.


http://ajpgi.physiology.org/cgi/content/abstract/282/4/G656

Regulation of bilirubin glucuronide transporters during hyperbilirubinemia in hepatic and extrahepatic tissues is not completely clear. In the present study, we evaluated the regulation of the bilirubin glucuronide transporters, multidrug resistance-associated proteins (MRP)2 and 3, in rats with obstructive jaundice. Bile duct ligation (BDL) or sham operation was performed in Wistar rats. Liver and kidneys were removed 1, 3, and 5 days after BDL (n = 4, in each group). Serum and urine were collected to measure bilirubin levels just before animal killing. MRP2 And MRP3 mRNA expressions were determined by real-time RT-PCR. Protein expression of MRP2 and MRP3 was determined by Western blotting. Renal MRP2 function was evaluated by para-aminohippurate (PAH) clearance. The effect of conjugated bilirubin, unconjugated bilirubin, human bile, and sulfate-conjugated bile acid on MRP2 gene expression was also evaluated in renal and hepatocyte cell lines. Serum bilirubin and urinary bilirubin excretion increased significantly after BDL. In the liver, the mRNA expression of MRP2 decreased 59, 86, and 82%, and its protein expression decreased 25, 74, and 93% compared with sham-operated animals after 24, 72, and 120 h of BDL, respectively. In contrast, the liver expression of MRP3 mRNA increased 138, 2,137, and 3,295%, and its protein expression increased 560, 634, and 612% compared with sham-operated animals after 24, 72, and 120 h of BDL, respectively. On the other hand, in the kidneys, the mRNA expression of MRP2 increased 162, 73, and 21%, and its protein expression increased 387, 558, and 472% compared with sham-operated animals after 24, 72, and 120 h of BDL, respectively. PAH clearance was significantly increased after BDL. The mRNA expression of MRP2 increased in renal proximal tubular epithelial cells after treatment with conjugated bilirubin, sulfate-conjugated bile acid or human bile. Upregulation of MRP2 in the

kidneys and MRP3 in the liver may be a compensatory mechanism to improve bilirubin clearance during obstructive jaundice.


http://ajpgi.physiology.org/cgi/content/abstract/282/1/G145

Endotoxemia causes an inflammatory response within the intestinal muscularis and gastrointestinal dysmotility. We hypothesize that the resident macrophage-derived chemokine monocyte chemoattractant protein-1 (MCP-1) plays a significant role in the recruitment of leukocytes into the lipopolysaccharide (LPS)-stimulated rat intestinal muscularis. MCP-1 mRNA expression was investigated by RT-PCR. Leukocyte extravasation and MCP-1 protein localization were determined by immunohistochemistry. Contractile activity was assessed by using a standard organ bath in rats that were treated with saline, recombinant MCP-1, LPS, LPS + nonspecific antibody, or LPS + MCP-1 antibody. Endotoxemia caused a significant 280-fold increase in MCP-1 mRNA expression in the muscularis, peaking at 3 h. MCP-1 protein was immunohistochemically located to muscularis macrophages. LPS application caused significant leukocyte recruitment into the muscularis and a 51% decrease in muscle contractility. MCP-1 antibody treatment significantly averted leukocyte recruitment and significantly prevented muscle dysfunction. These parameters were not significantly altered by the nonspecific antibody. Results show that resident muscularis macrophage-derived MCP-1 plays a major role in the recruitment of monocytes during endotoxemia, which then subsequently secrete kinetically active substances that cause ileus.


http://ajpgi.physiology.org/cgi/content/abstract/287/3/G555

Lactase-phlorizin hydrolase gene expression is spatially restricted along the anterior-posterior gut axis. Lactase gene transcription is maximal in the distal duodenum and jejunum in adult mammals and is barely detectable in the proximal duodenum. By contrast, pancreatic duodenal homeobox-1 (PDX-1) protein is expressed maximally in the proximal duodenum. This study aimed to determine the role of PDX-1 in regulating lactase gene promoter activity in intestinal epithelial cells. Caco-2 cells were cotransfected with lactase promoter-reporter constructs in the presence of a PDX-1 expression vector and assayed for luciferase activity. PDX-1 cotransfection results in repression of lactase promoter activity. Sequence analysis of the lactase promoter revealed a putative PDX-1 DNA binding site in the proximal 100-bp lactase gene promoter. EMSAs demonstrated that PDX-1 can interact with the lactase promoter binding site but not with a site in which the core PDX-1 binding sequence TAAT is mutated. Site-directed mutagenesis of the PDX-1 core binding site in the lactase promoter-reporter construct suggests that PDX-1 can function independently of DNA binding to its consensus binding site. Stable overexpression of PDX-1 results in repression of the endogenous human lactase gene in differentiated Caco-2 cells. Given the contrasting spatial expression pattern, PDX-1 may function to specify the anterior boundary of lactase expression in the small intestine and is thus a candidate regulator of anterior spatial restriction in the gut.

Zollner, G., P. Fickert, et al. (2002). "Induction of short heterodimer partner 1 precedes downregulation of

http://ajpgi.physiology.org/cgi/content/abstract/282/1/G184

Cholestasis is associated with retention of bile acids and reduced expression of the Na+/taurocholate cotransporter (Ntcp), the major hepatocellular bile acid uptake system. This study aimed to determine whether downregulation of Ntcp in obstructive cholestasis 1) is a consequence of bile acid retention and 2) is mediated by induction of the transcriptional repressor short heterodimer partner 1 (SHP-1). To study the time course for the changes in serum bile acid levels as well as SHP-1 and Ntcp steady-state mRNA levels, mice were subjected to common bile duct ligation (CBDL) for 3, 6, 12, 24, 72, and 168 h and compared with sham-operated controls. Serum bile acid levels were determined by radioimmunoassay. SHP-1 and Ntcp steady-state mRNA expression were assessed by Northern blotting. In addition, Ntcp protein expression was studied by Western blotting and immunofluorescence microscopy. Increased SHP-1 mRNA expression paralleled elevations of serum bile acid levels and was followed by downregulation of Ntcp mRNA and protein expression in CBDL mice. Maximal SHP-1 mRNA expression reached a plateau phase after 6-h CBDL (12-fold; P < 0.001) and preceded the nadir of Ntcp mRNA levels (12%, P < 0.001) by 6 h. In conclusion, bile acid-induced expression of SHP-1 may, at least in part, mediate downregulation of Ntcp in CBDL mice. These findings support the concept that downregulation of Ntcp in cholestasis limits intracytoplasmatic accumulation of potentially toxic bile acids.

Am J Physiol Heart Circ Physiol (15)


http://ajpheart.physiology.org/cgi/content/abstract/00320.2004v1

Heart failure (HF) is characterized by marked prolongation of the action potential duration and a reduction in cellular repolarization reserve. These changes are caused, in large part, by HF-induced Potassium (K)-current down regulation. Molecular mechanisms underlying these changes remain uncertain. We determined whether down regulation of K currents in a canine model of tachycardia-induced HF is caused by altered expression of underlying {alpha} and {beta} K channel subunits encoding these currents. K channel subunit expression was quantified in normal and failing dogs at the mRNA and protein levels in epicardial (EPI), midmyocardial (MID), and endocardial (ENDO) layers of the left ventricle. Results: Analysis of mRNA and protein levels of candidate genes encoding the transient-outward K-current (Ito) revealed marked reductions in cKv4.3 expression in HF in EPI (44% mRNA, 39% protein), MID (52% mRNA, 34% protein), and ENDO (49% mRNA, 73% protein) layers, a paradoxical enhancement (41% EPI, 97% MID, 113% ENDO) in cKv1.4 protein levels, without significant changes in cKChIP2 expression. Expression of cKir2.1, the gene underlying IK1, was unaffected by HF at mRNA and protein levels despite significant reduction in IK1, while remarkably canine-ERG, encoding IKr, exhibited increased protein expression. HF was not accompanied by significant changes in cKvLQT1 or cMinK mRNA and protein levels. Conclusions: These data indicate that: 1) Down-regulation of Ito in HF is associated with decreased cKv4.3, and not cKv1.4 or cKChIP2. 2) Alterations in IKr, IKs and IK1 in non-ischemic dilated cardiomyopathy are not caused by changes in either transcript or immunoreactive protein levels of relevant channel subunits, suggesting post-translational
modification of these currents by HF.


http://ajpheart.physiology.org/cgi/content/abstract/286/4/H1354

We studied molecular and functional characteristics as well as hormonal regulation of the Na-K-2Cl cotransporter (NKCC) in the isolated rat heart and cardiomyocytes. NKCC activity was measured as bumetanide-sensitive 86Rb+ influx in isolated perfused rat hearts and isolated cardiomyocytes. Stimulation of (alpha)1-adrenoceptors (AR) by phenylephrine (30 {micro}M) increased 86Rb+ influx. The NKCC inhibitor bumetanide (50 {micro}M) reduced the response to phenylephrine by 45 +/- 13% (n = 12, P < 0.01). PD-98059 (10 {micro}M), an inhibitor of the activation of the mitogen-activated protein kinases extracellular signal-regulated protein kinase 1 and 2 (ERK1/2), reduced the total response to phenylephrine by 51 +/- 13% (n = 10, P < 0.01) and eliminated the bumetanide-sensitive component, indicating that (alpha)1-AR mediated stimulation of NKCC is dependent on activation of ERK1/2. Inhibitors of protein kinase C or phosphatidylinositol 3-kinase had no effect. The presence of NKCC mRNA and protein was demonstrated in isolated rat cardiomyocytes. Phosphorylation of NKCC after (alpha)1-AR stimulation was shown by immunoprecipitation of the phosphoprotein from 32Pi prelabeled cardiomyocytes. Increased phosphorylation of the NKCC protein was also abolished by PD-98059. We conclude that the NKCC is present in rat cardiomyocytes and that ion transport by the cotransporter is regulated by (alpha)1-AR stimulation through phosphorylation of this protein involving the ERK pathway.


http://ajpheart.physiology.org/cgi/content/abstract/284/5/H1778

Arteries remodel in response to environmental changes. We investigated whether mechanical strain modulates production of matrix metalloproteinase (MMP)-2 and -9 by cultured vascular smooth muscle cells (SMC). MMP-2 and MMP-9 expression were tested using human saphenous vein SMC cultured on silicone membranes at rest or subjected to physiological levels (5%) of stationary or cyclical (1 Hz) uniaxial strain. Compared with control, stationary strain significantly increased MMP-2 mRNA levels at all time points, whereas cyclic strain decreased it after 48 h. Both secreted and cell-associated pro-MMP-2 levels were increased by stationary strain at all times (P < 0.01), whereas cyclic strain decreased secreted levels after 48 h (P < 0.02). MMP-9 mRNA levels and pro-MMP-9 protein were increased after 48 h of stationary stretch (P < 0.01) compared with both no strain and cyclic strain. Our study indicates that vascular SMC show a selective response to different types of strain. We suggest that local increases in stationary mechanical strain resulting from stenting, hypertension, or atherosclerosis may lead to enhanced matrix degradation by SMC.

Despite the important roles played by ventricular fibroblasts and myofibroblasts in formation and maintenance of the extracellular matrix, neither the ionic basis for membrane potential, nor the effect of modulating membrane potential on function, have been analyzed in detail. In this study, whole-cell patch clamp experiments were done using ventricular fibroblasts and myofibroblasts. Time- and voltage-dependent outward K+ currents were recorded at depolarized potentials, and an inwardly rectifying K+ (Kir) current was recorded near the resting membrane potential (RMP) and at more hyperpolarized potentials. The apparent reversal potential of Kir currents shifted to more positive potentials as external K+ concentration ([K+]o) was raised and this Kir current was blocked by 100 - 300 micromolar Ba2+. RT-PCR measurements showed that mRNA for Kir2.1 was expressed. Accordingly, we conclude that Kir current is the primary determinant of RMP in both fibroblasts and myofibroblasts. Changes in [K+]o influenced fibroblast membrane potential as well as proliferation and contractile functions. Recordings made with a voltage-sensitive dye, DiBAC3(4), showed that 1.5 mM [K+]o resulted in a hyperpolarization, while 20 mM [K+]o produced a depolarization. Low [K+]o (1.5 mM) enhanced myofibroblast number relative to control (5.4 mM [K+]o). In contrast, 20 mM [K+]o resulted in a significant reduction in myofibroblast number. In separate assays, 20 mM [K+]o significantly enhanced contraction of collagen I gels seeded with myofibroblasts, compared to control mechanical activity in 5.4 mM [K+]o. In combination these results show that ventricular fibroblasts and myofibroblasts express a variety of K+ channel alpha subunits and demonstrate that Kir current can modulate RMP and alter essential physiological functions.


Platelet-derived growth factor (PDGF)-BB, a potent mitogen for mesenchymal cells, also downregulates expression of multiple smooth muscle (SM) cell (SMC)-specific markers. However, there is conflicting evidence whether PDGF-BB represses SMC marker expression at a transcriptional or posttranscriptional level, and little is known regarding the mechanisms responsible for these effects. Results of the present studies provide clear evidence that PDGF-BB treatment strongly repressed SM α-actin, SM myosin heavy chain (MHC), and SM22α promoters in SMCs. Of major significance for resolving previous controversies in the field, we found PDGF-BB-induced repression of SMC marker gene promoters in subconfluent, but not postconfluent, cultures. Treatment of postconfluent SMCs with a tyrosine phosphatase inhibitor restored PDGF-BB-induced repression, whereas treatment of subconfluent SMCs with a tyrosine kinase blocker abolished PDGF-BB-induced repression, suggesting that a tyrosine phosphorylation event mediates cell density-dependent effects. On the basis of previous observations that Ets-1 transcription factor is upregulated within phenotypically modulated neointimal SMCs, we tested whether Ets-1 would repress SMC marker expression. Consistent with this hypothesis, results of cotransfection experiments indicated that Ets-1 overexpression reduced transcriptional activity of SMC marker promoter constructs in SMCs, whereas it increased activity of SM α-actin promoter in endothelial cells. PDGF-BB treatment increased expression of Ets-1 in cultured SMCs, and SM α-actin mRNA expression was reduced in multiple independent clones of SMCs stably transfected with an Ets-1-overexpressing construct. Taken together, results of these experiments provide novel insights regarding possible mechanisms whereby PDGF-BB and Ets-1 may contribute to SMC phenotypic switching associated with vascular injury.
We developed an RT-PCR assay to study both the time course and the mechanism for the triiodothyronine (T3)-induced transcription of the [alpha]- and [beta]-myosin heavy chain (MHC) genes in vivo on the basis of the quantity of specific heterogeneous nuclear RNA (hnRNA). The temporal relationship of changes in transcriptional activity to the amount of [alpha]-MHC mRNA and the coordinated regulation of transcription of more than one gene in response to T3 are demonstrated here for the first time. Quantitation of [alpha]-MHC hnRNA demonstrated that T3 induced [alpha]-MHC transcription in hypothyroid rats within 30 min of a single injection of T3 (0.5 \( \mu \)g/100 g body wt). Maximal transcription rates (135% +/- 15.8 of euthyroid values) occurred 6 h after injection and subsequently declined in parallel with serum T3 levels. The transcription of [beta]-MHC was reduced to 86% of peak hypothyroid levels 6 h after a single T3 injection and reached a nadir of 59% of hypothyroid levels at 36 h. Analysis of the time course of T3-mediated induction of [alpha]-MHC hnRNA and repression of [beta]-MHC hnRNA indicates that separate molecular mechanisms are involved in the coordinated regulation of these genes.

Angiogenesis, the growth of new blood vessels from existing ones, occurs in the skeletal muscle as an adaptive response to exercise that satisfies the increased requirement of this tissue for oxygen delivery and metabolic processes. Of the factors that have been identified to regulate this process, the endothelial cell mitogen vascular endothelial growth factor (VEGF) has been proposed to play a key role. The aim of this study was to measure the skeletal muscle VEGF mRNA content and arteriovenous protein balance across the working leg in response to a single bout of prolonged, submaximal exercise. Seven physically active males completed 3 h of two-legged kicking ergometry. Muscle biopsies were collected from the vastus lateralis muscle from both working legs, and blood samples were collected from one femoral artery and femoral vein before, during, and in recovery from exercise. We show that the exercise stimulus elicited a decrease in VEGF protein arteriovenous balance across the exercising leg (P = 0.007), and a ninefold elevation in skeletal muscle VEGF mRNA expression (P < 0.001). The changes in VEGF protein balance and mRNA content were most pronounced 1 h after the cessation of exercise. In conclusion, these findings demonstrate that submaximal exercise, suitable for humans with low CV fitness, induces a decrease in VEGF arteriovenous balance that is likely to be of clinical significance in promoting angiogenic effects.

Mitogenic effects of the extracellular nucleotides ATP and UTP are mediated by P2Y1, P2Y2, and P2Y4 receptors. However, it has not been possible to examine the highly expressed UDP-sensitive P2Y6 receptor because of the lack of stable, selective agonists. In rat aorta smooth muscle cells (vascular smooth muscle cells; VSMC), UDP and UTP stimulated 3H-labeled...
thymidine incorporation with similar pEC50 values (5.96 and 5.69). Addition of hexokinase did not reduce the mitogenic effect of UDP. In cells transfected with P2Y receptors the stable pyrimidine agonist uridine 5′-O-(2-thiodiphosphate) (UDP[β]S) was specific for P2Y6 with no effect on P2Y1, P2Y2, or P2Y4 receptors. UDP[β]S stimulated [3H]thymidine and [3H]leucine incorporation and increased cell number in VSMC. Flow cytometry demonstrated that UDP stimulated cell cycle progression to both the S and G2 phases. The intracellular signal pathways were dependent on phospholipase C, possibly protein kinase C-[delta], and a tyrosine kinase pathway but independent of Gi proteins, eicosanoids, and protein kinase A. The half-life of P2Y6 receptor mRNA was <1 h by competitive RT-PCR. The mitogen-activated protein kinase inhibitor PD-098059 significantly suppressed, whereas ATP and interleukin-1[β] upregulated, expression of P2Y6 receptor mRNA. The results demonstrate that UDP stimulates mitogenesis through activation of P2Y6 receptors and that the receptor is regulated by factors important in the development of vascular disease.


http://ajpheart.physiology.org/cgi/content/abstract/286/6/H2257

T-type Ca2+ channels are implicated in cardiac automaticity, cell growth, and cardiovascular remodeling. Two voltage-gated Ca2+ subtypes (Cav3.1 and Cav3.2) have been cloned for the pore-forming (alpha)1-subunit of the T-type Ca2+ channel in cardiac muscle, but their differential roles remain to be clarified. The aim of this study was to elucidate the relative contribution of the two subtypes in the normal development of mouse hearts. A whole cell patch clamp was used to record ionic currents from ventricular myocytes isolated from mice of early (E9.5) and late embryonic days (E18) and from adult 10-wk-old mice. Large T-type Ca2+ current (ICa,T) was observed at both E9.5 and E18, displaying similar voltage-dependence and kinetics of activation and inactivation. The current was inhibited by Ni2+ at relatively low concentrations (IC50 26-31 {micro}M). ICa,T was undetectable in adult myocytes. Quantitative PCR analysis revealed that Cav3.2 mRNA is the predominant subtype encoding T-type Ca2+ channels at both E9.5 and E18. Cav3.1 mRNA increased from E9.5 to E18, but remained low compared with Cav3.2 mRNA during the whole embryonic period. In the adulthood, in contrast, Cav3.1 mRNA is greater than Cav3.2 mRNA. These results indicate that Cav3.2 underlies the functional T-type Ca2+ channels in the embryonic murine heart, and there is a subtype switching of transcripts from Cav3.2 to Cav3.1 in the perinatal period.


http://ajpheart.physiology.org/cgi/content/abstract/288/4/H1997

Angiotensin receptor blockers (ARBs) reduce adverse left ventricular (LV) remodeling and improve LV function and survival when started postmyocardial infarction (MI). ARBs also reduce ventricular arrhythmias during ischemia-reperfusion injury when started pre-MI. No information exists regarding their efficacy and safety when started pre-MI and continued peri- and post-MI. We evaluated whether the ARB losartan improves the outcome when started pre-MI and continued peri- and post-MI. Male Wistar rats (n = 502) were treated for 7 days pre-MI with losartan at a high dose (30 mg{middle dot}kg-1{middle dot}day-1), progressively increasing dose (3 mg{middle dot}kg-1{middle dot}day-1 increased to 10 mg{middle dot}kg-1{middle dot}day-1 10 days and 30 mg{middle dot}kg-1{middle dot}day-1 20 days post-MI), or no treatment. Ambulatory systolic blood pressure and Holter monitoring were performed for 24 h post-MI. Echocardiography
was done 30 days post-MI, and LV remodeling, cardiac hemodynamics, and fetal gene expression were assessed 38 days post-MI. High-dose losartan reduced 24-h post-MI survival compared with the progressive dose and control (21.9% vs. 36.6% and 38.1%, P = 0.033 and P = 0.009, respectively). This was associated with greater hypotension in the high dose and no change in ventricular arrhythmias in all groups. In 24-h post-MI survivors, the progressive dose group had reduced mortality from 24 h to 38 days (8.5% vs. 28.6% for control vs. 38.9% for high dose, P = 0.032 and P = 0.01, respectively). Survivors of both losartan groups demonstrated improved LV remodeling, cardiac hemodynamics, preserved GLUT-4, and reduced cardiac fetal gene expression. Pretreatment with ARBs does not reduce 24-h post-MI ventricular arrhythmias or survival, and high doses increase mortality by causing excessive hypotension. In 24-h post-MI survivors, progressively increasing doses of losartan have multiple beneficial effects, including improved survival.


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We tested the hypothesis that TRPC3, a member of the canonical transient receptor potential (TRP) family of channels, mediates agonist-induced depolarization of arterial smooth muscle cells (SMCs). In support of this hypothesis, we observed that suppression of arterial SMC TRPC3 expression with antisense oligodeoxynucleotides significantly decreased the depolarization and constriction of intact cerebral arteries in response to UTP. In contrast, depolarization and contraction of SMCs induced by increased intravascular pressure, i.e., myogenic responses, were not altered by TRPC3 suppression. Interestingly, UTP-evoked responses were not affected by suppression of a related TRP channel, TRPC6, which was previously found to be involved in myogenic depolarization and vasoconstriction. In patch-clamp experiments, UTP activated a whole cell current that was greatly reduced or absent in TRPC3 antisense-treated SMCs. These results indicate that TRPC3 mediates UTP-induced depolarization of arterial SMCs and that TRPC3 and TRPC6 may be differentially regulated by receptor activation and mechanical stimulation, respectively.


http://ajpheart.physiology.org/cgi/content/abstract/288/2/H469

The goal of this study was to determine the role of estrogen receptor subtypes in the development of pressure overload hypertrophy in mice. Epidemiological studies have suggested gender differences in the development of hypertrophy and heart disease, but the mechanism and the role of estrogen receptor subtypes are not established. We performed transverse aortic constriction (TAC) and sham operations in male and female wild-type (WT) mice and mice lacking functional estrogen receptor-(alpha) [(alpha)-estrogen receptor knockout ((alpha)-ERKO)] and mice lacking estrogen receptor-(beta) ((beta)-ERKO). Body, heart, and lung weights were measured 2 wk postsurgery. WT male mice subjected to TAC showed a 64% increase in the heart weight-to-body weight ratio (HW/BW) compared with sham, and WT males have increased lung weight at 2 wk. WT female mice subjected to TAC showed a 31% increase in HW/BW compared with sham, which was significantly less than their male counterparts and with no evidence of heart failure. (alpha)-ERKO females developed HW/BW nearly identical to that seen in WT littermate females in response to TAC, indicating that estrogen receptor-(alpha) is not essential for the attenuation of hypertrophy observed in WT females. In contrast, (beta)-ERKO
females responded to TAC with a significantly greater increase in HW/BW than WT littermate females. \{beta\}-ERKO females have lower expression of lipoprotein lipase at baseline than WT or \{alpha\}-ERKO females. These data suggest an important role for estrogen receptor-{beta} in attenuating the hypertrophic response to pressure overload in females.


http://ajpheart.physiology.org/cgi/content/abstract/284/1/H268

Dilated cardiomyopathy, a disease of unknown etiology and pathogenesis, is associated with heart failure and compensatory hypertrophy. Although cell and animal models suggest a role for altered gene expression in the transition to heart failure, there is a paucity of data derived from the study of human heart tissue. In this study, we used DNA microarray profiling to investigate changes in the expression of genes involved in apoptosis that occur in human idiopathic dilated cardiomyopathic hearts that had progressed to heart failure. We observed altered gene expression consistent with a proapoptotic shift in the TNF-{alpha} signaling pathway. Specifically, we found decreased expression of TNF-{alpha}- and NF-{kappa}B-induced antiapoptotic genes such as growth arrest and DNA damage-inducible (GADD)45[beta], Flice inhibitory protein (FLIP), and TNF-induced protein 3 (A20). Consistent with a role for apoptosis in heart failure, we also observed a significant decrease in phosphorylation of BAD at Ser-112. This study identifies several pathways that are altered in human heart failure and provides new targets for therapy.


http://ajpheart.physiology.org/cgi/content/abstract/287/3/H1296

Ghrelin, a newly identified endogenous ligand for growth hormone secretagogue receptor 1a (GHSR-1a, i.e., ghrelin receptor), was recently demonstrated to be a potent vasoactive peptide. Although sepsis is characterized by an early, hyperdynamic phase, it remains unknown whether ghrelin or GHSR-1a plays a role in the cardiovascular response to sepsis. To determine this, polymicrobial sepsis was induced by cecal ligation and puncture in male adult rats. At 5 h (i.e., early sepsis) or 20 h (i.e., late sepsis) after cecal ligation and puncture, blood and tissue samples were collected. Ghrelin levels and ghrelin and GHSR-1a mRNA expression were assessed by RIA and RT-PCR, respectively. In addition, GHSR-1a protein levels in aorta, heart, and small intestine were determined by Western blotting. The vascular response to ghrelin was determined by using an isolated gut preparation. A primary rat aortic smooth muscle cell culture was used to determine the effects of LPS on GHSR-1a expression. The results indicate that although ghrelin levels decreased at early and late sepsis, its receptor was markedly elevated in early sepsis. Moreover, ghrelin-induced relaxation in resistance blood vessels of the isolated small intestine increased significantly during early sepsis but was not altered in late sepsis. Furthermore, GHSR-1a expression in smooth muscle cells was significantly increased at mRNA and protein levels with stimulation by LPS at 10 ng/ml. These results demonstrate that GHSR-1a expression is upregulated and vascular sensitivity to ghrelin stimulation is increased in the hyperdynamic phase of sepsis.

T-type Ca2+ channels may play a role in cardiac development. We studied the developmental regulation of the T-type currents (ICa,T) in cardiomyocytes (CMs) derived from mouse embryonic stem cells (ESCs). ICa,T was studied in isolated CMs by whole cell patch clamp. Subsequently, CMs were identified by the myosin light chain 2v-driven green fluorescent protein expression, and laser capture microdissection was used to isolate total RNA from groups of cells at various developmental time points. ICa,T showed characteristics of Cav3.1, such as resistance to Ni2+ block, and a transient increase during development, correlating with measures of spontaneous electrical activity. Real-time RT-PCR showed that Cav3.1 mRNA abundance correlated (r² = 0.81) with ICa,T. The mRNA copy number was low at 7+4 days (2 copies/cell), increased significantly by 7+10 days (27/cell; P < 0.01), peaked at 7+16 days (174/cell), and declined significantly at 7+27 days (25/cell). These data suggest that ICa,T is developmentally regulated at the level of mRNA abundance and that this regulation parallels measures of pacemaker activity, suggesting that ICa,T might play a role in the spontaneous contractions during CM development.


Transient receptor potential (TRP) cation channels are a critical pathway for Ca2+ entry during pulmonary artery (PA) smooth muscle contraction. However, whether canonical TRP (TRPC) subunits and which TRP channel isoforms are involved in store depletion-induced pulmonary vasoconstriction in vivo remain unclear. This study was designed to test whether overexpression of the human TRPC1 gene (hTRPC1) in rat PA enhances pulmonary vasoconstriction due to store depletion-mediated Ca2+ influx. The hTRPC1 was infected into rat PA rings with an adenoviral vector. RT-PCR and Western blot analyses confirmed the mRNA and protein expression of hTRPC1 in the arterial rings. The amplitude of active tension induced by 40 mM K+ (40K) in PA rings infected with an empty adenoviral vector (647 +/- 88 mg/mg) was similar to that in PA rings infected with hTRPC1 (703 +/- 123 mg/mg, P = 0.3). However, the active tension due to capacitative Ca2+ entry (CCE) induced by cyclopiazonic acid was significantly enhanced in PA rings overexpressing hTRPC1 (91 +/- 13% of 40K-induced contraction) compared with rings infected with an empty adenoviral vector (61 +/- 14%, P < 0.001). Endothelial expression of hTRPC1 was not involved since the CCE-induced vasoconstriction was also enhanced in endothelium-denuded PA rings infected with the adenoviral vector carrying hTRPC1. These observations demonstrate that hTRPC1 is an important Ca2+-permeable channel that mediates pulmonary vasoconstriction when PA smooth muscle cell intracellular Ca2+ stores are depleted.


To test the hypothesis that chronic intrauterine pulmonary hypertension (PHTN) compromises pulmonary artery (PA) smooth muscle cell (SMC) O2 sensing, fluorescence microscopy was used to study the effect of an acute increase in PO2 on the cytosolic Ca2+ concentration ([Ca2+]i) of chronically hypoxic subconfluent monolayers of PA SMC in primary culture. PA SMCs were derived from fetal lambs with PHTN due to intrauterine ligation of the ductus arteriosus. Acute normoxia decreased [Ca2+]i in control but not PHTN PA SMC. In control PA SMC, [Ca2+]i increased after Ca2+-sensitive (KCa) and voltage-sensitive (Kv) K+ channel blockade and decreased after diltiazem treatment. In PHTN PA SMC, KCa blockade had no effect, whereas Kv blockade and diltiazem increased [Ca2+]i. Inhibition of sarcoplasmic reticulum Ca2+ ATPase activity caused a greater increase in [Ca2+]i in controls compared with PHTN PA SMC. Conversely, ryanodine caused a greater increase of [Ca2+]i in PHTN compared with control PA SMC. KCa channel mRNA is decreased and Kv channel mRNA is unchanged in PHTN PA SMC compared with controls. We conclude that PHTN compromises PA SMC O2 sensing, alters intracellular Ca2+ homeostasis, and changes the predominant ion channel that determines basal [Ca2+]i from KCa to Kv.


Expression of cell adhesion molecule (CAM) in endothelial cells upon activation by human immunodeficiency virus (HIV) infection is associated with the development of atherosclerotic vasculopathy. We postulated that induction of vascular cell adhesion molecule-1 (VCAM-1) by
HIV-1 tat protein in endothelial cells might represent an early event that could culminate in inflammatory cell recruitment and vascular injury. We determined the role of HIV-1 tat protein in VCAM-1 expression in human pulmonary artery endothelial cells (HPAECs). HIV-1 tat protein treatment significantly increased cell-surface expression of VCAM-1 in HPAECs. Consistently, mRNA expression of VCAM-1 was also increased by HIV-1 tat protein as measured by RT-PCR. HIV-1 tat protein-induced VCAM-1 expression was abolished by the NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC) and the p38 MAPK inhibitor SB203580. Furthermore, HIV-1 tat protein enhanced DNA binding activity of NF-κB, facilitated nuclear translocation of NF-κB subunit p65, and increased production of reactive oxygen species (ROS). Similarly to VCAM-1 expression, HIV-1 tat protein-induced NF-κB activation and ROS generation were abrogated by PDTC and SB203580. These data indicate that HIV-1 tat protein is able to induce VCAM-1 expression in HPAECs, which may represent a pivotal early molecular event in HIV-induced vascular/pulmonary injury. These data also suggest that molecular mechanism underlying the HIV-1 tat protein-induced VCAM-1 expression may involve ROS generation, p38 MAPK activation and NF-κB translocation, which are the characteristics of pulmonary endothelial cell activation.


Surfactant protein D (SP-D) is a member of the collectin subfamily of C-type lectins, pattern recognition proteins participating in the innate immune response. Gene-targeted mice deficient in SP-D develop abnormalities in surfactant homeostasis, hyperplasia of alveolar epithelial type II cells, and emphysema-like pathology. Granulocyte/macrophage colony-stimulating factor (GM-CSF) is required for terminal differentiation and subsequent activation of alveolar macrophages, including the expression of matrix metalloproteinases and reactive oxygen species, factors thought to contribute to lung remodeling. Type II cells also express the GM-CSF receptor. Thus we hypothesized GM-CSF might mediate some or all of the cellular and structural abnormalities in the lungs of SP-D-deficient mice. To test this, SP-D (D-G+) and GM-CSF (D+G-) single knockout mice as well as double knockout mice deficient for both SP-D and GM-CSF (D-G-) were analyzed by design-based stereology. Compared with wild type, D-G+ as well as D+G- mice showed decreased alveolar numbers, increased alveolar sizes, and decreased alveolar epithelial surface areas. These emphysema-like changes were present to a greater extent in D-G- mice. D-G+ mice developed type II cell hyperplasia and hypertrophy with increased intracellular surfactant pools, whereas D+G- mice had smaller type II cells with decreased intracellular surfactant pools. In contrast to the emphysema-like changes, the type II cell alterations were mostly corrected in D-G- mice. These results indicate that GM-CSF-dependent macrophage activity is not necessary for emphysema development in SP-D-deficient mice, but that type II cell metabolism and proliferation are, either directly or indirectly, regulated by GM-CSF in this model.


Oei, Erwin, Thomas Kalb, Prarthana Beuria, Matthieu Allez, Atsushi Nakazawa, Miyuki Azuma, Michael Timony, Zanetta Stuart, Houchu Chen, and Kirk Sperber. Accessory cell function of airway epithelial cells. We previously demonstrated that airway epithelial cells (AECs) have many features of accessory cells, including expression of class II molecules CD80 and CD86 and
functional Fc(\(\gamma\)) receptors. We have extended these studies to show that freshly isolated AECs have mRNA for cathepsins S, V, and H [proteases important in antigen (Ag) presentation], invariant chain, human leukocyte antigen (HLA)-DM-(alpha) and HLA-DM-(beta), and CLIP, an invariant chain breakdown product. A physiologically relevant Ag, ragweed, was colocalized with HLA-DR in AECs, and its uptake was increased by granulocyte-macrophage colony-stimulating factor and IFN-(\(\gamma\)) treatments, which had no effect on CD80 and CD86 expression. We demonstrate the presence of other costimulatory molecules, including B7h and B7-H1, on AECs and the increased expression of B7-H1 on AECs after treatment with granulocyte-macrophage colony-stimulating factor and IFN-(\(\gamma\)). Finally, we compared T cell proliferation after allostimulation with AECs and dendritic cells (DCs). The precursor frequency of peripheral blood T cells responding to AECs was 0.264% compared with 0.55% for DCs. DCs stimulated CD45RO+, CD45RA+, CCR7+ and CCR7-CD4+, and CD8+ T cells, whereas AECs stimulated only CD45RO+, CD45RA-, CCR7-, CD4+, and CD8+ T cells. There was no difference in cytokine production, type of memory T cells stimulated (effector vs. long-term memory), or apoptosis by T cells cocultured with AECs and DCs. The localization of AECs exposed to the external environment may make them important in the regulation of local immune responses.


http://ajplung.physiology.org/cgi/content/abstract/282/3/L546

Mycobacterium tuberculosis (Mtbc) infection induces the expression of matrix metalloproteinase-9 (MMP-9) in mouse lungs. In cultured human mononuclear cells, Mtbc bacilli and the cell wall glycolipid lipoarabinomannan (LAM) stimulate high levels of MMP-9 activity. Here, we explore the cellular mechanisms involved in the induction of MMP-9 by Mtbc. We show that infection of THP-1 cells with Mtbc caused a fivefold increase in MMP-9 mRNA that was associated with increased MMP-9 activity. MMP-9 induction was dependent on microtubule polymerization and protein kinase activation and was associated with increased DNA binding by the transcription factor activator protein-1 (AP-1), which appeared to be important for MMP-9 expression. We then explored the surface molecules potentially involved in Mtbc induction of MMP-9, focusing on ligands of the mannose and [beta]-glucan receptors. MMP-9 activity was induced by the mannose receptor ligands mannan, zymosan, and LAM, whereas the [beta]-glucan receptor ligand laminarin was not effective. The most active inducers of MMP-9 activity were the particulate ligand zymosan and LAM. Pretreatment of cells with an anti-mannose receptor monoclonal antibody, but not anti-complement receptor 3, decreased the induction of MMP-9 activity by Mtbc bacilli. Together, these results suggest that MMP-9 induction by Mtbc occurs by receptor-mediated signaling mechanisms involving the binding of mannosylated ligands to mannose receptors, the modulation by cytoskeletal elements such as microtubules, the activation of protein kinases, and transcriptional activation by AP-1.


http://ajplung.physiology.org/cgi/content/abstract/282/2/L207

Staphylococcus aureus [alpha]-toxin is a pore-forming bacterial exotoxin that has been implicated as a significant virulence factor in human staphylococcal diseases. In primary cultures of rat pneumocyte type II cells and the human A549 alveolar epithelial cell line, purified [alpha]-toxin provoked rapid-onset phosphatidylinositol (PtdIns) hydrolysis as well as liberation of nitric oxide and the prostanoids PGE2, PGI2, and thromboxane A2. In addition, sustained upregulation of
proinflammatory interleukin (IL)-8 mRNA expression and protein secretion occurred. "Priming" with low-dose IL-1[beta] markedly enhanced the IL-8 response to [alpha]-toxin, which was then accompanied by IL-6 appearance. The cytokine response was blocked by the intracellular Ca2+-chelating reagent 1,2-bis(2-aminophenoxy)-ethane-N,N',N'-tetraacetic acid, the protein kinase C inhibitor bis-indolyl maleimide I, as well as two independent inhibitors of nuclear factor-[kappa]B activation, pyrrolidine dithiocarbamate and caffeic acid phenethyl ester. We conclude that alveolar epithelial cells are highly reactive target cells of staphylococcal [alpha]-toxin. [alpha]-Toxin pore-associated transmembrane Ca2+ flux and PtdIns hydrolysis-related signaling with downstream activation of protein kinase C and nuclear translocation of nuclear factor-[kappa]B are suggested to represent important underlying mechanisms. Such reactivity of the alveolar epithelial cells may be relevant for pathogenic sequelae in staphylococcal lung disease.


http://ajplung.physiology.org/cgi/content/abstract/285/5/L1026

In this study, we examined the sequential expression of several matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors as well as the presence of apoptosis in a model of pulmonary fibrosis induced in rats with paraquat and hyperoxia. Animals showing neither clinical nor morphological changes with this double aggression were classified as "resistant". Rats were killed at 1, 2, 3, and 6 wk, and lungs were used for collagen content, gene expression by real-time PCR, gelatinolytic activity by zymography, apoptosis by in situ DNA fragmentation, and protein localization by immunohistochemistry. Our results showed a significant decrease of collagenases MMP-8 and MMP-13, with an increase of TIMP-1 and transforming growth factor-(beta). Immuno reactive TIMP-1 was increased in experimental rats and primarily localized in alveolar macrophages. Expression of gelatinases MMP-2 and MMP-9 mRNAs was not affected, but lung zymography revealed an increase in progelatinase B, progelatinase A, and its active form. Epithelial apoptosis was evident from the first week, whereas at later periods, interstitial cell apoptosis was also noticed. Resistant animals behave as controls. These findings suggest that an imbalance between collagenases and TIMPs, excessive gelatinolytic activity, and epithelial apoptosis participate in the fibrotic response in this experimental model.


http://ajplung.physiology.org/cgi/content/abstract/282/3/L394

Surfactant protein B (SP-B) is a developmentally and hormonally regulated lung protein that is required for normal surfactant function. We generated transgenic mice carrying the human SP-B promoter ([-]1,039/+431 bp) linked to chloramphenicol acetyltransferase (CAT). CAT activity was high in lung and immunoreactive protein localized to alveolar type II and bronchiolar epithelial cells. In addition, thyroid, trachea, and intestine demonstrated CAT activity, and each of these tissues also expressed low levels of SP-B mRNA. Developmental expression of CAT activity and SP-B mRNA in fetal lung were similar and both increased during explant culture. SP-B mRNA but not CAT activity decreased during culture of adult lung, and both were reduced by transforming growth factor (TGF)-[beta]1. Treatment of adult mice with intratracheal bleomycin caused similar time-dependent decreases in lung SP-B mRNA and CAT activity. These findings indicate that the human SP-B promoter fragment directs tissue- and lung cell-specific transgene expression and contains cis-acting elements involved in regulated expression during development, fetal lung
explant culture, and responsiveness to TGF-[beta] and bleomycin-induced lung injury.


http://ajplung.physiology.org/cgi/content/abstract/283/1/L144

Pulmonary vascular medial hypertrophy due to proliferation of pulmonary artery smooth muscle cells (PASMC) greatly contributes to the increased pulmonary vascular resistance in pulmonary hypertension patients. A rise in cytosolic free Ca2+ concentration ([Ca2+]cyt) is an important stimulus for cell growth in PASMC. Resting [Ca2+]cyt, intracellularly stored [Ca2+], capacitative Ca2+ entry (CCE), and store-operated Ca2+ currents (ISOC) are greater in proliferating human PASMC than in growth-arrested cells. Expression of TRP1, a transient receptor potential gene proposed to encode the channels responsible for CCE and ISOC, was also upregulated in proliferating PASMC. Our aim was to determine if inhibition of endogenous TRP1 gene expression affects ISOC and CCE and regulates cell proliferation in human PASMC. Cells were treated with an antisense oligonucleotide (AS, for 24 h) specifically designed to cleave TRP1 mRNA and then returned to normal growth medium for 40 h before the experiments. Then, mRNA and protein expression of TRP1 was downregulated, and amplitudes of ISOC and CCE elicited by passive depletion of Ca2+ from the sarcoplasmic reticulum using cyclopiazonic acid were significantly reduced in the AS-treated PASMC compared with control. Furthermore, the rate of cell growth was decreased by 50% in AS-treated PASMC. These results indicate that TRP1 may encode a store-operated Ca2+ channel that plays a critical role in PASMC proliferation by regulating CCE and intracellular [Ca2+]cyt.


http://ajplung.physiology.org/cgi/content/abstract/286/4/L777

Retinoic acid (RA) is known to accelerate wound healing and induce cell differentiation. All-trans RA (ATRA) exerts its effect by binding retinoic acid receptors, which are members of the nuclear receptor family. We investigated whether RA can alter expression of eotaxin, a potent eosinophil chemoattractant that is regulated by the transcription factors signal transducer and activator of transcription 6 (STAT6) and NF-{kappa}B. We examined the effects of RA on eotaxin expression in a human bronchial epithelial cell line BEAS-2B. ATRA and its stereodimer 9-cis retinoic acid (9-cis RA) inhibited IL-4-induced release of eotaxin at 10-6 M by 78.0 and 52.0%, respectively (P < 0.05). ATRA and 9-cis RA also significantly inhibited IL-4-induced eotaxin mRNA expression at 10-6 M by 52.3 and 53.5%, respectively (P < 0.05). In contrast, neither ATRA nor 9-cis RA had any effects on TNF-{alpha}-induced eotaxin production. In transfection studies using eotaxin promoter luciferase plasmids, the inhibitory effect of ATRA on IL-4-induced eotaxin production was confirmed at the transcriptional level. Interestingly, ATRA had no effects on IL-4-induced tyrosine phosphorylation, nuclear translocation, or DNA binding activity of STAT6. Activating protein-1 was not involved in ATRA-mediated transrepression of eotaxin with IL-4 stimulation. The mechanism of the inhibitory effect of ATRA on IL-4-induced eotaxin production in human bronchial epithelial cells has not been elucidated but does not appear to be due to an effect on STAT6 activation. These findings raise the possibility that RA may reduce eosinophilic airway inflammation, one of the prominent pathological features of allergic diseases such as bronchial asthma.
Connective tissue growth factor (CTGF), a potent profibrotic mediator, acts downstream and in concert with transforming growth factor (TGF)-β to drive fibrogenesis. Significant upregulation of CTGF has been reported in fibrogenic diseases, including idiopathic pulmonary fibrosis (IPF), and is partly responsible for associated excessive fibroblast proliferation and extracellular matrix deposition, but no effective therapy exists for averting such fibrogenic events. Simvastatin has reported putative antifibrotic actions in renal fibroblasts; this study explores such actions on human IPF-derived and normal lung fibroblasts and examines associated driving mechanisms. Simvastatin reduces basal CTGF gene and protein expression in all fibroblast lines, overriding TGF-β induction through inhibition of the cholesterol synthesis pathway. Signaling pathways driving simvastatin's effects on CTGF/TGF-β interaction were evaluated using transient reporter transfection of a CTGF promoter construct. Inhibition of CTGF promoter activity by simvastatin was most marked at 10 μM concentration, reducing activity by 76.2 and 51.8% over TGF-β-stimulated cultures in IPF and normal fibroblasts, respectively. We also show that geranylgeranylpiprophosphate (GGPP), but not farnesylpyrophosphate, induces CTGF promoter activity following simvastatin inhibition by 55.3 and 31.1% over GGPP-negative cultures in IMR90 and IPF-derived fibroblasts, respectively, implicating small GTPase Rho involvement rather than Ras in these effects. Indeed, the specific Rho inhibitor C3 exotoxin significantly (P < 0.05) suppressed TGF-β-induced CTGF promoter activity in transfected lung fibroblasts, a finding further supported by transfection of dominant-negative and constitutively active RhoA constructs, thus demonstrating that simvastatin through a Rho signaling mechanism in lung fibroblasts can modulate CTGF expression and interaction with TGF-β.

Am J Physiol Regulatory Integrative Comp Physiol  (12)


In obesity-related hypertension, activation of the renin-angiotensin system (RAS) has been reported despite marked fluid volume expansion. Adipose tissue expresses components of the RAS and is markedly expanded in obesity. This study evaluated changes in components of the adipose and systemic RAS in diet-induced obese hypertensive rats. RAS was quantified in adipose tissue and compared with primary sources for the circulating RAS. Male Sprague-Dawley rats were fed either a low-fat (LF; 11% kcal as fat) or moderately high-fat (32% kcal as fat) diet for 11 wk. After 8 wk, rats fed the moderately high-fat diet segregated into obesity-prone (OP) and obesity-resistant (OR) groups based on their body weight gain (body weight: OR, 566 ± 10; OP, 702 ± 20 g; P < 0.05). Mean arterial blood pressure was increased in OP rats (LF: 97 ± 2; OR: 97 ± 2; OP: 105 ± 1 mmHg; P < 0.05). Quantification of mRNA expression by real-
time PCR demonstrated a selective increase (2-fold) in angiotensinogen gene expression in retroperitoneal adipose tissue from OP vs. OR and LF rats. Similarly, plasma angiotensinogen concentration was increased in OP rats (LF: 390 {+/-} 48; OR: 355 {+/-} 24; OP: 530 {+/-} 22 ng/ml; P < 0.05). In contrast, other components of the RAS were not altered in OP rats. Marked increases in the plasma concentrations of angiotensin peptides were observed in OP rats (angiotensin II: LF: 95 {+/-} 31; OR: 59 {+/-} 20; OP: 295 {+/-} 118 pg/ml; P < 0.05). These results demonstrate increased activity of the adipose and systemic RAS in obesity-related hypertension.


http://ajpregu.physiology.org/cgi/content/abstract/287/1/R69

Many mammals, nearing the end of life, spontaneously decrease their food intake and body weight, a stage we refer to as senescence. The spontaneous decrease in food intake and body weight is associated with attenuated responses to intracerebroventricular injections of neuropeptide Y (NPY) compared with old presenescent or with young adult rats. In the present study, we tested the hypothesis that this blunted responsiveness involves the number and expression of hypothalamic paraventricular nucleus (PVN) Y1 and/or Y5 NPY receptors, both of which are thought to mediate NPY-induced food intake. We found no significant difference in mRNA levels, via quantitative PCR, for Y1 and Y5 receptors in the PVN of senescent vs. presenescent rats. In contrast, immunohistochemistry indicated that the number of PVN neurons staining for Y1 receptor protein was greater in presenescent compared with senescent rats. We conclude that a decreased expression and number of Y1 or Y5 receptors in the PVN cannot explain the attenuated responsiveness of the senescent rats to exogenous NPY.


http://ajpregu.physiology.org/cgi/content/abstract/288/5/R1316

Burn injury has been shown to impair gut transit, but the exact mechanism remains unknown. The present study investigated whether nitric oxide synthase (NOS) and cyclooxygenase (COX) mediated changes in burn-induced colonic transit. After rats underwent 30% total body surface area burn injury, they were injected with S-methylisothiourea (SMT, selective inducible NOS inhibitor), 7-nitronitazol (7-NI, selective neuronal NOS inhibitor), and nimesulide (NIM, selective COX-2 inhibitor), respectively. The protein and mRNA of NOS and COX-2 were measured by Western blot analysis and real-time RT-RCR, and localization of NOS and COX-2 protein was determined by immunohistochemistry. Our results showed that colonic transit assessed by the geometric center was delayed from 3.47 {+/-} 0.28 in controls to 2.21 {+/-} 0.18 after burn (P < 0.009). SMT and NIM significantly improved colonic transit in burned rats but had no effect in sham-operated rats. 7-NI failed to modify delayed transit in burned rats but significantly delayed colonic transit in sham-operated rats. Both protein and mRNA of inducible NOS and COX-2 increased significantly but not neuronal NOS in burned rats. Inducible NOS protein expression was noted not only in epithelial cells but also in neurons of the myenteric ganglia in burned rats. These findings suggest that nitric oxide (NO) produced by neuronal NOS plays an important role in mediating colonic transit under the physiological condition. NO produced by inducible NOS and prostaglandins synthesized by COX-2 are both involved in the pathogenesis of delayed colonic transit after burn injury. Inducible NOS expression in neurons of the myenteric ganglia may contribute to dysmotility with burn injury.

http://ajpregu.physiology.org/cgi/content/abstract/287/2/R397

The present study investigated the effect of an acute exercise bout on the mRNA response of vascular endothelial growth factor (VEGF) splice variants in untrained and trained human skeletal muscle. Seven habitually active young men performed one-legged knee-extensor exercise training at an intensity corresponding to [~]70% of the maximal workload in an incremental test five times/week for 4 wk. Biopsies were obtained from the vastus lateralis muscle of the trained and untrained leg 40 h after the last training session. The subjects then performed 3 h of two-legged knee-extensor exercise, and biopsies were obtained from both legs after 0, 2, 6, and 24 h of recovery. Real-time PCR was used to examine the expression of VEGF mRNA containing exon 1 and 2 (all VEGF isoforms), exon 6 or exon 7, and VEGF165 mRNA. Acute exercise induced an increase (P < 0.05) in total VEGF mRNA levels as well as VEGF165 and VEGF splice variants containing exon 7 at 0, 2, and 6 h of recovery. The increase in VEGF mRNA was higher in the untrained than in the trained leg (P < 0.05). The results suggest that in human skeletal muscle, acute exercise increases total VEGF mRNA, an increase that appears to be explained mainly by an increase in VEGF165 mRNA. Furthermore, 4 wk of training attenuated the exercise-induced response in skeletal muscle VEGF165 mRNA.


http://ajpregu.physiology.org/cgi/content/abstract/283/6/R1450

Milk-borne insulin-like growth factors (IGFs) enhance nutrient absorption in the immature intestine, which is characterized by low levels of glucose oxidation. We therefore hypothesized that feeding a rat milk substitute (RMS) devoid of growth factors to rat pups would lower serum glucose levels relative to dam-fed control rats and that supplementation of RMS with physiological doses of either IGF-I or IGF-II would normalize serum glucose levels via increased jejunal glucose transporter 2 (GLUT2) and high-affinity Na+-glucose cotransporter (SGLT1) expression. We found lower serum glucose concentrations in RMS-fed pups; in contrast, serum glucose levels in the IGF-supplemented pups were similar to those of dam-fed controls. RT-PCR and laser scanning confocal microscopy similarly demonstrated that IGF supplementation increased expression of jejunal glucose transporters. Further experiments demonstrated that IGF supplementation altered mRNA levels of key mitochondrial enzymes without altering jejunal lactase activity. We conclude that IGF-I and IGF-II supplementation increases serum glucose levels in the immature rat pup fed artificial formula and alters gene expression of the jejunal glucose transporters.


http://ajpregu.physiology.org/cgi/content/abstract/282/6/R1762
Bacterial lipopolysaccharide (LPS) induces fever that is mediated by pyrogenic cytokines such as interleukin (IL)-1[beta]. We hypothesized that the anti-inflammatory cytokine IL-10 modulates the febrile response to LPS by suppressing the production of pyrogenic cytokines. In rats, intravenous but not intracerebroventricular infusion of IL-10 was found to attenuate fever induced by peripheral administration of LPS (10 {micro}g/kg iv). IL-10 also suppressed LPS-induced IL-1[beta] production in peripheral tissues and in the brain stem. In contrast, central administration of IL-10 attenuated the febrile response to central LPS (60 ng/rat icv) and decreased IL-1[beta] production in the hypothalamus and brain stem but not in peripheral tissues and plasma. Furthermore, intravenous LPS upregulated expression of IL-10 receptor (IL-10R1) mRNA in the liver, whereas intracerebroventricular LPS enhanced IL-10R1 mRNA in the hypothalamus. We conclude that IL-10 modulates the febrile response by acting in the periphery or in the brain dependent on the primary site of inflammation and that its mechanism of action most likely involves inhibition of local IL-1[beta] production.


http://ajpregu.physiology.org/cgi/content/abstract/283/2/R496

Exposure to chronic hypoxia induces erythropoietin (EPO) production to facilitate oxygen delivery to hypoxic tissues. Previous studies from our laboratory found that ovariectomy (OVX) exacerbates the polycythemic response to hypoxia and treatment with 17[beta]-estradiol (E2-[beta]) inhibits this effect. We hypothesized that E2-[beta] decreases EPO gene expression during hypoxia. Because E2-[beta] can induce nitric oxide (NO) production and NO can attenuate EPO synthesis, we further hypothesized that E2-[beta] inhibition of EPO gene expression is mediated by NO. These hypotheses were tested in OVX catheterized rats treated with E2-[beta] (20 {micro}g/day) or vehicle for 14 days and exposed to 8 or 12 h of hypoxia (12% O2) or normoxia. We found that E2-[beta] treatment significantly decreased EPO synthesis and gene expression during hypoxia. E2-[beta] treatment did not induce endothelial NO synthase (eNOS) expression in the kidney but potentiated hypoxia-induced increases in plasma nitrites. We conclude that E2-[beta] decreases hypoxic induction of EPO. However, this effect does not appear to be related to changes in renal eNOS expression.


http://ajpregu.physiology.org/cgi/content/abstract/00771.2004v1

The present study tested the hypothesis that exercise with a large compared with a small active muscle mass results in a higher contraction induced increase in Na+, K+ -ATPase mRNA expression, due to greater hormonal responses. Furthermore, the relative abundance of Na+, K+ -ATPase subunits {alpha}1, {alpha}2, {alpha}3, {alpha}4, {beta}1, {beta}2 and {beta}3 mRNA in human skeletal muscle was investigated. On two occasions, eight subjects performed one-legged knee-extension exercise (L) or combined one-legged knee-extension and bi-lateral arm cranking (AL) for 5.00, 4.25, 3.50, 2.75 and 2.00 min separated by 3 min of rest. Leg exercise power output was the same in AL and L (57{+/-}5 vs. 59{+/-}5 W), but heart rate (HR) at the end of each exercise interval was higher in AL compared with L (91{+/-}3% vs. 61{+/-}12% of maximal HR, P<0.001). One minute after exercise, arm venous blood lactate was higher (P<0.001) in AL than in L (11.4{+/-}4.2 vs. 4.2{+/-}2.2 mM). A higher (P<0.05) level of blood epinephrine (141%) and norepinephrine (380%) was evident three minutes after exercise in AL compared with L. Nevertheless, none of the exercise induced increases (P<0.001) in {alpha}1, {alpha}2, {beta}1
and {\(\beta\)3 mRNA expression levels were higher in AL compared with L. The most abundant Na\(^{+}\), K\(^{+}\) -ATPase subunit at the mRNA level was {\(\beta\)1}, which was 3.4 times more expressed than {\(\alpha\)2} (P<0.001). Expression of {\(\alpha\)1}, {\(\beta\)2} and {\(\beta\)3} was less than 5% of the {\(\alpha\)2} expression, and no reliable detection of {\(\alpha\)3} and {\(\alpha\)4} was possible. In conclusion, activation of additional muscle mass does not result in a higher exercise induced increase in Na\(^{+}\), K\(^{+}\) -ATPase subunit specific mRNA.


http://ajpregu.physiology.org/cgi/content/abstract/288/2/R482

The rev-erbA{\(\alpha\)} orphan protein belongs to the steroid nuclear receptor superfamily. No ligand has been identified for this protein, and little is known of its function in development or physiology. In this study, we focus on 1) the distribution of the rev-erbA{\(\alpha\)} protein in adult fast- and slow-twitch skeletal muscles and muscle fibers and 2) how the rev-erbA{\(\alpha\)} protein influences myosin heavy chain (MyHC) isoform expression in mice heterozygous (+/−) and homozygous (−/−) for a rev-erbA{\(\alpha\)} protein null allele. In the fast-twitch extensor digitorum longus muscle, rev-erbA{\(\alpha\)} protein expression was linked to muscle fiber type; however, MyHC isoform expression did not differ between wild-type, +/−, or −/− mice. In the slow-twitch soleus muscle, the link between rev-erbA{\(\alpha\)} protein and MyHC isoform expression was more complex than in the extensor digitorum longus. Here, a significantly higher relative amount of the {\(\beta\)/slow} (type I) MyHC isoform was observed in both rev-erbA{\(\alpha\)}−/− and +/− mice vs. that shown in wild-type controls. A role for the ratio of thyroid hormone receptor proteins {\(\alpha\)1} to {\(\alpha\)2} in modulating MyHC isoform expression can be ruled out because no differences were seen in MyHC isoform expression between thyroid hormone receptor {\(\alpha\)2}-deficient mice (heterozygous and homozygous) and wild-type mice. Therefore, our data are compatible with the rev-erbA{\(\alpha\)} protein playing an important role in the regulation of skeletal muscle MyHC isoform expression.


http://ajpregu.physiology.org/cgi/content/abstract/282/4/R1164

The present study was designed to examine whether changes in Ca\(^{2+}\) release by inositol-1,4,5-trisphosphate (IP3) in 8-, 15-, and 30-day-old rat skeletal muscles could be associated with the expression of IP3 receptors. Experiments were conducted in slow-twitch muscle in which both IP3-induced Ca\(^{2+}\) release and IP3-receptor (IP3R) expression have been shown to be larger than in fast-twitch muscle. In saponin-skinned fibers, IP3 induced transient contractile responses in which the amplitude was dependent on the Ca\(^{2+}\)-loading period with the maximal IP3 contracture being at 20 min of loading. The IP3 tension decreased during postnatal development, was partially inhibited by ryanodine (100 {\(\mu\)M}), and was blocked by heparin (20-400 {\(\mu\)g/ml}). Amplification of the DNA sequence encoding for IP3R isoforms (using the RT-PCR technique) showed that in slow-twitch muscle, the type 2 isoform is mainly expressed, and its level decreases during postnatal development in parallel with changes in IP3 responses in immature fibers. IP3-induced Ca\(^{2+}\) release would then have greater participation in excitation-contraction coupling in developing fibers than in mature muscle.
Previous reports implicate the orexins in eating and body weight regulation. This study investigated possible functional relationships between hypothalamic orexins and circulating hormones or metabolites. In situ hybridization and quantitative PCR were used to examine orexin expression in the perifornical hypothalamus (PF) of rats and mice on diets varying in fat content and with differential propensity toward obesity. The results showed that orexin gene expression was stimulated by a high-fat diet in close association with elevated triglyceride levels, suggesting a functional relationship between these measures. Results obtained in obesity-prone rats and mice revealed a similar increase in orexin in close relation to triglycerides. A direct test of this orexin-triglyceride link was performed with Intralipid, which increased PF orexin expression along with circulating triglycerides. Whereas PF galanin is similarly stimulated by dietary fat, double-labeling immunofluorescence studies showed that orexin and galanin neurons are anatomically distinct. This evidence suggests that the orexins, like galanin, are "fat-responsive" peptides that respond to circulating lipids.


http://ajpregu.physiology.org/cgi/content/abstract/283/5/R1198

Animals reared at 18(degrees)C exhibit enhanced innervation of brown adipose tissue (BAT) and greater cold tolerance as adults, yet gain more weight when fed an enriched diet compared with rats reared at 30(degrees)C. To explore this paradox, sympathoadrenal activity was examined using techniques of [3H]norepinephrine ([3H]NE) turnover and urinary catecholamine excretion in male and female rats reared until 2 mo of age at 18 or 30(degrees)C. Gene expression in BAT was also analyzed for several sympathetically related proteins. Although [3H]NE turnover in heart did not differ between groups, [3H]NE turnover in BAT was consistently elevated in the 18(degrees)C-reared animals, even 2 mo after removal from the cool environment. Gene expression for uncoupling proteins 1 and 3, GLUT-4, leptin, and the [alpha]1A-adrenergic receptor was more abundant in BAT and the increase in epinephrine excretion with fasting suppressed in 18(degrees)C-reared animals. These studies demonstrate that obesity consequent to exposure to 18(degrees)C in early life occurs despite tonic elevation of sympathetic input to BAT. Diminished adrenal epinephrine responsiveness to fasting may play a contributory role.

Mutations that disrupt a PY motif in epithelial Na+ channel (ENaC) subunits increase surface expression of Na+ channels in the collecting duct, resulting in greater Na+ reabsorption. Recently, Nedd4 and Nedd4-2 have been identified as ubiquitin ligases that can interact with ENaC via its PY motifs to regulate channel activity. To further understand the role of human Nedd4-2 (hNedd4-2), we cloned its cDNAs and determined its genomic organization using a bioinformatic approach. The gene is present as a single copy, spans at least 400 kb, and contains >40 exons. Multiple 5'-exons were identified by 5'-rapid amplification of cDNA ends, and tissue-specific expression of these transcripts was noted by RT-PCR and RNase protection assay. Alternate polyadenylation signal sequences led to varying lengths of the 3'-untranslated region. Alternate splicing events within internal exons were also noted. Open reading frame analysis indicates that hNedd4-2 encode multiple protein variants with and without a C2 domain, and with a variable number of WW domains. Coexpression, in Fischer rat thyroid epithelia, of ENaC and Nedd4-2 cDNAs leads to a significant reduction in amiloride-sensitive currents, confirming a role in Na+ transport regulation. In vitro binding studies demonstrated that individual PY motifs of \(\alpha\)-, \(\beta\)-, and \(\gamma\)-ENaC have strong affinity for WW domains 3 and 4 but not 1 and 2. These studies indicate that alternate transcripts of Nedd4-2 may interact with ENaC differently. Understanding the function of variant proteins will increase our knowledge of the role of hNedd4-2 in the regulation of ENaC and define protein domains important for Nedd4-2 function.


Evaluation of thick ascending limb (TAL) function has been hindered by the limited ability to selectively examine the function of this nephron segment in vivo. To address this, a Cre/loxP strategy was employed whereby the Tamm-Horsfall (THP) promoter was used to drive Cre recombinase expression in transgenic mice. The THP gene was cloned from a mouse genomic library, and 3.7 kb of the mouse THP 5'-flanking region containing the first noncoding exon of the THP gene were inserted upstream of an epitope-tagged Cre recombinase (THP-CreTag). THP-CreTag transgenic mice were bred with ROSA26-enhanced yellow fluorescent protein (eYFP) mice (contain a loxP-flanked “STOP” sequence 5' to eYFP), and doubly heterozygous offspring were analyzed. THP and eYFP were expressed in an identical pattern with predominant localization to the renal outer medulla without expression in nonrenal tissues. eYFP did not colocalize with thiazide-sensitive cotransporter (distal tubule) or neuronal nitric oxide synthase (macula densa) expression. THP mRNA expression was detected only in kidney, whereas CreTag mRNA was also present in testes. These data indicate that THP-CreTag transgenic mice can be used for TAL-specific gene recombination in the kidney.

Background: Compromised barrier function and intestinal inflammation are common complications of total parenteral nutrition (TPN). Objective: We tested the hypothesis that the lack of enteral nutrients in TPN might select commensal or pathogenic bacteria that use mucus as a substrate, thereby weakening the protection provided by the intestinal mucus layer. Design: Ileal microbiota profiles of piglets fed by total enteral nutrition (TEN; n = 6) or TPN (n = 5) were compared with the use of 16S ribosomal DNA polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis and with a PCR-based method developed to specifically measure Clostridium perfringens concentrations. Ileal bacteria from TEN and TPN piglets were also examined for their ability to grow on mucin or sulfated monosaccharides. Results: Bacterial community structure was equally complex in the ileum of TEN and TPN piglets, but profiles clustered according to mode of nutrition. Sixty-two percent of total mucus-associated bacteria (100 colonies tested) in TPN compared with 33% of mucus-associated bacteria (100 colonies tested) in TEN ileal samples grew on mucin. Bacteria capable of using sulfated monosaccharides were also enriched in TPN samples. C. perfringens, an opportunistic pathogen, was specifically enriched in the TPN ileum (P < 0.05). These results were corroborated by cultivation-based studies that showed rapid growth of C. perfringens on mucin-based substrates. Conclusions: Mucolytic potential is widespread among intestinal bacteria. Mucolytic bacteria in general and C. perfringens in particular were selected when enteral nutrients were withheld in this TPN piglet model. Similar enrichment processes may occur in humans nourished by TPN and may thereby contribute to intestinal dysfunction.


Background: The association between polymorphisms in the scavenger receptor class B type I (SRB-I) gene and variations in basal plasma concentrations of cholesterol in humans has recently been described. Objective: The objective of the study was to determine whether the exon 1 variant (G[-]A) at the SRB-I gene is associated with the lipid response to the content and quality of dietary fat in healthy subjects. Design: We studied 97 healthy volunteers with exon 1 polymorphism [65 homozygous for allele 1 (1/1) and 32 heterozygous for allele 2 (1/2)]. Both groups consumed 3 diets lasting 4 wk each. The first was a saturated fatty acid (SFA)-rich diet (38% fat, 20% SFA), which was followed by a carbohydrate (Cho)-rich diet (30% fat, < 10% SFA, 55% carbohydrate) or a monounsaturated fatty acid (MUFA), olive oil-rich diet (38% fat, 22% MUFA) according to a randomized crossover design. At the end of each dietary period, plasma concentrations of triacylglycerol and of total, LDL, and HDL cholesterol were measured. Results: Carriers of the 1/2 genotype had a trend toward higher concentrations of LDL cholesterol (P < 0.11) after the SFA-rich diet than did those who were homozygous for 1/1. Carriers of the mutation showed a significantly greater (P = 0.007) decrease in LDL-cholesterol concentrations (-23%) in changing from an SFA-rich diet to a Cho-rich diet than did noncarriers of the mutation (-16%). Conclusion: Carriers of the minority allele, 1/2, are more susceptible to the presence of SFA in the diet because of a greater increase in LDL cholesterol.

http://aje.oupjournals.org/cgi/content/abstract/159/3/269

The association between androgen receptor gene polymorphisms and benign prostatic hyperplasia was investigated among 510 men randomly selected from Olmsted County, Minnesota. From 1990 through 2000, lower urinary tract symptom severity was assessed by the American Urological Association Symptom Index (AUASI), and peak urinary flow rate, prostate volume, and serum prostate-specific antigen level were measured. Androgen receptor CAG and GGN genotyping was performed. A CAG repeat length of <21 was associated with an enlarged prostate (hazard ratio (HR) = 1.4, 95% confidence interval (CI): 1.0, 1.9) and a serum prostate-specific antigen level >1.4 ng/ml (HR = 1.5, 95% CI: 1.1, 2.0). A GGN repeat length of <16 was associated with an AUASI >7 (HR = 1.6, 95% CI: 1.1, 2.3) and a serum prostate-specific antigen level >1.4 ng/ml (HR = 1.5, 95% CI: 1.0, 2.3). Having <21 CAG repeats and <16 GGN repeats compared with having neither was associated with an enlarged prostate (HR = 2.5, 95% CI: 1.5, 4.2), a serum prostate-specific antigen level >1.4 ng/ml (HR = 2.8, 95% CI: 1.6, 4.7), a peak flow rate <12 ml/second (HR = 1.9, 95% CI: 1.1, 3.4), and an AUASI >7 (HR = 1.6, 95% CI: 1.0, 2.7). Androgen receptor gene polymorphisms may have a potential role in the pathogenesis of benign prostatic hyperplasia.

Am. J. Geriatr. Psychiatry (1)


http://ajgp.psychiatryonline.org/cgi/content/abstract/10/5/619

OBJECTIVE: A systematic genome survey was initiated to identify loci that affect the likelihood of reaching age 90 with preserved cognition. This communication describes the clinical characterization and comparison of the experimental groups, validation of the experimental method, and results for the Y chromosome. METHODS: The genome survey was conducted at 10 cM resolution for simple sequence tandem repeat polymorphisms (SSTRPs) that identify genes for successful aging by virtue of linkage disequilibrium. Efficiency was enhanced by genotyping pools of DNA from 100 cognitively intact elders (50 men/50 women) and 100 young (age 18-25 years) adults matched for sex, race, ethnicity, and geographic location. RESULTS: Elders (94 nonagenarians, 6 centenarians) manifested preserved cognition, as reflected by clinical and psychometric assessments; "good" average capacity to carry out their activities of daily living; and the majority were living independently despite multiple medical conditions. None had a history of mental disorders in early or middle adulthood, only one was a current smoker, and 80% consumed alcohol less than once each month. The genome survey method detected the expected elevation of the APOE {epsilon}2 allele frequency, and reciprocal reduction in the {epsilon}4 frequency, among the elders, compared with the young adults. It also detected significant differences in the allelic distributions of DYS389 and DYS390, which are separated by only 2.6 Mb near the centromere of Yq. CONCLUSIONS: These results suggest that several behavioral and genetic factors may contribute to the likelihood of achieving exceptional longevity.
with preserved cognition.


http://ajrcmb.atsjournals.org/cgi/content/abstract/27/3/329

Eotaxin is a critical chemokine eliciting migration of eosinophils and basophils in the pathogenesis of bronchial asthma. Recent studies have shown that the specific receptor for eotaxin, CCR3, is expressed in bronchial epithelial cells. Although mitogen-activated protein (MAP) kinases are involved in diverse cell functions of bronchial epithelial cells, their role in eotaxin signaling is unknown. In this study, we studied the activation and functional relevance of MAP kinases in bronchial epithelial cells stimulated with eotaxin. Eotaxin (1-100 nM) induced tyrosine/threonine phosphorylation and activation of extracellular regulated kinase (ERK) 1/2 and p38 in NCI-H292 cells and normal human bronchial epithelial cells. The phosphorylation of these MAP kinases was detectable after 30 s, and peaked at 5 min. Eotaxin stimulated production of interleukin-8 and granulocyte macrophage colony-stimulating factor. Pretreatment of Compound X (a specific CCR3 antagonist), pertussis toxin, genistein, and wortmannin reduced the MAP kinase phosphorylation and cytokine production. The eotaxin-induced cytokine production was inhibited by specific inhibitors for MAP/ERK kinase (PD98059) and p38 MAP kinase (SB202190). These results suggest that both ERK1/2 and p38 MAP kinase activated by eotaxin have a critical role in the pathogenesis of asthma.


http://ajrcmb.atsjournals.org/cgi/content/abstract/32/3/192

Many military personnel are at risk of lung damage or systemic toxicity as a result of exposure to the jet fuel JP-8. We have now used microarray analysis to characterize changes in the gene expression profile of lung tissue induced by exposure of rats to JP-8 at a concentration of 171 or 352 mg/m3 for 1 h/d for 7 d, with the higher dose estimated to mimic the level of occupational exposure in humans. The expression of 56 genes was significantly affected by a factor of [\&lt;=\] 0.6 or [\&gt;=\] 1.5 by JP-8 at the low dose. Eighty-six percent of these genes were downregulated by JP-8. The expression of 66 genes was similarly affected by JP-8 at the higher dose, with the expression of 42% of these genes being upregulated. Prominent among the latter genes was that for the centrosome-associated protein {gamma}-synuclein, whose expression was consistently increased. The expression of various genes related to antioxidant responses and detoxification, including those for glutathione S-transferases and cytochrome P450 proteins, were also upregulated. The microarray data were confirmed by quantitative RT-PCR analysis. Our extensive data set may thus provide important insight into the pulmonary response to occupational exposure to JP-8 in humans.

http://ajrcmb.atsjournals.org/cgi/content/abstract/30/4/585

Asthma is a chronic inflammatory disease of the airways. Mast cell-derived cytokines may mediate both airway inflammation and remodeling. It has also been shown that fibroblasts can be the source of proinflammatory cytokines. In the human airways, mast cell-fibroblast interactions may have pivotal effects on modulating inflammation. To study this further, we cocultured normal human lung fibroblasts (NHLF) with a human mast cell line (HMC-1) and assayed for production of interleukin (IL)-6, an important proinflammatory cytokine. When cultured together, NHLF/HMC-1 contact induced IL-6 secretion. Separation of HMC-1 and NHLF cells by a porous membrane inhibited this induction. HMC-1-derived cellular membranes caused an increase in IL-6 production in NHLF. Activation of p38 MAPK was also seen in cocultures by Western blot, whereas IL-6 production in cocultures was significantly inhibited by the p38 inhibitor SB203580. IL-6 production in cocultures was minimally inhibited by a chemical inhibitor of nuclear factor-κB (Bay11), indicating that nuclear factor-κB may have a minimal role in signaling IL-6 production in mast cell/fibroblasts cocultures. Blockade of inter-cellular adhesion molecule-1, tumor necrosis factor-RI, and surface IL-1β with neutralizing antibodies failed to significantly decrease IL-6 production in our coculture, indicating that other receptor-ligand associations may be responsible for this activation. These novel studies reveal the importance of cell-cell interactions in the complex milieu of airway inflammation.


http://ajrcmb.atsjournals.org/cgi/content/abstract/32/3/201

Respiratory tract infections result in wheezing in a subset of patients. Mycoplasma pneumoniae is a common etiologic agent of acute respiratory infection in children and adults that has been associated with wheezing in 20-40% of individuals. The current study was undertaken to elucidate the host-dependent pulmonary and immunologic response to M. pneumoniae respiratory infection by studying mice with different immunogenetic backgrounds (BALB/c mice versus C57BL/6 mice). After M. pneumoniae infection, only BALB/c mice developed significant airway obstruction (AO) compared with controls. M. pneumoniae-infected BALB/c mice manifested significantly elevated airway hyperresponsiveness (AHR) compared with C57BL/6 mice 4 and 7 d after inoculation as well as BALB/c control mice. Compared with C57BL/6 mice, BALB/c mice developed worse pulmonary inflammation, including greater peribronchial infiltrates. Infected BALB/c mice had significantly higher concentrations of tumor necrosis factor-α, interferon-γ, interleukin (IL)-1β, IL-6, IL-12, KC (functional IL-8), and macrophage inflammatory protein 1α in the bronchoalveolar lavage fluid compared with infected C57BL/6 mice. No differences in IL-2, IL-4, IL-5, IL-10, and granulocyte/macrophage colony-stimulating factor concentrations were found. The mice in this study exhibited host-dependent infection-related AO and AHR associated with chemokine and T-helper type (Th)1 pulmonary host response and not Th2 response after M. pneumoniae infection.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/5/720
We investigated the mechanisms of endogenous nitric oxide (NO) modulation of lung sodium (Na+) transport. C57BL/6 mice injected intraperitoneally with the specific inducible NO synthase (iNOS) inhibitor 1400W (10 mg/kg every 8 h for 72 h) exhibited decreased alveolar nitrite levels and Na+-dependent amiloride-sensitive alveolar fluid clearance as compared with mice injected with vehicle. Similarly, pretreatment of mouse tracheal epithelial cells with 1400W abolished the inhibitory effects of amiloride on their Na+ short circuit currents. On the other hand, mouse tracheal epithelial cells pretreated with 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, a specific inhibitor of guanylate cyclase, had lower levels of cGMP, but normal values of amiloride-sensitive Na+ currents. Amiloride also inhibited whole-cell Na+ currents across A549 cells treated with vehicle (Ki = 249 nM), but had no effect in A549 cells treated with 1400W. Western blotting studies showed significantly lower levels of {alpha} and {gamma}ENaC in lung tissues and alveolar type II (ATII) cells from iNOS-/- as well as iNOS+/+ mice treated with 1400W, as compared with the corresponding values from vehicle-treated iNOS+/+ mice. Similar values for ratios of {alpha}, {beta}, and {gamma}enac to gapdh were obtained by real-time polymerase chain reaction for iNOS+/+ mice and iNOS-/ mice. We concluded that NO derived from iNOS under basal conditions is necessary for amiloride-sensitive Na+ transport across lung epithelial cells and modulates the amount of {alpha} and {gamma}ENaC via post-transcriptional, cGMP-independent mechanisms.


http://ajrcmb.atsjournals.org/cgi/content/abstract/28/5/563

Tissue structural cells are known in some situations to play a role in the presentation of antigen and in immunoregulation. We assessed the expression of B7 homologs, known to be involved in antigen presentation and lymphocyte costimulation, in human airway epithelial cells. Flow cytometry performed on the airway epithelial cell line BEAS-2B, as well as primary bronchial epithelial cells (PBEC), showed that B7-H2 was constitutively expressed on both BEAS-2B and PBEC, whereas B7-1 and B7-2 were undetectable on either epithelial cell type. B7-H2 expression was confirmed by Western blot using a specific antibody. Stimulation with various cytokines, including tumor necrosis factor-{alpha}, interferon-{gamma}, and interleukin-4, slightly downregulated B7-H2 expression detected by flow cytometry, but did not significantly alter the apparent level of protein as assessed by Western blotting. Northern blotting detected mRNA for B7-H2 and B7-1, but not B7-2. B7-H2 was cloned from BEAS-2B cells and the sequence verified. Expression of B7-H2 mRNA was detected by real-time reverse transcriptase-polymerase chain reaction in PBEC from three independent donors. Immunohistochemical analysis of airway derived from autopsies revealed expression of B7-H2 in human airway epithelial cells. These results demonstrate that airway epithelial cells express the costimulatory molecule B7-H2, and suggest the possibility that B7-H2 may participate in antigen presentation by epithelial cells.


http://ajrcmb.atsjournals.org/cgi/content/abstract/31/3/283

Mast cells play pivotal roles in immunoglobulin (Ig) E-mediated airway inflammation, expressing interleukin (IL)-13 and monocyte chemoattractant protein-1 (MCP-1), which in turn regulate IgE synthesis and/or inflammatory cell recruitment. The molecular effects of IL-1{beta} on cytokine expression by human mast cells (HMC) have not been studied well. In this report, we provide
evidence that human umbilical cord blood-derived mast cells (CBDMC) and HMC-1 cells express the type 1 receptor for IL-1. We also demonstrate that IL-1β and tumor necrosis factor-alpha are able to induce, individually or additively, dose-dependent expression of IL-13 and MCP-1 in these cells. The induction of IL-13 and MCP-1 gene expression by IL-1β was accompanied by the activation of IL-1 receptor-associated kinase and translocation of the transcription factor, nuclear factor (NF) κB into the nucleus. Accordingly, Bay-11 7082, an inhibitor of NF-κB activation, inhibited IL-1β-induced IL-13 and MCP-1 expression. IL-1β also induced IL-13 promoter activity while enhancing the stability of IL-13 messenger RNA transcripts. Dexamethasone, a glucocorticoid, inhibited IL-1β-induced nuclear translocation of NF-κB and also the secretion of IL-13 from mast cells. Our data suggest that IL-1β can serve as a pivotal costimulus of inflammatory cytokine synthesis in human mast cells, and this may be partly mediated by IL-1 receptor-binding and subsequent signaling via nuclear translocation of NF-κB. Because IL-1β is a ubiquitously expressed cytokine, these findings have important implications for non-IgE-mediated signaling in airway mast cells as well as for innate immunity and airway inflammatory responses, such as observed in extrinsic and intrinsic asthma.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/1/51

Mechanical ventilation has been shown to cause ventilator-induced lung injury (VILI), probably by overdistending or stretching the lung. Hyaluronan (HA), a component of the extracellular matrix, in low molecular weight (LMW) forms has been shown to induce cytokine production. LMW HA is produced by hyaluronan synthase 3 (HAS 3). We found that HAS 3 mRNA expression was upregulated and that LMW HA accumulated in an animal model of VILI. We hypothesized that stretch-induced LMW HA production that causes cytokine release in VILI was dependent on HAS 3 mRNA expression. We explored this hypothesis with in vitro lung cell stretch. Cell stretch induced HAS 3 mRNA expression and LMW HA in fibroblasts. Nonspecific inhibitors of HAS 3 (cyclohexamide and dexamethasone), a nonspecific inhibitor of protein tyrosine kinases (genistein), and a janus kinase 2 inhibitor (AG490) blocked stretch-induced HAS 3 expression and synthesis of LMW HA. Stretch-induced LMW HA from fibroblasts caused a significant dose-dependent increase in interleukin-8 production both in static and stretched epithelial cells. These results indicated that de novo synthesis of LMW HA was induced in lung fibroblasts by stretch via tyrosine kinase signaling pathways, and may play a role in augmenting induction of proinflammatory cytokines in VILI.


http://ajrcmb.atsjournals.org/cgi/content/abstract/26/1/105

To examine the effects of acid exposure with moderate acidity (pH 3.0-5.0) on bactericidal activity of airway surface liquid (ASL), ASL was collected by washing the surface of primary cultures of human tracheal epithelial cells 24 h after treatment with phosphate-buffered saline (PBS) adjusted to a pH of 3.0, 4.0, or 5.0. In all ASL, bactericidal activity was sensitive to sodium concentration. Escherichia coli (500 colony forming units [CFU]) was incubated in ASL, and the number of surviving bacteria was examined. The number of surviving bacteria in ASL from cultured cells with acid exposure at pH 3.0-5.0 was significantly higher than that in control ASL. The minimum inhibitory dilution ratio of ASL against 500 CFU of E. coli was also examined by microdilution assays. According to this assay, the bactericidal activity in ASL with acid challenge
at a pH of 3.0 was less than half of that in control ASL. Reverse transcription-polymerase chain reaction and Western blot analysis showed that the production of mRNA and protein of human [beta]-defensin (HBD)-1 were significantly decreased by acid exposure at pH 3.0-5.0. In contrast, acid exposure did not change the production of mRNA and protein of HBD-2 and [beta]-actin mRNA. These results indicate that acid exposure, even with moderate acidity, may inhibit the production of bactericidal molecules, including HBD-1, in airway epithelial cells. Acid exposure may reduce bactericidal activity of ASL in human airway epithelial cells and may increase susceptibility of the airway to bacterial infection.


http://ajrcmb.atsjournals.org/cgi/content/abstract/26/3/348

Pulmonary inflammation increases nitric oxide (NO) production via inducible nitric oxide synthase (iNOS). This study was performed to determine some of the factors that affect the availability of the NOS substrate, L-arginine (L-arg), in the intact lung subjected to silica-induced inflammation. Nitrate production, as an index of NO production, was significantly greater in silica-exposed lungs (53.5 +/- 12.1 nmol/90 min) compared with controls (22.5 +/- 5.1 nmol/90 min, P < 0.05). This was accompanied by greater (P < 0.0001) 90-min [3H]L-arg uptake (62 +/- 3% control, 82 +/- 1% silica), a significantly (P < 0.005) increased permeability-surface area product for L-arg (0.28 +/- 0.05 ml/min control, 0.63 +/- 0.07 ml/min silica), and a significantly (P < 0.001) increased urea production (1.16 +/- 0.08 {micro}mol/90 min control, 1.77 +/- 0.06 {micro}mol/90 min silica). There was no difference in eNOS protein between groups and eNOS mRNA was not detectable in either group, whereas silica exposure resulted in the appearance of both iNOS protein and mRNA. Silica exposure increased CAT-1 and CAT-2 mRNA ~ 8-fold compared with controls. We conclude that the increase in NO production in silica-exposed lungs was associated with increased L-arg uptake from the vasculature, presumably resulting from increased CAT-1 and CAT-2, and by increased L-arg metabolism via arginase.


http://ajrcmb.atsjournals.org/cgi/content/abstract/28/4/473

The rate limiting step in neuronal acetylcholine (ACh) synthesis is the uptake of choline by the high-affinity choline transporter (CHT1). Here, we investigated the distribution of CHT1 in the rat trachea. CHT1-mRNA was detected by reverse transcriptase-polymerase chain reaction in trachea without epithelium, abraded tracheal mucosa, and in epithelial cells obtained by laser-assisted cell-picking. Accordingly, CHT1-mRNA could also be detected in tracheal epithelial cells by in situ hybridization. Recently obtained polyclonal rabbit and guinea-pig antisera against a synthetic peptide corresponding to amino acid residues 29-40 of the rat CHT1 sequence localized CHT1 protein in combination with antisera against the vesicular acetylcholine transporter in cholinergic fibers innervating tracheal glands and the tracheal muscle. In case of the tracheal epithelium, CHT1 was restricted to the apical membrane of the ciliated cells, as demonstrated by confocal laser scanning and electron microscopy using an affinity-purified CHT1 antiserum. The close apposition of CHT1 to reported sites of localization of choline acetyltransferase in these cells is strongly in favor of ACh synthesis being fuelled by choline uptake via CHT1 after release and breakdown of ACh at the luminal surface. Accordingly, cholinergic regulation of tracheal epithelial function is governed by local release and recycling of ACh by ciliated cells.

Keratinocyte growth factor (KGF or FGF-7) stimulates alveolar type II cell proliferation, but little is known about the signaling pathways involved. We investigated the role of the ERK (p42/44 mitogen activated protein [MAP] kinase) and phosphatidylinositol 3-OH kinase (PI3 kinase) pathways on alveolar type II cell proliferation and differentiation. Rat type II cells were cultured on tissue culture plastic and Matrigel in the presence or absence of KGF and specific chemical inhibitors PD98059, LY294002, and rapamycin at various concentrations. Proliferation was measured by thymidine incorporation and DNA quantitation, and differentiation was measured by expression of surfactant protein A and alkaline phosphatase. We demonstrate that KGF activates distal effectors of the PI3 kinase pathway, PKB/Akt, and p70S6 kinase, as well as p42/44 MAP kinase proteins. Inhibition of these pathways with PD98059, LY294002, or rapamycin inhibited type II cell proliferation but had no significant effect on differentiation. KGF did not activate the c-Jun kinase or p38 MAP kinase pathways. We conclude that the p42/44 MAP kinase and PI3 kinase pathways are important in regulating alveolar type II cell proliferation in response to KGF.


Extracellular matrix (ECM) expansion contributes to airway remodeling in asthma. This study examines the effect of leukotriene D4 (LTD4), combined with epidermal growth factor (EGF), on proteoglycan synthesis by cultured human bronchial smooth muscle cells (BSMCs). LTD4 plus EGF stimulated proliferation of BSMCs with increased versican synthesis. Further, versican mRNA splice variants, V0 and V1, were differently regulated in BSMCs by LTD4 plus EGF. Synthesis of [35S]-methionine labeled versican V0, as a percentage of total versican, was doubled. This upregulation was confirmed by Western analysis. Synthetic changes were paralleled by alterations in versican V0 mRNA. The effects of LTD4 and EGF on proteoglycan synthesis were inhibited by montelukast. Similar upregulation of versican V0 was observed in arterial smooth muscle cells (ASMCs) stimulated with LTD4 plus EGF as measured by western and reverse transcriptase-polymerase chain reaction analyses. Changes in ECM in the asthmatic airway may parallel those in atherosclerotic lesions where proliferating ASMCs synthesize a versican-rich expanded ECM. Inhibition of these processes could lead to reduced tissue expansion in the early phases of asthma progression.


The ADAM (A Disintegrin and Metalloprotease) family of Zn++-dependent metalloproteases are multidomain proteins involved in diverse cellular activities. Polymorphic variation in ADAM33 is strongly associated with asthma and bronchial hyperresponsiveness. Identification of those
isoforms of ADAM33 that are expressed in airways is fundamental to dissecting the role of ADAM33 in asthma. Analysis of primary human airways fibroblasts has shown the presence of a number of alternatively spliced forms of ADAM33, including one encoding a putative secreted variant, and many transcripts lacking the metalloproteinase domain. The relative abundance of these transcripts has been quantified using reverse transcription real-time polymerase chain reaction, in both nuclear and cytoplasmic fractions of RNA. These results demonstrate that a number of splice variants of ADAM33 are transported into the cytoplasm. Ninety percent of ADAM33 mRNA is retained in the nucleus and the subtle differences in the composition of nuclear and cytoplasmic RNA suggest important events in the splicing and selection of ADAM33 transcripts. Western blot analysis confirmed that several protein isoforms of ADAM33 are expressed in primary airways fibroblasts. These findings demonstrate that ADAM33 exists in multiple isoforms, suggesting that it is a complex molecule that plays multiple roles within mesenchymal cells.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/1/12

Neurotrophins (NTs) promote survival and differentiation of central and peripheral neurons, and display several activities also in non-neuronal cells. Human lungs synthesize and release NTs, which are probably involved in the pathophysiology of pulmonary disturbances. In this article the expression and anatomic localization of nerve growth factor, brain-derived neurotrophic factor, and NT-3 and of corresponding high-affinity receptors TrkA, TrkB (full-length and truncated [TR-] isoforms), TrkC, and of the low-affinity p75 receptor, were assessed in surgical samples from adult human lung by reverse transcriptase-polymerase chain reaction, Western blot, and immunohistochemistry. NTs and their cognate receptor mRNA and protein transcripts were detected by reverse transcriptase-polymerase chain reaction and immunoblotting, respectively, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) mRNA and corresponding protein transcripts being the most expressed. High levels of TrkB-[TR-] mRNA and of its protein transcript were also demonstrated, whereas a low expression of p75 mRNA and of corresponding protein transcript were found. Microanatomic analysis of immunohistochemical study revealed that bronchial epithelial cells were immunoreactive for different NTs, with a higher intensity of BDNF immune staining compared with other NTs, but did not express NT receptor immunoreactivity. Alveolar cells were immunoreactive for TrkA and TrkC receptor protein, but did not display immunoreactivity for NTs or other receptors investigated. Gland cells expressed NT and high-affinity NT receptor immunoreactivity, but not p75 receptor immunoreactivity. NT and low-affinity receptor immunoreactivity was observed within neurons and satellite cells of parasympathetic ganglia as well as in nerve fiber-like structures supplying the bronchopulmonary tree. An obvious immunoreactivity for NTs and NT receptor protein was also observed in intrapulmonary branches of pulmonary artery. Pulmonary lymphocytes and macrophages express nerve growth factor and high-affinity NT receptor immunoreactivity. The role of NTs in non-neuronal tissue including lung has not been clarified yet. The widespread expression of NTs and their receptors in different components of the lung suggests that these factors may contribute to regulate cell function in human lung.


http://ajrcmb.atsjournals.org/cgi/content/abstract/28/2/199
We investigated the pharmacologic effects of the antioxidant Vitamin E ([alpha]-tocopherol ([alpha]-toc)) in airway inflammation induced by inhaled endotoxin. A preparation of [alpha]-toc incorporated in liposomes was administered intraperitoneally in mice 1 h after exposure of aerosolized endotoxin. Injection of 50 mg [alpha]-toc/kg significantly decreased the number of neutrophils in airspaces and prevented lung injury, monitored both as decreased lactate dehydrogenase activity in airways and reduced lung edema when compared with animals treated with plain liposomes. Immunofluorescence staining of lung tissue revealed that treatment with [alpha]-toc decreased the number of neutrophils in lung interstitium, whereas the number in lung blood vessels and peripheral blood did not differ between mice treated with [alpha]-toc and control mice. Our results indicate that [alpha]-toc downmodulates the migration of neutrophils across the endothelial barrier, but in contrast to strong anti-inflammatory drugs such as corticosteroids, without inhibition of transcription factors involved in the early inflammatory response (nuclear factor-(kappa)B/activator protein-1). Neither was the endotoxin-induced expression of proinflammatory cytokines in lung tissue downregulated. Treatment with a combination of [alpha]-toc and a suboptimal dose of 0.5 mg/kg dexamethasone enhanced the effect, suggesting that [alpha]-toc, in combination with low doses of corticosteroids, might be effective for therapeutic treatment of acute lung injury.


Beryllium (Be) presentation to CD4+ T cells from patients with chronic beryllium disease (CBD) results in T cell activation, and these Be-specific CD4+ T cells undergo clonal proliferation and T-helper 1-type cytokine production. In exposed workers, genetic susceptibility to this granulomatous disorder is associated with particular HLA-DPB1 alleles. We hypothesized that these HLA-DP molecules could mediate Be-stimulated tumor necrosis factor-(alpha) (TNF-(alpha)) messenger RNA (mRNA) and protein production. Using intracellular cytokine staining, we found that treatment with an anti-HLA-DP, but not anti-HLA-DR, monoclonal antibody inhibited Be-stimulated TNF-(alpha) expression in lung CD3+ CD4+ T cells. This monoclonal antibody also blocked Be-specific T cell proliferation, increased production of TNF-(alpha) mature-mRNA transcripts, and increased TNF-(alpha) protein production by Be-stimulated CBD peripheral blood mononuclear cells and bronchoalveolar lavage (BAL) cells. The Be-stimulated upregulation of TNF-(alpha) mature-mRNA levels with TNF-(alpha) protein production was a unique property of CBD BAL cells, and did not occur in BAL cells from Be-sensitized patients without CBD, or sarcoidosis BAL cells. This study identifies HLA-DP as a regulatory component in the activation of T cell receptors on Be-specific CD4+ T cells from CBD patients resulting in proliferation and proinflammatory cytokine production.


Human prostasin is a membrane-anchored serine peptidase hypothesized to regulate lung epithelial sodium transport. It belongs to a unique family of genes on chromosome 16p11.2/13.3. Here we describe genomic cloning, promoter analysis, and expression of prostasin's mouse ortholog. The 4.3-kb mouse prostasin gene (prss8) has a six-exon organization identical to human prostasin. Prss8 spans two signal tagged-sites localized to chromosome 7. Multiple mRNA transcripts arise from two consensus initiator elements of a TATA-less promoter and an alternatively spliced, 5' untranslated region intron. Reporter assay establishes that the initiator
elements and a GC-rich domain comprise the core promoter and identifies 5' flanking regions with strong enhancer and repressor activity. The 3' untranslated region overlaps the 3' untranslated region of the Myst1 gene oriented tail-to-tail at this locus. Prss8 is highly transcribed in pancreas, kidney, submaxillary gland, lung, thyroid, prostate, and epididymis, and is developmentally regulated. Using selective riboprobes and antibodies to mouse prostasin, we localized its expression to lung airway epithelial and alveolar type II cells and kidney cortical tubule epithelium. Mouse prostasin highly resembles its human ortholog in gene organization and tissue specificity, including strong expression in pulmonary epithelium, suggesting that mice will be useful for probing prostasin's functions in vivo.


http://ajrcmb.atsjournals.org/cgi/content/abstract/26/4/484

Cytokines derived from lymphocytes are believed to play key roles in a variety of diseases, including airway diseases such as asthma. The current study was designed to evaluate the hypothesis that cytokines derived from Th2 cells, interleukin (IL)-4 and IL-13, might contribute to tissue remodeling by modulating the production of transforming growth factor (TGF)-[beta]. In addition, the ability of interferon (IFN)-[gamma], a cytokine derived from Th1 cells that can antagonize many effects of IL-4 and IL-13, was also assessed for its effects on TGF-[beta] production. IL-4 and IL-13 both stimulated production of TGF-[beta]2 release from human bronchial epithelial cells in a time- and concentration-dependent manner. Both with and without acidification, TGF-[beta]2 were detected. Neither TGF-[beta]1 nor TGF-[beta]3 was released. In contrast to the stimulatory effect on human bronchial epithelial cells, neither IL-4 nor IL-13 stimulated release of any TGF-[beta] isoform from human lung fibroblasts. IFN-[gamma] reduced both basal, IL-4-, and IL-13-stimulated release of TGF-[beta]2 in human bronchial epithelial cells. The stimulatory effects of IL-4 and IL-13 and the inhibitory effect of IFN-[gamma] on TGF-[beta]2 release were paralleled by mRNA levels, as assessed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). In summary, the Th2-derived cytokines, IL-4 and IL-13, can stimulate production of TGF-[beta] from airway epithelial cells but not from lung fibroblasts. IFN-[gamma], in contrast, can inhibit TGF-[beta]2 release both under basal conditions and following IL-4 or IL-13 stimulation. The ability of these cytokines to modulate TGF-[beta] release may contribute to both normal airway repair and to the development of subepithelial fibrosis in asthma.


http://ajrcmb.atsjournals.org/cgi/content/abstract/31/1/92

Hyaluronan (HA) is an important constituent of the extracellular matrix and accumulates during inflammatory lung diseases like asthma. Little is known about the factors that regulate HA synthesis by lung cells. Accordingly, we investigated the effect of T-helper 1 (TH1) and 2 (TH2) cytokines and the anti-inflammatory agents fluticasone and salmeterol on HA synthesis in human lung fibroblasts. Interleukin-[beta]1 (IL-1[beta]) and tumor necrosis factor (TNF)-[alpha] were the most potent stimulators of HA synthesis and when combined, caused synergistic increases in HA accumulation. Time-course analysis of HA accumulation and [3H]-glucosamine incorporation into HA demonstrated continued synthesis over the 24 h of stimulation. Peak synthesis at 6-12 h coincided with an increased proportion of high molecular weight HA. Reverse transcriptase polymerase chain reaction (RT-PCR) revealed that IL-1[beta] and TNF-[alpha] induced HA synthase-2 messenger RNA (mRNA) 3 h following stimulation and remained elevated throughout
the 24-h stimulation period. Fluticasone inhibited IL-1β and TNF-α induced HA synthesis (44.5%) whereas salmeterol had no effect. When combined, fluticasone and salmeterol inhibited HA synthesis to a greater extent (85.2%). Further, fluticasone attenuated IL-1β and TNF-α stimulated hyaluronan synthase-2 messenger RNA (mRNA), and the addition of salmeterol cooperatively enhanced this inhibition. These results indicate that enhanced synthesis of HA by the proinflammatory cytokines IL-1β and TNF-α can be abrogated by specific corticosteroid and β2 blocker combinations shown to be effective in the treatment of asthma.

http://ajrcmb.atsjournals.org/cgi/content/abstract/2005-0034OCv1

IL-25, a novel Th2 cytokine, is capable of amplifying allergic inflammation. We investigated the modulation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPK) pathways in IL-25-activated eosinophils, the principal effector cells of allergic inflammation, for the in vitro release of chemokines including monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-8, and macrophage inflammatory protein (MIP)-1α, and inflammatory cytokine IL-6. Gene expression of chemokines and IL-6 was evaluated by RT-PCR and concentrations of chemokines and cytokine were measured by cytokine protein array, cytometric bead array and ELISA. NF-κB, c-Jun amino-terminal kinase (JNK) and p38 MAPK activities in eosinophils were assessed by electrophoretic mobility shift assay and Western blot. IL-25 was found to up-regulate the gene expression of chemokines MCP-1, MIP-1α, IL-8 and cytokine IL-6 in eosinophils; and significantly increase the release of the above chemokines and IL-6 from eosinophils. IL-25 could also activate the JNK, p38 MAPK and NF-κB pathways of eosinophils, while inhibitor of IκB-α phosphorylation (BAY11-7082), JNK (SP600125) and p38 MAPK (SB203580) could suppress the release of IL-8, MIP-1α, MCP-1 and IL-6. Together, the above results showed that the induction of MCP-1, MIP-1α, IL-8 and IL-6 in IL-25 activated eosinophils are regulated by JNK, p38 MAPK and NF-κB pathways.

http://ajrcmb.atsjournals.org/cgi/content/abstract/29/1/133

We investigated the intracellular signaling mechanisms for cytokine interleukin (IL)-3, IL-5, or granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced expression of adhesion molecules including very late antigen 4 (CD49 d), macrophage antigen-1 (CD11b), leukocyte function-associated antigen-1 (CD11a/CD18), intercellular adhesion molecule (ICAM)-1, and ICAM-3 on eosinophils. The expression of adhesion molecules and nuclear factor (NF)-κB activity were measured by flow cytometry and cDNA expression array, respectively. The phosphorylation of inhibitor (κB)-α and p38 mitogen-activated protein kinase (MAPK) was detected by Western blot, whereas NF-κB activity was measured by electrophoretic mobility shift assay. IL-3, IL-5, and GM-CSF could enhance p38 MAPK and NF-κB activity and induce ICAM-1, CD11b, and CD18 expressions on eosinophils. They could suppress ICAM-3 expression, but had no effect on CD49 d expression. Either SB 203580 or MG-132 was able to offset the cytokine-induced expression of ICAM-1. Only SB 203580 could reverse the effect on CD11b, CD18, and ICAM-3 expressions. Therefore, the expression of ICAM-1 might involve both p38 MAPK and NF-κB activities, whereas the regulation of CD11b, CD18, and ICAM-3
expressions might be mediated through p38 MAPK but not NF-κB. These cytokines therefore play a crucial role, via the p38 MAPK and NF-κB pathways, in the expression of important adhesion molecules on eosinophils in allergic inflammation.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/4/428

Primary ciliary dyskinesia (PCD) is an autosomal recessive disease caused by mutations that affect the proper function of cilia. Recently, deletion of DNA polymerase λ (Poll) in mice produced a phenotype characteristic of PCD (Kobayashi et al., 2002, Mol. Cell. Biol. **22**:2769-2776). Because it is unclear how a mutation in a DNA polymerase would result in a specific defect in axonemes, the targeting construct was examined further. Analysis of the genomic region surrounding the Poll gene revealed an uncharacterized gene, named Dpcd, that is predicted to be transcribed from the opposite strand relative to Poll. The deletion of Poll would also remove the first exon of Dpcd. Because it is possible that the PCD phenotype observed is due to the absence of either gene, the expression of these genes during ciliogenesis of human airway epithelial cells was examined. Northern analysis demonstrated that DPCD expression increases during ciliated cell differentiation; the expression of POLL decreases. To examine directly whether DPCD is mutated in cases of human PCD, the complete coding sequence of DPCD was sequenced from 51 unrelated PCD patients. No disease-causing mutations were confirmed; however, one variant could not be excluded. Therefore, DPCD remains a novel candidate gene for PCD.


http://ajrcmb.atsjournals.org/cgi/content/abstract/30/2/174

Alveolar type II cells increase lipogenesis and convert glycogen into the phospholipids of surfactant in the late term fetal lung. Recent studies suggest that CCAAT/enhancing-binding protein (C/EBP) isoforms and sterol regulatory element binding protein (SREBP)-1c regulate fatty acid synthesis in adult type II cells in vitro. To define the temporal relationships and enzymes involved in lipogenesis in fetal rat lung, the mRNA levels of selected transcription factors and enzymes were determined. There was an increase in the mRNA levels of C/EBPα, C/EBPβ, C/EBPδ, peroxisomal proliferator-activated receptor (PPAR)γ, and SREBP-1c, but not SREBP-1a or SREBP-2 from fetal Days 19-21. There was also an increase in the mRNA levels of fatty acid synthase, stearoyl-CoA desaturase 1 (SCD-1), fatty acid translocase, glycerol-3-P acyl transferase, and phosphatidate cytidylyltransferase. By in situ hybridization, there was detectible expression of fatty acid synthase, SCD-1, and C/EBPα along the alveolar septae with the same distribution pattern as surfactant protein-C, whereas PPARγ expression appeared to be restricted to macrophages. Regulation of lipogenesis at the mRNA level is predominately on enzymes of fatty acid synthesis and appears to be regulated by C/EBPα and SREBP-1c. SCD-1 and phosphatidate cytidylyltransferase are important components of the lipogenic response in the fetal lung that have not been recognized previously.

http://ajrccm.atsjournals.org/cgi/content/abstract/169/9/1007

Recent evidence suggests that deficiency in the Th1 cytokine pathway may underlie the susceptibility to allergic asthma. This study examined whether (1) single-nucleotide polymorphisms exist in the promoter region of the two interleukin (IL)-12 subunit genes in patients with asthma; (2) messenger RNA and protein expressions of signal transducers and activators of transcription, IL-12, IFN-\(\gamma\), and their receptors are altered in asthma; and (3) linkage to genes in the Th1 pathway is present in families with asthma in Iceland. The promoter regions of the IL-12 subunit genes were sequenced in 94 patients with asthma and 94 control subjects without asthma. Linkage was examined in 169 families that included over 570 patients with asthma and 950 of their unaffected relatives. The results demonstrate no evidence of linkage to microsatellite markers in close association with genes within the Th1 pathway, and no polymorphism was detected in the promoter regions of the two IL-12 subunit genes in the cohort with asthma patients. Moreover, we found no differences in the messenger RNA or protein expression signals of genes in the IL-12 pathway between the patients and control subjects. We conclude that decrease in Th1 type cytokine response is unlikely to present a primary event in asthma.


http://ajrccm.atsjournals.org/cgi/content/abstract/167/3/444

Investigators have intensively evaluated the major histocompatibility (MHC) complex for sarcoidosis susceptibility genes with the majority of reports implicating the human leukocyte antigen (HLA)-DRB1 gene. Because most studies have been performed in white and Asian populations, we sought to determine which MHC genes might be risk factors for sarcoidosis in African Americans. We genotyped six microsatellite markers spanning 11.6 megabases that overlapped the MHC region on chromosome 6p21-22 in 225 nuclear families ascertained by African American probands with a history of sarcoidosis. Using a family-based association methods approach, we performed multiallelic tests of association between each marker and sarcoidosis. A statistically significant association was detected between sarcoidosis and the DQCAR marker \((p = 0.002)\) less than two kilobases telomeric from the HLA-DQB1 gene. Typing two additional markers in this region revealed that DQCAR-G51152 haplotypes, spanning a 38-kilobase region across the HLA-DQB1 gene, were associated with sarcoidosis on a global level \((p = 0.022)\). Analysis of individual DQCAR and G51152 alleles showed that the DQCAR 178 (expected = 21.0; observed = 10; \(p = 0.0005\)) and G51152 217 (expected = 25.6; observed = 14; \(p = 0.0009\)) alleles were transmitted to affected offspring less often than expected; whereas the DQCAR 182 allele was transmitted more often than expected (expected = 52.6; observed = 66; \(p = 0.002\)). Our results indicate that HLA-DQB1 and not HLA-DRB1 plays an important role in sarcoidosis susceptibility in African Americans. Identification of the specific HLA-DQB1 alleles that influence sarcoidosis susceptibility in African Americans and the study of their antigenic-binding properties may reveal why African Americans suffer disproportionately from this disease.

http://www.ajronline.org/cgi/content/abstract/183/2/383

OBJECTIVE. Our purpose was to find out whether percutaneous biopsy of hepatocellular carcinoma will cause significant dissemination of tumor into the circulation by quantitative analysis of circulating tumor DNA. SUBJECTS AND METHODS. In this prospective study of 32 patients with suspected hepatocellular carcinoma who underwent sonographically guided liver biopsy, a peripheral venous blood sample was obtained before and 5 min after the procedure. Biopsy was performed using an 18-gauge biopsy gun. DNA was extracted from the plasma of the blood samples for methylation-specific polymerase chain reaction. Quantitative measures of the plasma tumor DNA were determined with real-time quantitative polymerase chain reaction, and the amount was expressed as a methylation index (%) in plasma. RESULTS. Nineteen (59.4%) of 32 patients did not have detectable p16 tumor suppressor gene marker (p16M) in plasma before biopsy, and they showed no detectable plasma p16M after biopsy. Thirteen (65%) of 20 patients had p16M identified in the plasma before liver biopsy. Quantitative analysis of the plasma tumor DNA in these 13 patients showed no statistically significant difference in the methylation index before and after biopsy (p = 0.345, Wilcoxon's signed rank test). CONCLUSION. No evidence exists that percutaneous liver biopsy results in hematogenous dissemination of hepatocellular carcinoma as shown by quantitative analysis of circulating tumor DNA (p16M) using methylation-specific real-time polymerase chain reaction.


http://www.sciencedirect.com/science/article/B6W9H-4D6XH23-22/2/07d705ef5da22c1982223a35d91bb701

Enteroviruses (EVs), especially group B coxsackieviruses, have been implicated in the pathogenesis of myocarditis and dilated cardiomyopathy (DCM). To determine whether a specific type of EV is present in DCM hearts, we examined the genotypes of EVs detected in endomyocardial biopsies and pericardial effusions by polymerase chain reaction--single-strand conformation polymorphism (PCR-SSCP) analysis. Positive PCR results were obtained from biopsies in 6 (19%) of 31 patients with DCM, 5 (18%) of 28 with myocarditis, 5 (22%) of 23 with other cardiac diseases, and from pericardial effusions in 4 (57%) of 7 patients with pericarditis. SSCP profiles of most of the clinical samples were different and were not identical to any of the standard group B coxsackie viruses. Our findings suggest that EV genomes are involved in the
myocardium of patients with various cardiac conditions and that a particular type of EV is not present in DCM hearts.

http://www.sciencedirect.com/science/article/B6W9H-4C53GWY-1T2/2/d09cb97952ad8c775768d0ef164467c6

http://www.sciencedirect.com/science/article/B6W9H-4BTH8F4-3S/2/05019a662dedc4758b5cccf0c41d5d44

American Journal of Hypertension (9)

http://www.sciencedirect.com/science/article/B6T0Y-4F4HJMW-J/2/587556a82a08097672fc28e19c35e905

The dopamine receptor type 1 (DRD1) has been implicated in the development of hypertension in humans as well as in animal models of spontaneous hypertension. We screened the entire coding and promoter region of the human DRD1 receptor for polymorphisms to analyze their association with hypertension. The allele frequencies of two common single-nucleotide polymorphisms, A-48G and G-94A were determined in 493 hypertensive patients and 209 normotensive controls. Allele frequencies did not differ for both polymorphisms between the two groups (-48 G-allele in hypertension = 0.37; -48 G-allele in normotension = 0.36; -94 A-allele in hypertension = 0.14; -94 A-allele in normotension = 0.10). Our findings in these Caucasian patients are in contrast to a recent Japanese study that revealed a significant association of the -48 G-allele with hypertension. Thus, racial differences may play an important role concerning the association of variants in the dopamine receptor type 1 gene with essential hypertension.

http://www.sciencedirect.com/science/article/B6T0Y-411X9FF-7/2/75d17231585456b87c42e39f173d1b70

Endothelial nitric oxide synthase (eNOS), encoded by NOS3, is a potent regulator of vasomotor
tone and peripheral resistance. Congenic experiments indicate that a chromosomal segment containing the rat eNOS gene contributes to rat spontaneous hypertension (HT). A role for NOS3 in onset of essential hypertension (HT) is, however, controversial. We therefore decided to test NOS3 polymorphisms in a set of patients who have an accentuated ability to show an existing genetic association. The 112 HT subjects had two HT parents and the normotensive (NT) subjects had two NT parents. All were Anglo-Celtic whites. The two most promising polymorphisms, viz, a biallelic variable number of tandem repeats (VNTR) in intron 4 and an exon 7 variant that leads to an amino acid change (Glu298Asp), were genotyped by PCR (and BanII digestion in the case of the latter). Frequency of the minor allele of the VNTR was 0.11 in the NT and 0.10 in the HT subjects (P = .9). For the exon 7 variant, Asp298 frequency was 0.30 and 0.32 in each respective group (P = .6). Tracking was seen for the Asp298 allele with elevation in body mass index (P = .034), and the minor allele of the VNTR with elevation in LDL (P = .007) and reduction in HDL (P = .048). In conclusion, we saw no association of NOS3 markers with HT in the population studied. However, possible genotypic effects on plasma lipids and body mass index might warrant further studies, especially in view of possible associations with heart disease.


http://www.sciencedirect.com/science/article/B6T0Y-411X9FF-C/2/39d828a8490e0256281de00cdcf4f4e

[beta]2-Adrenergic receptors ([beta]2-AR) contribute to cardiovascular regulation by influencing several functions and previous studies suggest that a decreased function of the [beta]2-AR may be involved in essential hypertension. [beta]2-AR are polymorphic and certain polymorphisms of these receptors are of functional importance. We focus here on the Arg16->Gly16 [beta]2-AR polymorphism, which shows enhanced agonist-promoted downregulation of the receptor and which, in two recent studies, yielded opposite results in terms of association with essential hypertension: an increased frequency of the Gly16 variant in African-Caribbean hypertensives and of the Arg16 variant in offspring of Norwegian white hypertensive parents. In the current study, we genotyped 243 subjects, including both African-American and white individuals, for codon 16 polymorphism and assessed blood pressure and cardiovascular function using impedance cardiography and pressor sensitivity to phenylephrine. We found similar patterns of cardiovascular function and expression of hypertension with the two genotypes of codon 16. There was no statistically significant difference in the overall allelic distribution of the two genotypes: among African-Americans, 51% of the hypertensives and 50% of the normotensives carried the Arg16 allele, whereas among the white subjects 40% of the hypertensives and 47% of the normotensives were carriers of the Arg16 allele. Although we observed a statistically significant increase in the Arg16/Gly16 heterozygotes in the African-American population, the Gly16 allele was not significantly increased in the African-Americans compared to whites. These findings indicate that the codon 16 polymorphisms are not associated with hypertension in a mixed American study population nor do they appear to substantially impact on a variety of hemodynamic variables.


http://www.sciencedirect.com/science/article/B6T0Y-45PJYJX-2/2/76e54e3a9643b3345e638931e5315a2d
Background

Our aim was to determine whether the aldosterone synthase (CYP11B2) -344 C/T polymorphism was associated with the blood pressure (BP)-lowering response to antihypertensive treatment.

Methods

Patients with mild-to-moderate primary hypertension and left ventricular hypertrophy were randomized in a double-blind study to receive treatment with either the angiotensin II type 1 (AT1) receptor antagonist irbesartan (n = 43), or the [beta]1-adrenergic receptor blocker atenolol (n = 43). The aldosterone synthase (CYP11B2) -344 C/T polymorphism was analyzed using solid-phase minisequencing and related to BP reduction after 3 months treatment. Serum aldosterone levels were measured.

Results

After 3 months treatment the mean reductions in BP were similar for both treatment groups. When assessing the systolic BP reduction in the irbesartan group, patients with the TT variant had a more pronounced reduction (-21 +/- 19 SD mm Hg, n = 17) than both the TC (-14 +/- 18 mm Hg, n = 18) and CC (0 +/- 17 mm Hg, n = 8) genotypes (P =.04). There was no association between this polymorphism and the diastolic BP response. The -344 C/T polymorphism was not associated with the BP response to atenolol. Nor was it related to the baseline serum aldosterone level.

Conclusions

The aldosterone synthase -344 C/T polymorphism was related to the BP-lowering response in hypertensive patients treated with the AT1-receptor antagonist irbesartan.


http://www.sciencedirect.com/science/article/B6T0Y-3XXCXNY-7/2/470ee14a954abf8c136983dcfe89f5a0

To evaluate the effects of nongenetic factors, aging, and salt-loading on the quantitative trait loci (QTLs) for blood pressure (BP), we conducted a genome-wide linkage analysis using multiple sets of BP measurements in 125 male F2 generation cross derived from stroke-prone spontaneously hypertensive rats and normotensive Wistar-Kyoto rats. The experiment was arranged in two stages. In the first stage, corresponding to the developing period of the rats, BP was measured repeatedly without loading of salt; this continued until the rats were 5 months of age. In the second stage, after the baseline BP leveled off, 1% salt water was given to the rats and BP was monitored for the subsequent 7 months. Genome scanning was performed using 201 markers. In the developing period, three QTLs were identified on chromosomes 1, 3, and 4 (logarithmic odds [LOD] scores of 5.6, 3.1, and 3.2, respectively), which had peaks at 8 or 10 weeks of age. In the latter salt-loading stage, QTLs for BP were detected on chromosomes 1 and 10 (LOD scores 4.6 and 4.5, respectively). When the BP increase during salt-loading was analyzed as a phenotype, however, only the region on chromosome 10 showed linkage at a suggestive level (LOD score 3.2). The present study provides experimental evidence that QTLs for BP could be modulated by nongenetic factors, such as aging and salt-loading.


http://www.sciencedirect.com/science/article/B6T0Y-44XDT90-H/2/e46919b5dcd3521ef1f3d0bc42a32b0

Background Earlier studies from this laboratory showed that central angiotensin II (AngII) receptors are upregulated by chronic cold exposure. The purpose of this study was to determine whether central AngII receptors may play a role in the development of cold-induced hypertension. Methods Four groups of rats (six rats each) were used. Two groups were exposed to cold (5[deg]C) and the other two groups were kept at 25[deg]C. One cold-exposed and one warm-adapted group were treated chronically, via osmotic minipumps, with AngII type 1 (AT1) receptor blocker (losartan, 6.0 [mu]g/2.5 [mu]L/h, intracerebroventricularly) at the beginning of cold exposure. Results Systolic blood pressure (BP) of the cold-exposed untreated group increased during week 1 of cold exposure and rose to 160 +/- 4 mm Hg by week 4, whereas BP of the losartan-treated group in cold did not increase and remained at 121 +/- 3 mm Hg. Cold-induced increases in drinking response to AngII, plasma renin activity, and urine norepinephrine output disappeared in the treated rats, indicating blockade of central AngII receptors. Withdrawal of losartan at 4 weeks resulted in an increase in BP of this group to the cold-exposed untreated level, which was accompanied by an increase in the above parameters. Significant increases in AngII-induced drinking response and hypothalamic AT1 receptor mRNA content of the cold-exposed rats indicate upregulation of AngII receptors during chronic cold exposure. Hypothalamic AngII level was not affected by cold exposure. Conclusion Upregulation of brain AT1 receptors plays a role in the development of cold-induced hypertension.

A Gly460Trp variant of the cytoskeletal protein [alpha]-adducin has recently been implicated in the etiology of essential hypertension (HT) in a study involving southern European whites. We attempted to replicate this finding in a well-characterized, extensively studied group of 112 white Australians with essential HT, with strong family history (two HT parents), early-onset, moderate to severe disease, and of British extraction. Controls were 196 normotensive (NT) white subjects whose parents were both NT older than age 50 years. A mismatch polymerase chain reaction method involving BanII was developed for genotyping. Frequency of the Trp460 allele was 0.23 in the HT and 0.24 in the NT groups ($\chi^2 = 0.2, P = .7$). No association was observed with blood pressure, body mass index, age, plasma renin, angiotensinogen, angiotensin converting enzyme, cholesterol, triglycerides, or HDL or LDL cholesterol. Our results therefore provide no support for a role for the [alpha]-adducin variant in hypertension, at least in our severely affected Anglo-white group with strong family history of HT.

American Journal of Obstetrics and Gynecology  (5)


ObjectiveOur purpose was to determine the presence of [alpha]1-adrenoceptor messenger RNA subtypes and extend the pharmacologic characterization of [alpha]1-adrenoceptors involved in human umbilical vein (HUV) contraction.Study designCords (n = 124) from healthy patients after term vaginal or cesarean deliveries were used. The vein was carefully dissected out of cords and used for reverse transcription combined with polymerase chain reaction (RT-PCR) to amplify [alpha]1-adrenoceptor transcripts. In isolated organ baths, HUV rings were mounted and cumulative concentration-response curves were constructed either for epinephrine or the selective [alpha]1A-adrenoceptor agonist, A-61603. In other series of experiments, the effects of the selective [alpha]1A- and [alpha]1B-adrenoceptor antagonists (RS-100329 or B8805-033 or spiperone, AH11110A and cyclazosin, respectively) were evaluated to estimate its blocking potencies on epinephrine concentration-response curves.ResultsBy means of RT-PCR technique [alpha]1a- and [alpha]1b-adrenoceptor transcripts were detected in the HUV. The blocking potency values of RS-100329 or B8805-033 against responses mediated by epinephrine were not consistent with the activation of an [alpha]1A-adrenoceptor population. Moreover, the low potency of the agonist A-61603 was not in accordance with an [alpha]1A-adrenoceptor interaction. On the other hand, the antagonist potencies of spiperone, AH11110A and cyclazosin were in agreement with an interaction on [alpha]1B-adrenoceptor subtype.ConclusionAlthough [alpha]1a- and [alpha]1b-adrenoceptor messenger RNAs are detected in the HUV, only [alpha]1B-adrenoceptors are involved in epinephrine vasoconstrictor action.

OBJECTIVE: The purpose of this study was to identify the factors that affect the quality of cytologic cervical cancer screening conducted in public sector clinics in Sonora, Mexico. STUDY DESIGN: We assessed the quality of cervical cytology before and after a 2-hour training session and the implementation of cervical spatulas and endocervical brushes. Additionally, we conducted a cross-sectional study of reproductive aged women who attended public clinics in paired border communities in Sonora and Arizona. Cervical cytologic specimens (n = 2436) were collected and compared for adequacy and cytologic diagnosis and for the prevalence of human papillomavirus infection. RESULTS: The training intervention achieved significantly improved the rates of satisfactory but limited smears and unsatisfactory smears. The cross-sectional study revealed comparable quality indicators for cytologic specimens that were collected in Sonora and Arizona clinics. CONCLUSION: A high-quality cytology-based cervical cancer screening program is possible in public sector clinics in Sonora, with indicators similar to those achieved in comparable Arizona clinics.


Objective Ovarian cancer cell lines and tissues express gonadotropin receptors. Conjugation of cytostatics to ligands of these receptors may increase the specificity of cytotoxic drugs. Study design Toxicity of doxorubicin-human chorionic gonadotropin conjugates was determined in 4 ovarian cancer cell lines. Expression and regulation of luteinizing hormone/human chorionic gonadotropin conjugates was analyzed before and after treatment with human chorionic gonadotropin, epidermal growth factor, and 8-bromo-cyclic adenosine monophosphate with a nested reverse transcriptase-polymerase chain reaction approach. Results Toxicity of human chorionic gonadotropin-doxorubicin conjugates was increased compared with unconjugated doxorubicin in OVCAR-3 cells. However, drug conjugates failed to demonstrate increased toxicity in other cell lines, especially after preincubation with human chorionic gonadotropin. All cell lines expressed luteinizing hormone/human chorionic gonadotropin receptors. Receptor expression in OVCAR-3 cells was not effected by human chorionic gonadotropin, endothelial growth factor, or 8-bromo-cyclic adenosine monophosphate treatment. In other cell lines, receptor expression was down-regulated by these agents. Conclusion Cytotoxic activity of doxorubicin was increased specifically by conjugation to human chorionic gonadotropin. However, the regulation of luteinizing hormone/human chorionic gonadotropin receptor expression and other compounds may reduce the drug-uptake of the conjugates.

Objective
Our purpose was to show the effects of pre-B-cell colony-enhancing factor on the genes that are expressed by the human fetal membranes.

Study design
Explants of fetal membranes (amnion, chorion, and decidua) from three term patients were treated with 100 ng/mL recombinant human pre-B-cell colony-enhancing factor for 4 hours. RNAs were hybridized to gene chips that contained >18,000 known genes. One experiment was done in triplicate to assess replication. Data were analyzed to quantitate the signal intensities of each complementary DNA on the array. Confirmation of the results was carried out on tissues from nine other patients by the measurement of the proteins or quantitative real-time reverse transcriptase-polymerase chain reaction.

Results
Replication gave
Conclusion
Pre-B-cell colony-enhancing factor appears to be at the proximal end of the pathway to labor initiation and may link sterile distention-induced labor with that of infection-induced labor.


http://www.sciencedirect.com/science/article/B6W9P-4CDHKM9-9J/2/89c71ba0330f704cf4e9bde559b1202b

OBJECTIVE: Our purpose was to determine the frequency of allele loss in the region of the BRCA1 gene in cancers of women who have both breast and ovarian cancer.

STUDY DESIGN: Four polymorphic microsatellite markers on chromosome 17q11-21 were examined by the polymerase chain reaction in deoxyribonucleic acid from paraffin blocks of normal tissues, breast cancers, and ovarian cancers in 24 women who had primary cancers in both sites. RESULTS: Loss of heterozygosity was seen in one or more markers on chromosome 17q11-q21 in 46% of breast cancer and 78% of ovarian cancers. In 38% of cases allele loss was seen in both cancers, and in all these cases the same allele was lost in both cancers. Significantly younger ages at diagnosis of both breast and ovarian cancer were noted among cases with allele loss in both cancers compared with cases in which allele loss was found only in the ovarian cancer (p CONCLUSIONS: Because cases in which 17q11-21 allele loss was seen in both cancers had a young age of onset and the same allele was always deleted in both cancers, hereditary alterations in BRCA1 may play a role in this subset. The older age of onset in cases in which allele loss was seen only in the ovarian cancer suggests that the development of these cancers is not related to an inherited defect in BRCA1.

American Journal of Ophthalmology (9)


http://www.sciencedirect.com/science/article/B6VK5-4DPKVWX-B/2/12269a6c98af636442767ef27751b984

Purpose
To report a phenotypic variant of lattice corneal dystrophy associated with two missense changes, Ala546Asp and Pro551Gln, in the transforming growth factor-[beta]-induced gene (TGFBI).

Design
Experimental study.
Methods
Genomic DNA was obtained from the proband as
well as affected and unaffected family members. Exons 4, 11, 12, and 14 of the TGFBI gene were
amplified and sequenced. Additionally, a corneal button excised from the proband was examined
by light and transmission electron microscopy. Haplotype analysis was performed on the
proband's family and members of a previously identified pedigree with the same TGFBI gene
missense changes.

Results
Bilateral, symmetric, radially arranged, branching refractile lines within
and surrounding an area of central anterior stromal haze were noted in the proband. Multiple
polymorphic, refractile deposits were noted in the mid and posterior stroma in both the proband
and her daughter. Light and electron microscopic analyses demonstrated amyloid and excluded
the presence of deposits characteristic of granular corneal dystrophy. Screening of TGFBI exon
12 in the proband and her affected daughter revealed two missense changes, Ala546Asp and
Pro551Gln (both absent in 250 control chromosomes). Haplotype analysis suggested that the
mutations in this family and in a previously identified pedigree reflect a founder effect, rather than
an independent occurrence.

Conclusions
We present a phenotypic variant of lattice corneal
dystrophy associated with the Ala546Asp and Pro551Gln missense changes in exon 12 of the
TGFBI gene. A common ancestor appears to account for the missense mutations observed in this
pedigree and in a previously reported family.

(CHST6) in American patients with macular corneal dystrophy." American Journal of

http://www.sciencedirect.com/science/article/B6VK5-4BVK5C0-
C/2/ffff9d2e1e8522533dc942b4db2bda860

Purpose
To further characterize the mutations within the CHST6 gene responsible for causing
macular corneal dystrophy in a cohort of affected patients from the United
States. Design
Experimental study. Methods
Genomic DNA was extracted from buccal epithelium of
16 affected patients (14 families), 17 unaffected relatives, and 127 controls, followed by
polymerase chain reaction amplification and direct sequencing of the CHST6 coding region.
Subtyping of affected patients into type I and II macular corneal dystrophy was performed by
measuring antigenic keratan sulfate (AgKS) serum levels. Haplotype analysis was performed in
families that demonstrated common mutations. Results
CHST6 coding region analysis in 10
patients identified as having type I macular corneal dystrophy revealed 10 sequence changes:
eight missense mutations, four of which are novel (Met104Val, Tyr110Cys, Gln122Pro, and
Leu276Pro) and four of which have been reported previously (Ser51Leu, Pro72Ser, Cys102Gly,
and Leu200Arg); one novel homozygous nonsense mutation in two patients from a single family
(c. 1683C>T, Gln331X); and one frameshift mutation in a heterozygous state in a single patient
(c.1744_1751dupGTGCAGCTG). Mutation analysis in the four patients identified as having type II
macular corneal dystrophy (serum samples were not obtained from two affected patients)
revealed three patients heterozygous for either the c.923G>C, c.969C>A, or c.1519T>C
sequence changes. The fourth patient was compound heterozygous for c.969C>A and
c.1291T>G. None of these changes was observed in 127 control individuals. Haplotype analysis
using microsatellite markers flanking the CHST6 gene did not reveal a common founder for the
Leu200Arg (1291T>G) missense mutation, present in five families, identifying this position as a
mutation hot-spot. Conclusions
A variety of previously unreported mutations in the coding region of
the CHST6 gene are associated with type I macular corneal dystrophy in a cohort of patients from
the United States.

and retinal pigmented epithelium: implications for the process of drusen formation." American
PURPOSE: The inheritance of specific apolipoprotein E alleles has been linked to atherosclerosis, Alzheimer disease, and, most recently, to the incidence of age-related macular degeneration. Apolipoprotein E is a common component of the extracellular plaques and deposits characteristic of these disorders, including drusen, which are a hallmark of age-related macular degeneration. Accordingly, we assessed the potential biosynthetic contribution of local ocular cell types to the apolipoprotein E found in drusen.

METHODS: We measured apolipoprotein E mRNA levels in human donor tissues using a quantitative assay of apolipoprotein E transcription, and we localized apolipoprotein E protein to specific cell types and compartments in the neural retina, retinal pigmented epithelium, and choroid using laser scanning confocal immunofluorescence microscopy.

RESULTS: Apolipoprotein E immunoreactivity is associated with photoreceptor outer segments, the retinal ganglion cell layer, the retinal pigmented epithelium basal cytoplasm and basal lamina, and with both collagenous layers of Bruch membrane. Apolipoprotein E appears to be a ubiquitous component of drusen, irrespective of clinical phenotype. It also accumulates in the cytoplasm of a subpopulation of retinal pigmented epithelial cells, many of which overlie or flank drusen. Mean levels of apolipoprotein E mRNA in the adult human retina are 45% and 150% of the levels measured in liver and adult brain, the two most abundant biosynthetic sources of apolipoprotein E. Apolipoprotein E mRNA levels are highest in the inner retina, and lowest in the outer retina where photoreceptors predominate. Significant levels of apolipoprotein E mRNA are also present in the retinal pigmented epithelium/choroid complex and in cultured human retinal pigmented epithelial cells.

CONCLUSIONS: Apolipoprotein E protein is strategically located at the same anatomic locus where drusen are situated, and the retinal pigmented epithelium is the most likely local biosynthetic source of apolipoprotein E at that location. Age-related alteration of lipoprotein biosynthesis and/or processing at the level of the retinal pigmented epithelium and/or Bruch membrane may be a significant contributing factor in drusen formation and age-related macular degeneration pathogenesis.


PURPOSE: Meesmann corneal dystrophy is an autosomal dominant disorder characterized by fragility of the anterior corneal epithelium. We have previously demonstrated that this disease can be caused by mutations in the genes encoding keratins K3 or K12, the major intermediate filament proteins expressed in corneal epithelial cells. Here, we have carried out mutation analysis in a United States kindred presenting with typical features of Meesmann corneal dystrophy.

METHODS: Exons 1 and 6 of the K12 gene (KRT12) were polymerase chain reaction amplified from the proband's and control DNA and subjected to direct automated sequencing.

RESULTS: A heterozygous missense mutation 1300A->G was detected in exon 6 of KRT12, predicting amino acid substitution I426V in the helix termination motif of the K12 polypeptide. The mutation was confirmed in the proband and excluded from 50 normal individuals by restriction enzyme analysis of polymerase chain reaction products.

CONCLUSION: We report a novel mutation in a critical molecular overlap region of K12 in a United States family with Meesmann corneal dystrophy. The results confirm that mutations in the corneal keratins (K3 or K12) can underlie Meesmann corneal dystrophy.


http://www.sciencedirect.com/science/article/B6VK5-42G0MJX-C/2/6555ab38842b29a386b1e95a9a380e6c

**PURPOSE:** To search for patients with Usher syndrome type IC among those with Usher syndrome type I who reside in New England.

**METHODS:** Genotype analysis of microsatellite markers closely linked to the USH1C locus was done using the polymerase chain reaction. We compared the haplotype of our patients who were homozygous in the USH1C region with the haplotypes found in previously reported USH1C Acadian families who reside in southwestern Louisiana and from a single family residing in Lebanon.

**RESULTS:** Of 46 unrelated cases of Usher syndrome type I residing in New England, two were homozygous at genetic markers in the USH1C region. Of these, one carried the Acadian USH1C haplotype and had Acadian ancestors (that is, from Nova Scotia) who did not participate in the 1755 migration of Acadians to Louisiana. The second family had a haplotype that proved to be the same as that of a family with USH1C residing in Lebanon. Each of the two families had haplotypes distinct from the other.

**CONCLUSION:** This is the first report that some patients residing in New England have Usher syndrome type IC. Patients with Usher syndrome type IC can have the Acadian haplotype or the Lebanese haplotype compatible with the idea that at least two independently arising pathogenic mutations have occurred in the yet-to-be identified USH1C gene.


http://www.sciencedirect.com/science/article/B6VK5-47GDKVG-4/2/1f13e2d2a6a5314973ea44700d4a0e70

**Purpose**

To determine whether cytomegalovirus (CMV) retinitis that responded poorly to intravenous ganciclovir but responded to the ganciclovir implant was caused by virus with resistance mutations in the viral UL97 and UL54 genes.

**Design**

Retrospective chart review and laboratory-based experimental study.

**Methods**

Regions of the CMV UL97 and UL54 were amplified from the vitreous and analyzed for resistant mutations by a combination of DNA sequencing and restriction digestion. Vitreous from patients with AIDS and retinal infections other than CMV retinitis served as negative controls.

**Results**

We amplified all target regions of UL97 DNA and most target regions of UL54 DNA from eight eyes with CMV retinitis. In one eye we found a ganciclovir resistance mutation at base 1781 of the UL97 gene, predicting an alanine to valine mutation at codon 594. In a second eye we found a ganciclovir resistance mutation at base 2960 of the UL54 gene, predicting an alanine to glycine mutation at codon 987. In two additional eyes, both from patients with bilateral retinitis, we found UL54 mutations that are likely to confer resistance to ganciclovir but have not been previously described. In both of these patients the UL54 genotype differed between the two diseased eyes.

**Conclusions**

Failure to control CMV retinitis with intravenous ganciclovir does not necessarily imply infection with a virus having a known mutation that confers drug resistance. The ganciclovir implant can be an effective therapy for CMV retinitis caused by virus with certain UL97 and UL54 resistance mutations. Cytomegalovirus UL54 genotypes can differ between eyes in patients with bilateral retinitis.


http://www.sciencedirect.com/science/article/B6VK5-44R37H5-
PURPOSE: To test the utility of DNA Taq polymerase (Taq) treated with 8-methoxypsoralen with ultraviolet irradiation and Taq treated with restriction endonuclease in a nested polymerase chain reaction test for the diagnosis of bacterial endophthalmitis. METHODS: Prospective, comparative study. Vitreous biopsy fluid from 32 cases of clinically diagnosed bacterial endophthalmitis and 10 noninfected controls were cultured for aerobic/anaerobic bacteria and tested by nested polymerase chain reaction using two sets of universal eubacterial primers in triplicate with untreated Taq, Taq treated with 8-methoxypsoralen with ultraviolet irradiation, and Taq treated with Sau 3A1. RESULTS: Using untreated Taq, false-positive results were obtained in nested polymerase chain reaction with all 10 control samples, which were not seen with the other two methods of nested polymerase chain reaction. However, the sensitivity of nested polymerase chain reaction using Sau 3A1 was the same sensitivity as the conventional culture (34.4%), whereas the sensitivity of the nested polymerase chain reaction using 8-methoxypsoralen was 46.9% higher than in the conventional culture. CONCLUSION: To eliminate the problem of false positives in bacterial nested polymerase chain reaction, we recommend the routine utilization of Taq treated with 8-methoxypsoralen and ultraviolet irradiation.


http://www.sciencedirect.com/science/article/B6VK5-417WDMG-1P/2/73452d192c76856d7cda501d5fa88571

PURPOSE: To screen a population with primary open-angle glaucoma for mutations in the gene that encodes the trabecular meshwork inducible glucocorticoid response protein (TIGR), also known as myocilin (MYOC). METHODS: Ophthalmologic information was collected for study subjects with primary open-angle glaucoma and their relatives. Mutation screening of 74 primary open-angle glaucoma probands was conducted by sequencing TIGR/MYOC coding sequence and splice sites. RESULTS: In 23 families we detected 13 nonsynonymous sequence changes, nine of which appear to be mutations likely to cause or contribute to primary open-angle glaucoma. Two mutations, Arg272Gly and Ile499Ser, and one nonsynonymous sequence variant, Asn57Asp, are novel. We found mutations in nine of 25 juvenile glaucoma probands (36%) and two of 49 adult-onset glaucoma probands (4%). Age classification of families rather than individual probands revealed mutations in three of nine families with strictly juvenile primary open-angle glaucoma (33%), and no mutations in 39 families with strictly adult-onset primary open-angle glaucoma (0%). In families with mixed-onset primary open-angle glaucoma containing both juvenile primary open-angle glaucoma and adult-onset primary open-angle glaucoma cases, we found mutations in eight of 26 families (31%). CONCLUSIONS: Our data suggest that Gly252Arg, Arg272Gly, Glu323Lys, Gln368STOP, Pro370Leu, Thr377Met, Val426Phe, Ile477Asn, and Ile499Ser are likely to play roles that cause or contribute to the etiology of autosomal dominant primary open-angle glaucoma. Our finding of more TIGR/MYOC mutations in families with mixed-onset primary open-angle glaucoma than in the families with strictly adult-onset primary open-angle glaucoma implies that the presence of relatives with juvenile primary open-angle glaucoma in a family could be used as a basis for identifying a subset of the population with adult-onset primary open-angle glaucoma with higher prevalence of TIGR/MYOC mutations. To address this issue, and to refine estimations of mutation prevalence in these age-defined subpopulations, prospective study of a larger population ascertained entirely through adult-onset primary open-angle glaucoma probands will be needed.

Purpose
To describe an American family with lattice corneal dystrophy type I, which associates with a novel mutation, Leu569Arg, of the TGFBI (BIGH3) gene.

Design
Experimental study.

Methods
Genomic DNA was extracted from buccal epithelial cells of four affected members of an American family with lattice corneal dystrophy type I. All 17 exons of the TGFBI gene were evaluated by PCR amplification and direct sequencing. Clinical and histologic data were also collected.

Results
Three generations of this family have been positively diagnosed with lattice corneal dystrophy, indicating autosomal dominant inheritance. We identified a heterozygous point mutation that associates with the disease phenotype. The single base-pair substitution (T1753G) results in an amino acid substitution (Leu569Arg) in exon 13 of the TGFBI gene.

Conclusions
Substitution of arginine for leucine at position 569 of the TGFBI gene results in a form of lattice corneal dystrophy that is phenotypically similar to other genetically distinct forms of type I disease. This is the first report of disease correlated with changes in exon 13 of the TGFBI gene.

Anesth. Analg. (1)


We sought to determine whether local administration of pentoxifylline (PTF) or propentofylline (PPTF), which hinders cytokine production, influences pain threshold and formalin-induced pain behavior in rats or the level of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) messenger RNA (mRNA) concentrations in the inflamed paw tissue. PTF (0.5, 1, or 2 mg) and PPTF (1 or 2 mg) injected intraplantarly (i.pl.) had no significant effect on pain threshold. Injection of 0.1 mL of a 12% formalin solution subcutaneously into the dorsal surface of the left hindpaw induced pain behavior (47.6 \(\pm\) 4.6 incidents per 5 min), and PTF injected at doses of 1 and 2 mg/100 \(\mu\)L i.pl. before (but not after) formalin was effective in antagonizing (33.6 \(\pm\) 2.5 and 23.6 \(\pm\) 3.4 incidents per 5 min, respectively) formalin-induced pain behavior. A similar antagonistic effect was observed after PPTF treatment at a dose of 2 mg/100 \(\mu\)L; however, in contrast to PTF, at a later time point (85-90 min) after the formalin challenge, this effect was independent of the scheme of PPTF administration, before or after formalin. The effect of PTF on formalin-induced pain behavior did not parallel paw volume as measured by plethysmometer; however, PTF per se significantly increased the paw volume. Formalin injection significantly increased the TNF-\(\alpha\) mRNA level in the inflamed tissue of the rat hind paw (150%). PTF administered before, but not after, formalin significantly antagonized (by approximately 40%) the observed increase in the level of TNF-\(\alpha\) mRNA. Our study demonstrates and provides biochemical evidence that preemptive inhibition of proinflammatory cytokine synthesis by the use of PTF and PPTF, phosphodiesterase, and glial activation inhibitors is useful in antagonizing hyperalgesia in formalin-induced pain. Moreover, local administration of PTF may be a valuable approach to the treatment of inflammatory pain. IMPLICATIONS: This study demonstrates and provides biochemical evidence that preemptive inhibition of proinflammatory cytokine synthesis by local...
administration of pentoxifylline and propentofylline is useful in antagonizing hyperalgesia in formalin-induced pain. Moreover, local administration of pentoxifylline could be regarded as a valid approach to the treatment of inflammatory pain.


http://www.annclinlabsci.org/cgi/content/abstract/34/3/314

(α)(1,2)-Fucosyltransferase catalyzes the transfer of fucose to the C-2 position of galactose on type II precursor substrate Gal[β]1-4GlcNAc[β]1-R. It plays an important biological role in the formation of H antigen, a precursor oligosaccharide for both A and B antigens on red blood cells. Aberration of (α)(1,2)-fucosyltransferase activity by gene mutations results in decreased synthesis of H antigen, leading to the para-Bombay phenotype. In this study, we collected about 250,000 blood samples in Taiwan during 5 yr and identified the subjects with para-Bombay phenotype. Then we analyzed the sequence of the (α)(1,2)-fucosyltransferase gene by direct sequencing and gene cloning methods, using the blood samples of 30 para-Bombay individuals and 30 control subjects who were randomly selected. The goals of this study were to search for new h alleles, to determine the h allele frequencies, and to test whether the sporadic theory is applicable in Taiwan. Six different h alleles (ha, 547~548 AG-del; hb, 880~881 TT-del; hc, R220C; hd, R220H; he, F174L; and hf, N327T) were observed. Two h alleles, he and hf, were newly discovered in Taiwan. The he allele has a nucleotide 522C>A point mutation, predicting the amino acid 174 substitution of Phe to Leu; the hf allele has missense mutation of nucleotide 980A>C, predicting the amino acid 327 substitution of Asn to Thr. Frequencies of the 6 alleles are ha 46.67%, hb 38.33%, hc 5.00%, hd 1.67%, he 3.33%, and hf 5.00%, respectively. These findings in the Taiwanese population confirm previous observations in other populations that the Bombay and para-Bombay phenotypes are due to diverse, sporadic, nonfunctional alleles, predominantly ha and hb, leading to H deficiency of red blood cells. In contrast to previous reports of non-prevalent associations of h alleles with para-Bombay phenotype, our results suggest a regional allele preference associated with para-Bombay individuals in Taiwan.


http://www.annclinlabsci.org/cgi/content/abstract/34/1/63

The ABO system is one of the major blood groups that have significant impact on blood transfusion and paternity testing. We have found a new ABO allele by analyses of the ABO genotype of the Taiwanese population. Exons 6 and 7 of the ABO gene were amplified by the polymerase chain reaction and analyzed by direct sequencing. The results indicated that the ABO gene in the Taiwanese population consists mainly of the A1, A1v, B, O1, and O1v alleles. In addition, a novel O allele designated as OTaiwan was identified that has G [GT] T substitution at the nucleotide 801 of the O1 allele. The OTaiwan allele is inheritable, since it is also present in an offspring of the OTaiwan-carrying individual. The information presented herein is valuable for population research and for analyses of evolutionary lineage.
Trisomy 21 is the most common chromosomal aberration in live births. In this study we employed human chromosome 21-specific short tandem repeat (STR) DNA markers to determine the numbers of chromosome 21 present in fetal cells. Forty amniotic fluid samples from pregnancies complicated with fetal Down syndrome and 98 samples from euploid pregnancies were analyzed for D21S11 and interferon-(alpha) receptor (IFNAR) gene intervening sequence. Fluorescent dye-labeled primers were used in PCR amplification of these 2 markers. The PCR amplicon was analyzed with an automatic DNA sequence analyzer. The results showed that 35 of 40 fetal Down syndrome samples analyzed for IFNAR showed 3 distinct peaks, while 24 of 30 cases analyzed for D21S11 showed 3 distinct peaks. Two Down syndrome samples showed two uneven peaks. By analyzing 98 euploid pregnancies as controls, the ratios of area under the peaks were determined to be 1.31 {+/-} 0.22 and 1.96 {+/-} 0.18 (mean {+/-} SD) for the euploid pregnancies and pregnancies complicated by fetal Down syndrome with 2 peaks, respectively. Our data showed that altogether 39 of 40 (97.5%) Down syndrome cases were correctly identified based on either the 3-peak pattern in one or more of the DNA markers or the relative peak area ratio calculation. In conclusion, polymorphic STR DNA markers are useful for determining the numbers of chromosome 21 in fetal cells. The high sensitivity and automation of the procedures suggest a good prospect for use of this method in prenatal detection of fetal Down syndrome. However, this is a preliminary investigation and a large-scale study is necessary to validate the clinical application of this protocol.
combined phenotype "diabesity" gave the strongest evidence for linkage (LOD = 5.2). Our aim is to positionally clone the gene(s) responsible for the linkage. Linkage disequilibrium mapping is being used to narrow the chromosomal region. Single nucleotide polymorphisms (SNPs) are being systematically identified and genotyped at 50-kb intervals across the region of linkage. To date, 455 SNPs have been genotyped in 1229 Pimas. A region containing a cluster of SNPs strongly associated with BMI and a second region, approximately 2 Mb telomeric, containing a cluster of SNPs associated with diabetes have been preliminarily identified.


http://www.annalsnyas.org/cgi/content/abstract/1007/1/17

Estrogen plays an important role during midbrain development. This is indicated by the presence of nuclear estrogen receptors and the transient expression of the estrogen-forming enzyme aromatase. A number of recent studies have shown that estrogen promotes the differentiation and survival, as well as physiological performance, of midbrain dopaminergic cells. In addition, we have reported that both ways of cellular estrogen signaling (classical and nonclassical) as well as interactions with nonneuronal target cells are involved in the transmission of intra- and intercellular estrogen effects in this brain region. This study provides additional evidence that (i) estrogen is capable of regulating gene expression in cultured embryonic neurons and astrocytes differently and (ii) both signaling mechanisms, i.e., classically through nuclear receptors and nonclassically through the stimulation of membrane-estrogen receptors, which are coupled to distinct intracellular signal transduction cascades, contribute diversely to gene regulation. These data reveal a high degree of complexity of estrogen action at the genomic level in the developing brain. Further studies are warranted to unravel the exact contribution of the differently regulated genes for developmental estrogen action.


http://www.annalsnyas.org/cgi/content/abstract/990/1/118

Vector-borne diseases are a potential public health threat to U.S. Forces Korea (USFK). Ehrlichia and Anaplasma spp., transmitted by ticks, are only two of several diseases that may affect military readiness and operations. Rodents were collected at selected U.S. military installations and training sites in the Republic of Korea. DNA was extracted from spleen tissues and assayed by PCR methods for Ehrlichia and Anaplasma species. From rodents and mustelids collected during 1999 and 2000, a total of 196 Apodemus agrarius (striped field mouse), 2 Mustela sibirica (weasel), and 1 Cricetulus triton nestor (Korean greater long-tailed hamster) were assayed for Ehrlichia and Anaplasma species-specific DNA fragments. Rodent surveillance indicated a very high prevalence of Ehrlichia and Anaplasma spp. at selected training sites. Ehrlichia/Anaplasma DNA were identified from spleen tissue from 157 Apodemus agrarius, 1 Mustela sibirica, and 1 Cricetulus riton nestor. Species-specific DNA fragments of E. canis (45), E. ewingii (16), A. phagocytophila (5), and A. platys (62) were amplified by PCR techniques. Seventy-one striped field mice had single infections, while 24 had mixed infections of 2 (17 specimens), 3 (7 specimens), or 4 (1 specimen) pathogens. The striped field mouse plays a role as a reservoir for latent infections of various Ehrlichia or Anaplasma species.

http://www.annalsnyas.org/cgi/content/abstract/1025/1/345

Analysis by differential display of genes induced in response to acute cocaine administration to rats revealed the significant downregulation of several mitochondrial genes in the cingulate cortex, including the subunits 1, 2, 4, 5, and 6 of NADH dehydrogenase and the subunit 2 of cytochrome c oxidase. Although the mechanism of the downregulation of expression of these mitochondrial genes by cocaine is presently not well understood, one can envisage that it involves an increased production of reactive oxygen species in cells of the cerebral cortex.


http://www.annalsnyas.org/cgi/content/abstract/1022/1/140

Bronchoscopy is a standard procedure in the workup of patients with suspicious pulmonary lesions. We wondered whether it is possible to isolate malignancy-associated mRNA from cell-free lavage supernatant. Extracellular mRNA from cell-free lavage supernatant of 25 patients with lung cancer (23 with non-small cell lung cancer, 2 with small cell lung cancer) was isolated, reverse-transcribed, and amplified by reverse transcriptase polymerase chain reaction. The quantity and quality of the isolated RNA were checked after cDNA synthesis by amplification with {beta}-actin-specific primers. Afterwards, a panel of eight genes known to be expressed in lung tumors was used for the detection of tumor-associated mRNA expression in lavage supernatant and serum. mRNA coding for {beta}-actin could be isolated from lavage supernatant of all 25 patients. In addition, the expression of at least one tumor-associated gene was detectable in all patients. These results show that intact mRNA can be isolated from cell-free lavage supernatant and that its quantity and quality are sufficient for the detection of tumor-associated gene expression alterations. This may open new possibilities for the diagnosis of lung cancer.


http://www.annalsnyas.org/cgi/content/abstract/962/1/332

Immune stimulants, such as the bacterial endotoxin, lipopolysaccharide (LPS), the human immunodeficiency virus-1 coat protein gp120, or {beta}-amyloid peptides, lead to glial activation and production of various immune mediators, such as nitric oxide (NO) and proinflammatory cytokines in the brain. These mediators appear to contribute to neuronal cell death in neurodegenerative diseases. However, the signaling pathways, which mediate the neurotoxic effect by the endotoxin, are not understood. The purpose of this study was to determine the role of mitogen-activated protein kinase (MAPK) in LPS-induced neurodegeneration using mesencephalic dopaminergic neuron/glia cultures. We have found that the p38 MAPK is important in LPS-induced death of mesencephalic neurons in rat neuron-glia mixed cultures. Upon treatment with 10 ng/ml LPS, the number of dopaminergic neurons decreased by 80% within 48 h, preceded by a significant production of NO by glia. Neuroprotection by selective inhibition of p38 MAPK activity paralleled a decrease in LPS-induced inducible nitric oxide.
synthase (iNOS) expression. These events were significantly reduced by the selective p38 MAPK inhibitor, SB202190, but not by the inactive analogue SB202474. Inhibition of iNOS activity and NO production by treatment with GW274150 was also neuroprotective. Although the p38 MAPK inhibitor afforded significant neuroprotection from LPS toxicity in the neuron-glia mixed culture, it failed to protect dopaminergic neurons from 6-hydroxy-dopamine-induced toxicity, which acts directly on dopaminergic neurons by inducing hydroxyl radical formation from the mitochondria. The results suggest that p38 MAPK in glia plays a significant role in the LPS-induced death of mesencephalic neurons through induction of nitric oxide synthase and resulting NO production.


http://www.annalsnyas.org/cgi/content/abstract/990/1/302

*Rickettsia prowazekii*, the etiologic agent for epidemic typhus, and *Borrelia recurrentis*, the etiologic agent of relapsing fever, both utilize the same vector, the human body louse (*Pediculus humanus*), to transmit human disease. We have developed an assay to detect both bacterial pathogens in a single tube utilizing real-time PCR. Assays for both agents are specific. The *R. prowazekii* and *B. recurrentis* assays do not detect nucleic acid from *R. typhi*, *R. canada*, or any of eight spotted fever rickettsiae. In addition they did not react with *Neorickettsia risticiii*, *N. sennetsu*, *Franciscella persica*, *Bartonella quintana*, *Legionella pneumophila*, *Proteus mirabilis*, *Salmonella enterica*, *Escherichia coli*, and *Staphylococcus aureus*. Moreover, the *B. recurrentis* assay did not detect *B. duttonii*, *B. coriaceae*, *B. afzelii*, *B. garinii*, *B. hermsii*, or *B. burgdorferi* nucleic acid. Both assays detected repeatedly only *R. prowazekii* or *B. recurrentis* either when tested alone or together in one test tube.


http://www.annalsnyas.org


http://www.annalsnyas.org/cgi/content/abstract/1011/1/332

Long-term exposure to cadmium (Cd) induces perturbation of kidney proximal tubular epithelial cells. Mitochondrial dysfunction in renal cortical cells may contribute to the pathogenesis of Cd-induced nephropathy. In this study, we examined the accumulation of mitochondrial DNA (mtDNA) with a large deletion and cellular senescence in the renal cortex. Wistar rats at 8 weeks of age were intraperitoneally injected with 1 mL of 1 mM CdCl2 or saline, 3 times/week for 5, 20, 40, or 80 weeks. Mitochondrial Cd content in the renal cortex was quantified by atomic absorption analysis. Cytochrome c oxidase (CCO) and senescence-associated beta-galactosidase (SA-\{beta\}-gal) activity were determined in renal cortex by enzyme-histochemistry. mtDNA in total DNA extracted from the renal cortex was amplified by PCR, and mtDNA deletions, including 4,834-bp (nt8118-nt12937) deletion, were determined and semiquantified. After 40 weeks of Cd injection, Cd levels in the renal cortex reached a saturation level, and 30% of the level of the whole-cell fraction was found in the mitochondria. CCO activity in the renal cortex, which was
predominantly found in proximal tubular cells, decreased after 40 weeks of Cd exposure. Expression of SA-(beta)-gal was detected primarily in the proximal tubular cells and significantly increased after 80 weeks of Cd exposure. After 40 weeks of study, accumulation of 4,834-bp deletion in mtDNA was evident in both groups of rats; however, the amount of the deletion was significantly greater in Cd-treated rats than in control rats. Our results indicate that long-term Cd exposure induced a post-regenerative state of proximal tubular cells, which accelerated accumulation of 4,834-bp mtDNA deletions in the renal cortex, suggesting that Cd may be a senescence acceleration factor for kidney proximal tubular epithelial cells, which results in Cd-induced nephropathy.


http://www.annalsnyas.org/cgi/content/abstract/967/1/71

We observed earlier that increased skeletal muscle lipid content in the hereditary hypertriglyceridemic (hHTg) rat is accompanied by a decline in plasma leptin. Leptin has recently been shown to enhance peripheral insulin sensitivity by decreasing the tissue triglyceride accumulation, possibly through regulation of fatty acid oxidation and lipogenesis. Thus, to test the hypothesis that insulin resistance and increased skeletal muscle lipid accumulation in hHTg rats are due to a defect in lipid catabolism, we measured mitochondrial and peroxisomal fatty acid oxidation and malonyl-CoA and acetyl-CoA carboxylase-2 content in skeletal muscles of these animals. In addition, we investigated possible molecular mechanisms responsible for the lower leptin levels in hHTg rats by measuring leptin and leptin-receptor (Ob-Ra) mRNA levels. We found the following: (1) in spite of a higher skeletal muscle malonyl-CoA content and an increased sensitivity of carnitine palmitoyltransferase-1 to malonyl-CoA, carnitine palmitoyltransferase-1 activity in muscle of hHTg rats was normal; (2) increased peroxisomal fatty acid oxidation did not seem to be sufficient to prevent the tissue lipid accumulation in these animals; (3) both lower leptin production by white adipose tissue and increased leptin uptake seem to be responsible for lower circulating leptin levels and therefore lower fatty acid catabolism.


http://www.annalsnyas.org/cgi/content/abstract/967/1/558

Variability in the number of tandem repeats of the insulin gene (INS VNTR) is known to influence several phenotypes, including polycystic ovary syndrome (PCOS), diabetes mellitus type 1, diabetes mellitus type 2, and birth weight. The presence of the class III allele of INS VNTR has been reported to be protective in diabetes mellitus type 1, but in contrary it increases the disease risk of PCOS and diabetes mellitus type 2. PCOS is a very common endocrinopathy in women of reproductive age. The etiology of PCOS is uncertain, but family history of this syndrome suggests a major genetic cause. The aim of this pilot study was to investigate the possible association of INS VNTR polymorphism with PCOS in Czech women. In PCOS, significantly higher WHR, BMI, G0, G180, I30, Cp0, Cp30, Cp60, AUC-I, AUC-Cp, and insulinogetic index and significantly lower AUC-G/AUC-I were found. No significant differences in INS VNTR genotype, phenotype, or allele frequencies were found between PCOS and controls. In spite of several differences in anthropometric and biochemical parameters (abdominal fat localization, increased (beta)-cell function, and lower insulin sensitivity in PCOS women), no effect of INS VNTR polymorphism was found on insulin secretion, insulin action, or any other screened parameter.
Serine racemase (SRace) is an enzyme that catalyzes the conversion of L-serine to pyruvate or D-serine, an endogenous agonist for NMDA receptors. Our previous studies showed that inflammatory stimuli such as A\(\beta\) could elevate steady-state mRNA levels for SRace, perhaps leading to inappropriate glutamatergic stimulation under conditions of inflammation. We report here that a proinflammatory stimulus (lipopolysaccharide) elevated the activity of the human SRace promoter, as indicated by expression of a luciferase reporter system transfected into a microglial cell line. This effect corresponded to an elevation of SRace protein levels in microglia, as well. By contrast, dexamethasone inhibited the SRace promoter activity and led to an apparent suppression of SRace steady-state mRNA levels. A potential binding site for NF\(\kappa\)B was explored, but this sequence played no significant role in SRace promoter activation. Instead, large deletions and site-directed mutagenesis indicated that a DNA element between -1382 and -1373 (relative to the start of translation) was responsible for the activation of the promoter by lipopolysaccharide. This region fits the consensus for an activator protein-1 binding site. Lipopolysaccharide induced an activity capable of binding this DNA element in electrophoretic mobility shift assays. Supershifts with antibodies against c-Fos and JunB identified these as the responsible proteins. An inhibitor of Jun N-terminal kinase blocked SRace promoter activation, further implicating activator protein-1. These data indicate that proinflammatory stimuli utilize a signal transduction pathway culminating in activator protein-1 activation to induce expression of serine racemase.


Background: Angiogenesis is essential for development, growth and advancement of solid tumors. ETS-1 has been recognized as a candidate for tumor angiogenic transcription factor. This prompted us to study the clinical implications of ETS-1-related angiogenesis in uterine cervical cancers. Patients and methods: Fifty patients underwent curative resection for uterine cervical cancers. The patients' prognoses were analyzed with a 24-month survival rate. In the tissue of 60 uterine cervical cancers, the levels of ets-1 mRNA, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF) and interleukin (IL)-8 were determined by competitive reverse transcription-polymerase chain reaction using recombinant RNA and enzyme immunoassay, and the localization and counts of microvessels were determined by immunohistochemistry. Results: There was a significant correlation between microvessel counts and ets-1 gene expression levels in uterine cervical cancers. Immunohistochemical staining revealed that the localization of ETS-1 was similar to that of vascular endothelial cells. The level of ets-1 mRNA correlated with the levels of PD-ECGF and IL-8 among angiogenic factors. Furthermore, the prognosis of the 25 patients with high ets-1 mRNA expression in uterine cervical cancers was extremely poor, while the 24-month survival
rate of the other 25 patients with low ets-1 mRNA expression was 92%. Conclusions: ETS-1 might be a prognostic indicator as an angiogenic mediator in uterine cervical cancers.


http://annonc.oupjournals.org/cgi/content/abstract/15/3/489

Background: Hypermethylation is studied as a new, relevant mechanism for silencing tumor suppressor genes. It is a potentially reversible epigenetic change and it is the target of novel anticancer compounds with demethylating activity. In this perspective, we investigated E-cadherin gene (CDH1) promoter hypermethylation in gastric carcinomas and its correlation with E-cadherin protein expression. Methods: Consecutive cases of gastric carcinoma with assessable paraffin-embedded tumor blocks and paired normal mucosa were considered eligible for study entry. CDH1 promoter hypermethylation and E-cadherin protein expression were determined by methylation-specific polymerase chain reaction and immunohistochemistry, respectively. Results: CDH1 promoter hypermethylation was found in 20 out of 70 gastric carcinomas and the epigenetic change occurred in the early, as well as in the locally advanced disease. In five cases, hypermethylation was also detected in the normal mucosa. Eighteen out of 20 hypermethylated tumors were of the diffuse histotype (P = 0.0001). Of 24 tumors with reduced or negative E-cadherin expression, 19 were hypermethylated and 5 were unmethylated (P = 0.0001). Conclusions: CDH1 promoter hypermethylation frequently occurs in gastric carcinomas of the diffuse histotype and it is significantly associated with downregulated E-cadherin expression. The knowledge on the hypermethylation status of tumor suppressor genes may be relevant to the development of demethylating drugs and novel chemopreventive strategies in solid tumors.


http://annonc.oupjournals.org/cgi/content/abstract/14/5/704

Background: Mutations in the p53 gene are the most common genetic alterations in human primary breast carcinoma and these mutations are often associated with worse prognosis and chemo/radioresistance. Patients and methods: The analysis of the p53 gene was performed by fluorescence-assisted mismatch analysis in 13 consecutive high-risk primary breast cancer (HR-BC) patients with 10 or more involved axillary nodes to evaluate its prognostic value. Results: Three p53 mutations (23%) and four allelic variants were detected. After a median follow-up of 52 months the HR-BC disease-free survival (DFS) was 51% and overall survival 79%. All patients harboring a p53 mutation (p53mut) relapsed within 10 months of the median DFS while 67% of those showing a wild-type p53 status (p53wt) survive disease-free at a median follow-up of 43 months. One p53mut patient is still alive while all the p53wt patients survive at 56 months median follow-up. Two out of the four p53wt relapsing breast cancer patients showed the Arg72Pro allelic variant; one of these died at 75 months. Conclusions: p53 mutations may help identify a subset of very high risk breast cancer patients (vHR-BC) with worse prognosis.

Background: To evaluate the impact of dihydrofolate reductase (DHFR) and reduced folate carrier (RFC) genes on methotrexate (MTX) resistance in osteosarcoma cells in relation to retinoblastoma (RB1) gene status. Materials and methods: A series of human osteosarcoma cell lines—either sensitive or resistant to MTX—and 16 osteosarcoma tumour samples were used in this study. Results: In U-2OS MTX-resistant variants, and in other RB1-positive cell lines, MTX resistance was associated with increased levels of DHFR and with a slight decrease of RFC gene expression. In Saos-2 MTX-resistant variants, and in another RB1-negative cell line, development of MTX resistance was associated with a decrease in expression of RFC, without any significant involvement of DHFR. In osteosarcoma clinical samples, amplification of the DHFR gene at clinical onset appeared to be more frequent in RB1-positive compared with RB1-negative tumours. Conclusions: Amplification of the DHFR gene may occur more frequently in the presence of RB1-mediated negative regulation of its activity and can be present at clinical onset in osteosarcoma patients. Simultaneous evaluation of RFC, DHFR and RB1 gene status at the time of diagnosis may become the basis for the identification of potentially MTX-unresponsive osteosarcoma patients, who could benefit from treatment protocols with alternative antifolate drugs.

Ann. Rheum. Dis (8)


Objective: To evaluate the role of parvovirus B19 (B19), varicella zoster virus (VZV), and human herpes virus 6 (HHV-6) in the aetiopathology of giant cell arteritis (GCA). Methods: Temporal artery biopsy specimens from 57 patients with GCA and 56 controls were investigated. DNA was obtained by biopsy, and quantitative real time polymerase chain reaction assay performed to establish the prevalence and viral load of B19, VZV, and HHV-6. Amplification of the human β-globin gene was used as internal positive control. Results: (a) B19 was detected in 31/57 (54%) patients (median viral load 45.2 (25th-75th centiles 0-180.2) copies/μg DNA) v 21/56 (38%) controls (median viral load 0 (0-66.7) copies/μg DNA; p = 0.07 for DNA prevalence, p = 0.007 for viral load. Among 31 B19 positive samples, 21 (68%) patients with biopsy proven GCA had >102 B19 copies/μg of DNA v 5/21 (24%) controls; p = 0.001. (b) No significant difference was found for VZV (p = 0.94 for DNA prevalence; p = 0.76 for viral load) and HHV-6 (p = 0.89 for DNA prevalence; p = 0.64 for viral load) in the GCA group compared with controls. Conclusion: B19 may have a role in the aetiopathology of GCA, particularly in those patients with high viral load; no evidence was found for VZV and HHV-6.

Background: Transgenic deficiency in interferon-\(\gamma\) (IFN-\(\gamma\)) or IFN-\(\gamma\) receptor makes resistant strains of mice bearing H-2b or H-2d susceptible to collagen induced arthritis (CIA). Objective: To determine whether the escape from regulation of disease susceptibility at the major histocompatibility complex level involves a new use of autoimmune T cells expressing T cell receptor (TCR) V\(\beta\) that vary from the cell populations previously identified within arthritic joints. Methods: Arthritis was induced by a standard protocol with type II bovine collagen (CII) in complete Freund's adjuvant. Clinical features, histopathology, immunological responses, and TCR profile in arthritic joints in IFN-\(\gamma\) knockout C57BL/6 (B6.IFN-\(\gamma\) KO) mice (H-2b) were compared directly with those in DBA/1 mice (H-2q). Results: 60-80% of B6.IFN-\(\gamma\) KO mice developed a progressive arthritis with a similar clinical course to classical CIA in DBA/1 mice. The affected joints in B6.IFN-\(\gamma\) KO mice had an erosive form of arthritis with similar features to joint disease in DBA/1 mice. B6.IFN-\(\gamma\) KO mice produced significantly higher levels of IgG2b and IgG1 autoantibodies to murine CII and showed increased proliferative response to CII compared with B6 mice. Comparable levels of interleukin 1\(\beta\) and tumour necrosis factor \(\alpha\) expression were detected in arthritic joints from B6.IFN-\(\gamma\) KO and DBA/1 mice. B6.IFN-\(\gamma\) KO mice used predominantly TCR V\(\beta\)6 and V\(\beta\)8 in arthritic joints. This TCR V\(\beta\) profile is similar to that found in DBA/1 mice with CIA. Conclusions: C57BL/6 mice deficient in IFN-\(\gamma\) production can develop arthritis that resembles classical CIA. These data suggest that IFN-\(\gamma\) is a key factor mediating susceptibility to CIA.


Background: Antiphospholipid antibodies reacting with \(\beta\)2-glycoprotein I (\(\beta\)2GPI) have been associated with recurrent fetal loss and pregnancy complications. Objective: To investigate whether specific mutations in the phospholipid binding site of \(\beta\)2GPI might affect its binding to trophoblast and in turn the anti-\(\beta\)2GPI antibody induced functional effects. Methods: \(\beta\)2GPI adhesion to trophoblast was evaluated as human monoclonal IgM or polyclonal IgG anti-\(\beta\)2GPI antibody binding to trophoblast monolayers cultured (1) in complete medium; (2) in serum-free medium; (3) after serum starvation in the presence of purified human \(\beta\)2GPI; or (4) in the presence of \(\beta\)2GPI with single or multiple mutations in the amino acid loop Cys281-Lys-Lys-Glu-Lys-Cys288. The effect of anti-\(\beta\)2GPI binding to trophoblast was evaluated as chorionic gonadotropin (hCG) mRNA expression, and protein release by RT-PCR and radioimmunoassay, respectively. Results: \(\beta\)2GPI adhesion to trophoblast and its consequent recognition by the specific antibodies were inversely proportional to the mutation number in the phospholipid binding site. Anti-\(\beta\)2GPI antibodies reduced gonadotropin release, hormone dependent hCG mRNA expression, and protein synthesis in the presence of \(\beta\)2GPI, while the addition of the mutants or the absence of \(\beta\)2GPI had no effect. Conclusions: \(\beta\)2GPI binds to trophoblast in vitro through its fifth domain, as reported for endothelial cells, and can be recognised by anti-\(\beta\)2GPI antibodies; the antibody binding downregulates trophoblast hCG synthesis and secretion. Such a mechanism might contribute to defective placentation in women with fetal loss associated with the antiphospholipid syndrome.

Background: Chronic recurrent multifocal osteomyelitis (CRMO) in children is a chronic non-suppurative inflammation involving multiple sites. Some children affected by chronic non-bacterial osteomyelitis (CNO) do not have multiple lesions or a recurrent course. Objective: To characterise the long term outcome of children with the full spectrum of CNO. Methods: 30 children diagnosed with CNO were followed up for a mean of 5.6 years and their disease assessed using a clinical score, multiple imaging, and a diagnostic biopsy, including extensive microbial analysis. Results: 9 patients had unifocal non-relapsing disease, 3 unifocal lesions with relapses, 9 multifocal lesions without relapses, and 9 multifocal lesions with relapses (CRMO). Granulocytes were present significantly more often in CRMO than in unifocal and non-recurrent lesions. Pustulosis was more common in multifocal cases regardless of recurrence. Mean duration of treatment in 15 children with a single occurrence was 9.2 months. Naproxen treatment was generally effective. Naproxen treatment in 12 patients with relapses lasted 25 months. However, 7 of these were not effectively treated with naproxen alone. Five were treated with oral glucocorticoids for 27 days in addition to naproxen, which induced remission in four, lasting for at least 1.5 years. Longitudinal growth of affected bones was not altered, except for the development of hyperostosis. Conclusion: CNO is a spectrum of inflammatory conditions, with CRMO being the most severe. Most children with CNO have a favourable outcome of the disease. Oral glucocorticoids may be necessary in severe recurrent cases.


Background: Expression of aggrecan is reduced during aging and osteoarthritic cartilage degeneration. CpG methylation may have a role in the down regulation of aggrecan transcriptions. Objective: To investigate whether a correlation between gene methylation and expression of aggrecan in chondrocytes exists. Methods: The human aggrecan promoter region was analysed computationally for CpG-rich regions. These were investigated for the methylation of C residues in normal (aged) and osteoarthritic chondrocytes by the bisulphite method for modifying DNA as well as sequence analysis using DNA directly extracted from normal and osteoarthritic cartilage tissue. Additionally, chondrocytic cell lines were investigated for methylation within the aggrecan promoter region. Results: The CpG-rich promoter region of the human aggrecan gene contains a 0.6 kb region that meets the criteria of a CpG island as defined by prediction programmes. A significant correlation of aggrecan mRNA expression levels and methylation status in normal (aged) and osteoarthritic chondrocytes as well as in different chondrocytic cell lines was not found. Conclusions: Expression of aggrecan in normal cartilage and diseased states is not modulated by gross changes of CpG methylation of its promoter region. CpG methylation does not have a central role in the switch off of aggrecan promoter activity in human adult articular chondrocytes.

Del1 mouse model for osteoarthritis. Methods: Northern analysis was used to measure mRNA levels of MMP-2, -3, -8, -9, -13, and -14, and TIMP-1, -2, and -3 in total RNA extracted from knee joints of transgenic Del1 mice, harbouring a 15 amino acid deletion in the triple helical domain of the \( \alpha_1(II) \) collagen chain, using their non-transgenic littermates as controls. Immunohistochemistry was used to study the presence of cleavage products (neoepitopes) of type II collagen, and the distribution of MMP-13 and TIMP-1 in degenerating cartilage. Results: Each of the MMP and TIMP mRNAs analysed exhibited distinct expression patterns during development and osteoarthritic degeneration of the knee joint. The most striking change was up regulation of MMP-13 mRNA expression in the knee joints of Del1 mice at the onset of cartilage degeneration. However, the strongest immunostaining for MMP-13 and its inhibitor TIMP-1 was not seen in the degenerating articular cartilage but in synovial tissue, deep calcified cartilage, and subchondral bone. The localisation of type II collagen neoepitopes in chondrocytes and their pericellular matrix followed a similar pattern; they were not seen in cartilage fibrillations, but in adjacent unaffected cartilage. Conclusion: The primary localisation of MMP-13 and TIMP-1 in hyperplastic synovial tissue, subchondral bone, and calcified cartilage suggests that up regulation of MMP-13 expression during early degeneration of articular cartilage is a secondary response to cartilage erosion. This interpretation is supported by the distribution of type II collagen neoepitopes. Synovial production of MMP-13 may be related to removal of tissue debris released from articular cartilage. In the deep calcified cartilage and adjacent subchondral bone, MMP-13 probably participates in tissue remodelling.


http://ard.bmjjournals.com/cgi/content/abstract/64/4/575

Objectives: To analyse the association of interleukin 10 (IL10) promoter polymorphisms, which have been shown to be related to IL10 secretion capacity, with the response to long term treatment with etanercept in patients with rheumatoid arthritis (RA). Methods: Fifty patients with active RA were treated for up to 4 years (median 39 months, range 3-52) with stable doses of etanercept as monotherapy. Treatment response was assessed as defined by the EULAR criteria in an intention to treat analysis, with the last observation carried forward. IL10 promoter microsatellite polymorphisms IL10.R and IL10.G were genotyped by fragment length analysis in patients and 189 healthy controls matched for ethnicity, age, and sex. Haplotypes were reconstructed using a method based on bayesian, coalescent theory with the PHASE software. Results: IL10 microsatellite polymorphisms were not associated with susceptibility to RA. When patients with good treatment response (n = 25) were compared with patients with moderate (n = 17) or no response (n = 8), a significantly different distribution of the prevailing alleles R2, R3 and G9, G13, respectively, became evident. Good treatment response was associated with carriage of the R3 allele or R3-G9 haplotype, whereas the allele G13 and the haplotype R2-G13 predominated in patients with moderate or no response. Conclusion: Genotyping of the IL10 promoter microsatellites may be useful in predicting the clinical response to etanercept in patients with RA. The high prevalence of the presumptive IL10 low producer allele R3 in patients with a favourable response suggests that IL10 promotes disease activity in RA under the specific condition of tumour necrosis factor antagonism.


http://ard.bmjjournals.com/cgi/content/abstract/61/8/741
Objective: To gain a better understanding of how iron accumulates in human rheumatoid synovium. Methods: The distribution of ferritin, transferrin receptor, and non-specific resistance associated macrophage proteins 1 and 2 (Nramp1 and Nramp2) in the human rheumatoid synovium was investigated by immunocytochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR). Results: Both heavy and light ferritin subunit types were detected in the lining layer and the subinitimal zone of rheumatoid synovium, heavy ferritin generally being more abundant than light. Both heavy and light ferritin were detected in isolated synovial macrophages and fibroblasts. Transferrin receptor expression was largely confined to fibroblasts of the synovial lining layer. Nramp2 was detected by PCR in both isolated synovial macrophages and fibroblasts, whereas Nramp1 was detected by PCR and immunocytochemistry in macrophages and neutrophils in the lining and subinitimal zone, and in inflammatory infiltrates, but was absent from fibroblasts. Conclusion: A complex chain of events, perhaps initiated by proinflammatory cytokines, may culminate in a toxic build up of iron in the rheumatoid joint.

Ann. Surg. Oncol. (7)


http://www.annalssurgicaloncology.org/cgi/content/abstract/11/9/861

Background: Theory holds that the upper outer quadrant of the breast develops more malignancies because of increased tissue volume. This study evaluated genomic patterns of loss of heterozygosity (LOH) and allelic imbalance (AI) in non-neoplastic tissues from quadrants of diseased breasts following mastectomy to characterize relationships between genomic instability and the propensity for tumor development. Methods: Tissues from breast quadrants were collected from 21 patients with various stages of breast carcinoma. DNA was isolated from non-neoplastic tissues using standard methods and 26 chromosomal regions commonly deleted in breast cancer were examined to assess genomic instability. Results: Genomic instability was observed in breast quadrants from patients with ductal carcinomas in situ and advanced carcinomas. Levels of instability by quadrant were not predictive of primary tumor location (P = .363), but outer quadrants demonstrated significantly higher levels of genomic instability than did inner quadrants (P = .017). Marker D8S511 on chromosome 8p22-21.3, one of the most frequently altered chromosomal regions in breast cancer, showed a significantly higher level of instability (P = .039) in outer compared with inner quadrants. Conclusions: Non-neoplastic breast tissues often harbor genetic changes that can be important to understanding the local breast environment within which cancer develops. Greater genomic instability in outer quadrants can partially explain the propensity for breast cancers to develop there, rather than simple volume-related concepts. Patterns of field cancerization in the breast appear to be complex and are not a simple function of distance from a developing tumor.


http://www.annalssurgicaloncology.org/cgi/content/abstract/10/3/297

Background: Heparan sulfate proteoglycans, the main components of the extracellular matrix, are
recognized as important components of signal transduction and play an important role in tumor progression. Heparanase (hep) degrades heparan sulfate proteoglycans, but the clinical importance of hep is unclear. In this study, we investigated the clinicopathologic importance of hep messenger RNA (mRNA) expression in esophageal squamous cell carcinoma (ESCC).

Methods: Fresh tumors and noncancerous epithelia were obtained from 57 ESCC patients after esophagectomy. Expression levels of hep and glyceraldehyde-3-phosphate dehydrogenase mRNA were quantitatively analyzed by real-time reverse transcriptase-polymerase chain reaction. Apoptotic cancer cells and microvessel density were evaluated immunohistochemically. Results: The relative hep mRNA expression level (hep:glyceraldehyde-3-phosphate dehydrogenase ratio) in ESCC was lower than in noncancerous tissue (P <.001). Tumor hep expression decreased according to tumor progression and correlated with the occurrence of apoptotic cancer cells, but not with tumor microvessel density. Moreover, low hep expression correlated with poor patient survival. Conclusions: Reduced hep mRNA expression might result in abnormal cell growth and correlate with ESCC progression.


http://www.annalssurgicaloncology.org/cgi/content/abstract/10/9/1086

Background: This study evaluated the relationship between DNA aneuploidy and loss of heterozygosity (LOH) at different genetic loci in colorectal adenocarcinoma. Methods: A total of 112 patients with surgically removed colorectal adenocarcinoma in Taipei Veterans General Hospital from January 1999 to July 2001 were included in this study. The pattern of DNA ploidy was determined with DNA flow cytometry, and the LOH of various genetic loci was determined with fluorescence polymerase chain reaction and denaturing gradient gel electrophoresis. The relationship between DNA ploidy, LOH of various genetic loci, and clinicopathologic variables was analyzed with the (chi)2 test with Yates' correction as well as by multivariate binary logistic regression analysis. Results: Seventy-one (63.4%) of the 112 carcinomas had DNA aneuploidy. The DNA aneuploidy was not associated with any clinicopathologic variable. Ninety-one tumors (81.3%) exhibited LOH in at least one genetic locus. In the univariate analysis, the DNA aneuploidy was associated with LOH of Tp53-penta, D8S254, D5S346, and high-frequency LOH (P =.001, P =.016, P =.041, and P <.001, respectively). In the multivariate analysis, the most significant factor influencing DNA aneuploidy was D8S254, followed by Tp53-penta, high-frequency LOH, and D5S346. Conclusions: DNA aneuploidy is strongly associated with LOH at specific genetic loci.


http://www.annalssurgicaloncology.org/cgi/content/abstract/10/2/136

Background: The aim of this study was to more precisely map the region of 16q loss of heterozygosity (LOH) in Wilms' tumors and to examine the expression of putative tumor suppressor. Methods: We performed polymerase chain reaction-based LOH analysis on the 185 sample pairs from 21 to 80 megabases (Mb) on chromosome 16q. Expression of two candidate tumor suppressor genes located within the identified consensus region of 16q LOH was examined by immunohistochemistry. Results: We identified 16q LOH in 7 (4%) of 185 Wilms' tumors not previously thought to demonstrate such genetic loss. The smallest common region of genetic loss was located between 67.3 and 74.0 Mb on chromosome 16. Within this 6.7-Mb region, there reside only three recognized tumor suppressor genes: E-cadherin, P-cadherin, and
E-cadherin demonstrates statistically significantly reduced expression in Wilms' tumors with 16q LOH. Conclusions: We have localized the consensus region of 16q LOH in Wilms' tumor to a 6.7-Mb locus and have identified three candidate Wilms' tumor suppressor genes within this narrowed region. Our data support E-cadherin as a candidate tumor suppressor gene in Wilms' tumor; however, further studies are needed to definitively prove its role as the tumor suppressor gene associated with 16q LOH.


http://www.annalssurgicaloncology.org/cgi/content/abstract/9/1/71

Background: We investigated tumor DNA changes before and after mastectomy in the plasma of breast cancer patients with no disseminated disease and eventually investigated these changes' relationship to specific pathological parameters of the tumors. Methods: We studied 41 patients. DNA extracted from tumor and normal breast tissues, mononuclear blood cells, and plasma was used for molecular studies. Alterations in the microsatellite markers D17S855, D17S654, D16S421, TH2, D10S197, and D9S161, as well as point mutations in the p53 gene and aberrant methylation of p16INK4a, were used to identify and characterize tumor and plasma DNA. A number of tumor clinicopathological parameters were analyzed in each patient. Results: We found that 18 (44%) of the 27 patients with alterations in tumor DNA presented the same plasma DNA alteration before mastectomy, and persistence of the same molecular features was detected in plasma DNA 4 to 6 weeks postmastectomy in 8 (19.5%) patients. Patients with vascular invasion, more than three lymph node metastases, and higher histological grade at diagnosis displayed plasma DNA after mastectomy with a significant difference. Conclusions: Persistence of plasma DNA with features of tumor DNA may be present after mastectomy in breast cancer patients, and its relation to bad-prognosis histological parameters may suggest undetectable micrometastatic disease.


http://www.annalssurgicaloncology.org/cgi/content/abstract/9/1/88

Background: T-cell receptor {gamma} (TCR-{gamma}) is involved in maintaining host cell integrity and homeostasis of the human immune system. We hypothesize that polymorphism of the TCR-{gamma} complex may be involved in the pathogenesis of colorectal cancer. Methods: The microsatellite markers D7S1818 and D7S2206 located within the TCR-{gamma} antigen locus on chromosome 7p were amplified by polymerase chain reaction, and genotypes were determined for 22 patients with early onset of colorectal cancer (<60 years old) and for 38 population-based control subjects. Results: Genotype BC of D7S1818 (P =.049) and haplotype AC of D7S1818/D7S2206 (P [<=].003) were associated with colorectal cancer as compared with the control population (extended Fisher's exact test). Conclusions: This study identifies a novel genetic and clinical association between TCR-{gamma} and early-onset colorectal cancer. Many young patients do not fulfill the criteria for hereditary colorectal cancer syndromes and are therefore not identified by established screening programs. Markers such as D7S1818 and D7S2206 may become useful in the identification of patients at risk of developing colorectal cancer and permit earlier therapeutic intervention.
Background: Cancer-testis antigens (CTA), such as MAGE, are selectively expressed in various types of human neoplasms but not in normal tissues other than testis. This characteristic feature of CTA makes them promising antigens for cancer-specific immunotherapy. Methods: We investigated the expression of five genes, including MAGE-1, MAGE-3, NY-ESO-1, SCP-1, and SSX-4, in 20 surgical samples of intrahepatic cholangiocarcinomas (IHCC) using reverse transcription-polymerase chain reaction. To visualize the localization of MAGE proteins, we performed immunohistochemical studies. Furthermore, the correlation between the CTA expression and DNA methylation status was studied in three bile duct cancer cell lines. Results: Expression of MAGE-1, MAGE-3, NY-ESO-1, SCP-1, and SSX-4 was recognized in 4, 4, 2, 6, and 3 of all 20 cases, respectively. In contrast, the expressions of five genes were not recognized at all in the corresponding normal tissues. In 10 cases (50%), the tumors expressed at least one of the five CTA. An immunohistochemical analysis of MAGE proteins demonstrated homogenous or focal distributions in cytoplasm of the IHCC. Using a demethylating agent, MAGE-1, NY-ESO-1, SCP-1, and SSX-4 were induced in two of three cell lines, whereas MAGE-3 was not. Conclusions: Half of the tumor tissues of IHCC expressed at least one of the CTA. Some of the patients with IHCC, therefore, should be candidates for potentially useful cancer-specific immunotherapy.


PurposeTo investigate whether the three single nucleotide polymorphisms (SNPs), SNP-43, -56, and -63 of CAPN10 were associated with type 2 diabetes in a West African cohort. MethodsA total of 347 diabetic subjects and 148 unaffected controls from four ethnic groups in two West African countries were enrolled in this study. After genotyping three SNPs of CAPN10 and one SNP from CYP19, the allele, genotype, and haplotype frequencies as well as the odds ratios were calculated to test their association with type 2 diabetes. Results None of the alleles or genotypes was associated with type 2 diabetes. Although statistical analysis indicated that haplotype 221 was associated with type 2 diabetes (OR, 3.765; 95% CI, 1.577-8.989) in the two ethnic groups of Nigeria, the same haplotype did not show any association with type 2 diabetes in the two ethnic groups in Ghana (OR, 0.906; 95% CI, 0.322-2.552). Conclusion Considering the relatively low frequency of haplotype 221 and that none of the haplotypes including 221 was associated with any of the diabetes-related quantitative traits tested, it is concluded that SNP-43, -56, and -63 of the CAPN10 gene variants may play a limited role in the risk of type 2 diabetes risks in this cohort of West Africans.
Purpose
Genetically determined mixture information can be used as a surrogate for physical or behavioral characteristics in epidemiological studies examining research questions related to socially stigmatized behaviors and horizontally transmitted infections. A new measure, the probability of mixture discrimination (PMD), was developed to aid mixture analysis that estimates the ability to differentiate single from multiple genomes in biological mixtures.

Methods
Four autosomal short tandem repeats (STRs) were identified, genotyped and evaluated in African American, European American, Hispanic, and Chinese individuals to estimate PMD. Theoretical PMD frameworks were also developed for autosomal and sex-linked (X and Y) STR markers in potential male/male, male/female and female/female mixtures.

Results
Autosomal STRs genetically determine the presence of multiple genomes in mixture samples of unknown genders with more power than the apparently simpler X and Y chromosome STRs. Evaluation of four autosomal STR loci enables the detection of mixtures of DNA from multiple sources with above 99% probability in all four racial/ethnic populations.

Conclusions
The genetic-based approach has applications in epidemiology that provide viable alternatives to survey-based study designs. The analysis of genes as biomarkers can be used as a gold standard for validating measurements from self-reported behaviors that tend to be sensitive or socially stigmatizing, such as those involving sex and drugs.

Antimicrob. Agents Chemother. (40)


In 1999, 39 of 2,599 isolates of the family Enterobacteriaceae (1.5%) collected by eight private laboratories in the Aquitaine region in France produced an extended-spectrum (β)-lactamase (ESBL). Among these were 19 Enterobacter aerogenes isolates; 8 Klebsiella pneumoniae isolates; 6 Escherichia coli isolates; 3 Proteus mirabilis isolates; and 1 isolate each of Serratia marcescens, Morganella morganii, and Providencia stuartii. ESBL producers were isolated from 38 patients, including 33 residents of 11 clinics or nursing homes and 5 ambulatory patients. Seven different ESBLs were characterized. These mainly consisted of TEM-24 (25 isolates) and TEM-21 (9 isolates), but TEM-15 (2 isolates) and TEM-3, TEM-19, SHV-4, and CTX-M-1 (1 isolate each) were also characterized. Seven strains showed the coexistence of different TEM- and/or SHV-encoding genes, including a new SHV-1 variant, SHV-44, defined by the substitution R205L previously reported for SHV-3 in association with S238G. The epidemiology of the ESBL producers was investigated by random amplification of polymorphic DNA, typing by enterobacterial repetitive intergenic consensus PCR, analysis of resistance cotransferred with the ESBL, and analysis of the restriction profiles of the ESBL-encoding plasmids. Of the TEM-24-expressing strains, 18 were E. aerogenes isolates, including 9 from the same clinic, that were representatives of the epidemic clone disseminating in France. Of the TEM-21-producing strains that belonged to different species of the family Enterobacteriaceae (E. coli, K. pneumoniae, and P. mirabilis), 8 were isolated in the same nursing home. Outbreaks due to strain and/or plasmid
dissemination in these clinic and nursing home were demonstrated. The presence of ESBL producers in five ambulatory patients probably resulted from nosocomial acquisition. Our data highlight the serious need to monitor patients for ESBL-producing Enterobacteriaceae in general practice.


http://aac.asm.org/cgi/content/abstract/46/5/1183

Enterobacter cloacae Ecl261 was isolated with Escherichia coli Ec257 from the urine of a patient living in a nursing home. Both isolates were resistant to ticarcillin (MICs, 1,024 {micro}g/ml), without significant potentiation of its activity by 2 {micro}g of clavulanate per ml (MICs, 512 {micro}g/ml), and susceptible to naturally active cephalosporins. This inhibitor-resistant phenotype was conferred in both strains by similar conjugative plasmids of 40 kb (Ecl261) and 30 kb (Ec257), which also conveyed resistance to sulfonamides and trimethoprim. Clinical and transconjugant strains produced a (beta)-lactamase with a pl of 5.2 which belonged to the TEM family, as indicated by specific PCR amplification. Compared with TEM-1, this enzyme exhibited lower catalytic efficiencies (14- and 120-fold less for amoxicillin and ticarcillin, respectively), and higher concentrations of (beta)-lactamase inhibitors were required to yield a 50% reduction in benzylpenicillin hydrolysis (750-, 82-, and 50-fold higher concentrations for clavulanate, sulbactam, and tazobactam, respectively). Gene sequencing revealed four nucleotide differences with the nucleotide sequence of blaTEM-1A. The first replacement (T32C), located in the promoter region, was described as being responsible for the increase in the level of (beta)-lactamase production. The three other changes led to amino acid substitutions that define a new inhibitor-resistant TEM (IRT) (beta)-lactamase, TEM-80 (alternate name, IRT-24). Two of them, Met69Leu and Asn276Asp, have previously been related to inhibitor resistance. The additional mutation, Ile127Val, was demonstrated by site-directed mutagenesis to have a very weak effect, at least alone, on the IRT phenotype. This is the first description of an IRT (beta)-lactamase in E. cloacae. The horizontal transfer of blaTEM-80 may have occurred either from Ec257 to Ecl261 or in the reverse order.


http://aac.asm.org/cgi/content/abstract/49/1/77

We assessed the sensitivities and specificities of three previously described PCR primers on enrichment broth cultures of feces for the accurate detection of fecal carriage of vancomycin-resistant enterococci (VRE). In addition, we investigated specimens that were vanB PCR positive but VRE culture negative for the presence of other vanB-containing pathogens. Feces from 59 patients (12 patients carrying vanB Enterococcus faecium strains and 47 patients negative for VRE carriage) were cultured for 36 h in aerobic brain heart infusion (BHI) broth, anaerobic BHI (AnO2BHI) broth, or aerobic Enterococcusocel (EC) broth. DNA was extracted from the cultures and tested for the presence of vanB by using the PCR primers of Dutka-Malen et al. (S. Dutka-Malen, S. Evers, and P. Courvalin, J. Clin. Microbiol. 33:24-27, 1995), Bell et al. (J. M. Bell, J. C. Paton, and J. Turnidge, J. Clin. Microbiol. 36:2187-2190, 1998), and Stinear et al. (T. P. Stinear, D. C. Olden, P. D. R. Johnson, J. K. Davies, and M. L. Grayson, Lancet 357:855-856, 2001). The sensitivity (specificity) of PCR compared with the results of culture on BHI, AnO2BHI, and EC broths were 67% (96%), 50% (94%), and 17% (100%), respectively, with the primers of Dutka-
Malen et al.; 92% (60%), 92% (45%), and 92% (83%), respectively, with the primers of Bell et al.; and 92% (49%), 92% (43%), and 100% (51%) respectively, with the primers of Stinear et al. The primers of both Bell et al. and Stinear et al. were significantly more sensitive than those of Dutka-Malen et al. in EC broth (P = 0.001 and P < 0.001, respectively). The poor specificities for all primer pairs were due in part to the isolation and identification of six anaerobic gram-positive bacilli, Clostridium hathewayi (n = 3), a Clostridium innocuum-like organism (n = 1), Clostridium bolteae (n = 1), and Ruminococcus lactaris-like (n = 1), from five fecal specimens that were vanB positive but VRE culture negative. All six organisms were demonstrated to contain a vanB gene identical to that of VRE. VanB-containing bowel anaerobes may result in false-positive interpretation of PCR-positive fecal enrichment cultures as VRE, regardless of the primers and protocols used.


http://aac.asm.org/cgi/content/abstract/46/3/680

The in vitro activities of ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin against a large collection of clinical isolates of Streptococcus pneumoniae (n = 4,650) obtained over a 5-year period, 1994-1995 through 1999-2000, were assessed as part of a longitudinal multicenter U.S. surveillance study of antimicrobial resistance. Three sampling periods were used during this investigation, the winter seasons of 1994-1995, 1997-1998, and 1999-2000; and 1,523, 1,596 and 1,531 isolates were collected during these three periods, respectively. The overall rank order of activity of the four fluoroquinolones examined in this study was moxifloxacin > gatifloxacin > levofloxacin = ciprofloxacin, in which moxifloxacin (MIC at which 90% of isolates are inhibited [MIC90], 0.25 {micro}g/ml; modal MIC, 0.12 {micro}g/ml) was twofold more active than gatifloxacin (MIC90, 0.5 {micro}g/ml; modal MIC, 0.25 {micro}g/ml), which in turn was fourfold more active than either levofloxacin (MIC90, 1 {micro}g/ml; modal MIC, 1 {micro}g/ml) or ciprofloxacin (MIC90, 2 {micro}g/ml; modal MIC, 1 {micro}g/ml). Changes in the in vitro activities of fluoroquinolones against S. pneumoniae strains in the United States over the 5-year period of the survey were assessed by comparing the MIC frequency distributions of the study drugs against the isolates obtained during the three sampling periods encompassing this investigation. These comparisons revealed no evidence of changes in the in vitro activities of the fluoroquinolones. In addition, the percentages of isolates in the three sampling periods for which MICs were above the resistance breakpoints were compared. Low percentages of resistant strains were detected, and there was no evidence of resistance rate changes over time. For example, by use of a ciprofloxacin MIC of [>=]4 {micro}g/ml to define resistance, the proportions of isolates from the three sampling periods for which MICs were at or above this breakpoint were 1.2, 1.6, and 1.4%, respectively. A total of 164 unique isolates (n = 58 from 1994-1995, 65 from 1997-1998, and 42 from 1999-2000) were examined for evidence of mutations in the quinolone resistance-determining regions (QRDRs) of the parC and the gyrA genes. Forty-nine isolates harbored at least one mutation in the QRDRs of one or both genes (1994-1995, n = 15; 1997-1998, n = 19; 1999-2000, n = 15). Among the 4,650 isolates of S. pneumoniae examined in the study, we estimated that 0.3% had mutations in both the parC and gyrA loci. The majority of mutations (67.3% of the mutations in 49 isolates with mutations) were amino acid substitutions in the parC locus only. Four isolates had a mutation in the gyrA locus only, and 12 isolates had mutations in both genes (6.2 and 24.5% of isolates with mutations, respectively). There was no significant difference in the number of isolates with parC and/or gyrA mutations detected during each study period. Finally, because of the magnitude of the study, we had reasonably large numbers of pneumococcal isolates with genotypically defined mechanisms of fluoroquinolone resistance and were thus able to determine the effects of specific resistance mutations on the activities of different fluoroquinolones. In general, isolates with mutations in parC only were resistant to ciprofloxacin but remained susceptible to levofloxacin, gatifloxacin, and moxifloxacin, whereas isolates with mutations in gyrA only and isolates with mutations in both
parC and gyrA were resistant to all four fluoroquinolones tested.


http://aac.asm.org/cgi/content/abstract/46/9/2765

Isoniazid is a first-line antibiotic used in the treatment of infections caused by Mycobacterium tuberculosis. Isoniazid is a prodrug requiring oxidative activation by the catalase-peroxidase hemoprotein, KatG. Resistance to isoniazid can be obtained by point mutations in the katG gene, with one of the most common being a threonine-for-serine substitution at position 315 (S315T). The S315T mutation is found in more than 50% of isoniazid-resistant clinical isolates and results in an {approx}200-fold increase in the MIC of isoniazid compared to that for M. tuberculosis H37Rv. In the present study we investigated the hypothesis that superoxide plays a role in KatG-mediated isoniazid activation. Plumbagin and clofazimine, compounds capable of generating superoxide anion, resulted in a lower MIC of isoniazid for M. tuberculosis H37Rv and a strain carrying the S315T mutation. These agents did not cause as great of an increase in isoniazid susceptibility in the mutant strain when the susceptibilities were assessed by using the inhibitory concentration that causes a 50% decrease in growth. These results provide evidence that superoxide can play a role in isoniazid activation. Since clofazimine alone has antitubercular activity, the observation of synergism between clofazimine and isoniazid raises the interesting possibility of using both drugs in combination to treat M. tuberculosis infections.


http://aac.asm.org/cgi/content/abstract/48/12/4733

The development of novel antibacterial agents is decreasing despite increasing resistance to presently available agents among common pathogens. Insights into relationships between pharmacodynamics and resistance may provide ways to optimize the use of existing agents. The evolution of resistance was examined in two ciprofloxacin-susceptible Staphylococcus aureus strains exposed to in vitro-simulated clinical and experimental ciprofloxacin pharmacokinetic profiles for 96 h. As the average steady-state concentration (Cavg ss) increased, the rate of killing approached a maximum, and the rate of regrowth decreased. The enrichment of subpopulations with mutations in grlA and low-level ciprofloxacin resistance also varied depending on the pharmacokinetic environment. A regimen producing values for Cavg ss slightly above the MIC selected resistant variants with grlA mutations that did not evolve to higher levels of resistance. Clinical regimens which provided values for Cavg ss intermediate to the MIC and mutant prevention concentration (MPC) resulted in the emergence of subpopulations with gyrA mutations and higher levels of resistance. A regimen producing values for Cavg ss close to the MPC selected grlA mutants, but the appearance of subpopulations with higher levels of resistance was diminished. A regimen designed to maintain ciprofloxacin concentrations entirely above the MPC appeared to eradicate low-level resistant variants in the inoculum and prevent the emergence of higher levels of resistance. There was no relationship between the time that ciprofloxacin concentrations remained between the MIC and the MPC and the degree of resistance or the presence or type of ciprofloxacin-resistance mutations that appeared in grlA or gyrA. Regimens designed to eradicate low-level resistant variants in S. aureus populations may prevent the emergence of higher levels of fluoroquinolone resistance.
Mutations in the pfcrt and pfmdr1 genes have been associated with chloroquine resistance in Plasmodium falciparum. Ten and five mutations, respectively, have been identified in these genes from chloroquine-resistant parasites worldwide. Mutation patterns in pfcrt revealed that chloroquine resistance evolved independently in southeast Asia, South America, and Papua New Guinea. However, the evolution of chloroquine resistance in the rest of the Pacific region is unclear. In this study, we examined sequence polymorphisms in these genes in isolates from Morong, Philippines, and compared them to known chloroquine resistance sequences. Two novel mutations, A144T and L160Y, were identified outside of the 10 known mutations in pfcrt in Morong isolates. These novel mutations were identified only in parasites with K76T and N326D but without the common A220S mutation found in most chloroquine-resistant isolates. This represents a unique chloroquine resistance allelic type (K76T/A144T/L160Y/N326D) not previously found elsewhere in the world. One Morong isolate also had an additional C72S mutation, whereas only one isolate possessed an allelic type typical of chloroquine resistance in Asia. Parasites with the novel pfcrt allelic types were resistant to chloroquine in vitro and were unresponsive to verapamil (0.9 \text{ micro}M) chemosensitization, similar to chloroquine-resistant parasites from South America and Papua New Guinea. These results suggest that chloroquine resistance evolved independently in the Philippines and represents a second chloroquine resistance founder event in the South Pacific.

Three classes of macrolide resistance phenotypes and three different erythromycin resistance determinants were found among 127 erythromycin-resistant group A streptococcal (GAS) isolates recovered from 355 (35.8\%) pediatric pharyngitis patients in Rome, Italy. According to emm and sof sequence typing results, erythromycin-resistant isolates comprised 11 different clonal types. Remarkably, 126 of the 127 macrolide-resistant isolates were serum opacity factor (sof) gene positive. These data suggest a strong association between macrolide resistance and the presence of sof among GAS isolates recovered from Italian pediatric pharyngitis patients.

Abacavir is a potent new carbocyclic nucleoside analogue. We employed our hollow-fiber pharmacodynamic modeling system to examine the antiretroviral effects of different abacavir exposures, as well as the impact of the schedule of drug administration on efficacy. Dose ranging of abacavir revealed that a concentration of four times the 50\% effective concentration (EC50) (approximately the EC95) was required to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) (strain MN) either in a continuous-infusion hollow-fiber experiment or in a classical
tissue culture flask experiment. In contrast to earlier work with another drug class (HIV-1 protease inhibitors), addition of physiological amounts of the human drug binding proteins albumin and α1 acid glycoprotein revealed that there was little impact on the antiviral effect of the drug. Comparison of equivalent exposures (an area under the concentration-time curve [AUC] developed by approximately 500 mg per day of orally administered abacavir), either in a continuous-infusion mode or as a single oral dose of abacavir, demonstrated no difference in the ability to suppress either strain IIIB or strain MN. Comparison of administration of 250 mg every 12 h (q12h) versus once-daily administration of 500 mg for strain MN again showed no significant difference in suppressive effect. These experiments were carried out over 8 to 15 days. Because of these promising initial results, we extended the experiment to 30 days and examined three different schedules of administration that generated the same AUC at 24 h (AUC24): 300 mg q12h, 600 mg q24h, and 1,200 mg q48h. The aim of the last of these regimens was to definitively demonstrate schedule failure. There was little difference between the 1,200-mg q48h treatment group and the untreated control at 30 days. Likewise, there was little difference between the 600-mg q24h and 300-mg q12h treatment groups. However, at circa day 18 of the experiment, there was a small increase in viral output of p24 in the once-daily dosing unit. Examination of virus from all groups demonstrated no phenotypic or genotypic differences. The small difference in hollow-fiber unit p24 in the once-daily dosing group was not due to emergence of resistance over the 30-day single-drug exposure. We conclude that the dose of abacavir currently being studied in clinical trials (300 mg orally q12h) will be efficacious for the majority of sensitive clinical isolates of HIV-1. These in vitro data also suggest that this drug may be able to be administered to patients on a once-daily basis at a dose of 600 mg.


http://aac.asm.org/cgi/content/abstract/46/8/2684

A PCR-based technique using molecular beacons was developed to detect the chloroquine resistance-associated pfcr K76T point mutation in Plasmodium falciparum. One hundred thirty African clinical isolates were tested by the new method in comparison with the PCR-restriction fragment length polymorphism method. This rapid and inexpensive genomic assay could expand the possibilities for monitoring chloroquine resistance.


http://aac.asm.org/cgi/content/abstract/49/1/366

Methicillin-resistant Staphylococcus aureus (MRSA) isolates have previously been classified into major epidemic clonal types by pulsed-field gel electrophoresis in combination with multilocus sequence typing (MLST) and staphylococcal cassette chromosome mec typing. We aimed to investigate whether genetic variability in potentially polymorphic domains of virulence-related factors could provide another level of differentiation in a diverse collection of epidemic MRSA clones. The target regions of strains representative of epidemic clones and genetically related methicillin-susceptible S. aureus isolates from the 1960s that were sequenced included the R domains of clfA and clfB; the D, W, and M regions of fnbA and fnbB; and three regions in the agr operon. Sequence variation ranged from very conserved regions, such as those for RNAIII and the agr interpromoter region, to the highly polymorphic R regions of the clf genes. The sequences of the clf R domains could be grouped into six major sequence types on the basis of the sequences in their 3' regions. Six sequence types were also observed for the fnb sequences at
the amino acid level. From an evolutionary point of view, it was interesting that a small DNA stretch at the 3' clf R-domain sequence and the fnb sequences agreed with the results of MLST for this set of strains. In particular, clfB R-domain sequences, which had a high discriminatory capacity and with which the types distinguished were congruent with those obtained by other molecular typing methods, have potential for use for the typing of S. aureus. Clone- and strain-specific sequence motifs in the clf and fnb genes may represent useful additions to a typing methodology with a DNA array.


http://aac.asm.org/cgi/content/abstract/46/2/350

Most Aeromonas strains isolated from two European rivers were previously found to be resistant to nalidixic acid. In order to elucidate the mechanism of this resistance, 20 strains of Aeromonas caviae (n = 10), A. hydrophila (n = 5), and A. sobria (n = 5) complexes, including 3 reference strains and 17 environmental isolates, were investigated. Fragments of the gyrA, gyrB, parC, and parE genes encompassing the quinolone resistance-determining regions (QRDRs) were amplified by PCR and sequenced. Results obtained for the six sensitive strains showed that the GyrA, GyrB, ParC, and ParE QRDR fragments of Aeromonas spp. were highly conserved ([≥]96.1% identity), despite some genetic polymorphism; they were most closely related to those of Vibrio spp., Pseudomonas spp., and members of the family Enterobacteriaceae (72.4 to 97.1% homology). All 14 environmental resistant strains carried a point mutation in the GyrA QRDR at codon 83, leading to the substitution Ser-83[->]Ile (10 strains) or Ser-83[->]Arg. In addition, seven strains harbored a mutation in the ParC QRDR either at position 80 (five strains), generating a Ser-80[->]Ile (three strains) or Ser-80[->]Arg change, or at position 84, yielding a Glu-84[->]Lys modification. No amino acid alterations were discovered in the GyrB and ParE QRDRs. Double gyrA-parC missense mutations were associated with higher levels of quinolone resistance compared with the levels associated with single gyrA mutations. The most resistant strains probably had an additional mechanism(s) of resistance, such as decreased accumulation of the drugs. Our data suggest that, in mesophilic Aeromonas spp., as in other gram-negative bacteria, gyrase and topoisomerase IV are the primary and secondary targets for quinolones, respectively.


http://aac.asm.org/cgi/content/abstract/46/5/1410

A newly discovered gene, designated tcrB, which is located on a conjugative plasmid conferring acquired copper resistance in Enterococcus faecium, was identified in an isolate from a pig. The tcrB gene encodes a putative protein belonging to the CPx-type ATPase family with homology (46%) to the CopB protein from Enterococcus hirae. The tcrB gene was found in E. faecium isolated from pigs (75%), broilers (34%), calves (16%), and humans (10%) but not in isolates from sheep. Resistant isolates, containing the tcrB gene, grew on brain heart infusion agar plates containing up to 28 mM CuSO4 compared to only 4 mM for the susceptible isolates. Copper resistance, and therefore the presence of the tcrB gene, was strongly correlated to macrolide and glycopeptide resistance in isolates from pigs, and the tcrB gene was shown to be located on the same conjugative plasmid as the genes responsible for resistance to these two antimicrobial agents. The frequent occurrence of this new copper resistance gene in isolates from pigs, where
copper sulfate is being used in large amounts as feed additive, suggests that the use of copper has selected for resistance.


Mutations in the dihydrofolate reductase (dhfr) genes of Plasmodium falciparum and P. vivax are associated with resistance to the antifolate antimalarial drugs. P. vivax dhfr sequences were obtained from 55 P. vivax isolates (isolates Belem and Sal 1, which are established lines originating from Latin America, and isolates from patient samples from Thailand [n = 44], India [n = 5], Iran [n = 2], and Madagascar [n = 2]) by direct sequencing of both strands of the purified PCR product and were compared to the P. vivax dhfr sequence from a P. vivax parasite isolated in Pakistan (isolate ARI/Pakistan), considered to represent the wild-type sequence. In total, 144 P. vivax dhfr mutations were found at only 12 positions, of which 4 have not been described previously. An F[-&gt;L] mutation at residue 57 had been observed previously, but a novel codon (TTA) resulted in a mutation in seven of the nine mutated variant sequences. A new mutation at residue 117 resulted in S[-&gt;T] (S[-&gt;N has been described previously). These two variants are the same as those observed in the P. falciparum dhfr gene at residue 108, where they are associated with different levels of antifolate resistance. Two novel mutations, I[-&gt;L] at residue 13 and T[-&gt;M] at residue 61, appear to be unique to P. vivax. The clinical, epidemiological, and sequence data suggest a sequential pathway for the acquisition of the P. vivax dhfr mutations. Mutations at residues 117 and 58 arise first when drug pressure is applied. Highly mutated genes carry the S[-&gt;T] rather than the S[-&gt;N] mutation at residue 117. Mutations at residues 57 and 61 then occur, followed by a fifth mutation at residue 13.


In order to track the evolution of primary protease inhibitor (PI) resistance mutations in human immunodeficiency virus type 1 (HIV-1) isolates, baseline and follow-up protease sequences were obtained from patients undergoing salvage PI therapy who presented initially with isolates containing a single primary PI resistance mutation. Among 78 patients meeting study selection criteria, baseline primary PI resistance mutations included L90M (42% of patients), V82A/F/T (27%), D30N (21%), G48V (6%), and I84V (4%). Despite the switching of treatment to a new PI, primary PI resistance mutations present at the baseline persisted in 66 of 78 (85%) patients. D30N persisted less frequently than L90M (50% versus 100%, respectively; P < 0.001) and V82A/F/T (50% versus 81%, respectively; P = 0.05). HIV-1 isolates from 38 (49%) patients failing PI salvage therapy developed new primary PI resistance mutations including L90M, I84V, V82A, and G48V. Common combinations of primary and secondary PI resistance mutations after salvage therapy included mutations at amino acid positions 10, 82, and 46 and/or 54 in 16 patients; 10, 90, and 71 and/or 73 in 14 patients; 10, 73, 84, 90, and 46 and/or 54 in 5 patients; 10, 48, and 82 in 5 patients; and 30, 88 and 90 in 5 patients. In summary, during salvage PI therapy, most HIV-1 isolates with a single primary PI resistance mutation maintained their original mutations, and 49% developed additional primary PI resistance mutations. The persistence of L90M, V82A/F/T, G48V, and I84V during salvage therapy suggests that these mutations play a role in clinical resistance to multiple PIs.

http://aac.asm.org/cgi/content/abstract/47/9/2971

It is generally thought that there is full cross-resistance in Mycobacterium tuberculosis between the aminoglycoside drugs kanamycin and amikacin. However, kanamycin resistance and amikacin susceptibility were seen in 43 of 79 (54%) multidrug-resistant Estonian isolates, indicating that there might be a need to test the resistance of M. tuberculosis isolates to both drugs.


http://aac.asm.org/cgi/content/abstract/48/7/2355

Resistance to quinolone antibiotics has been associated with single-nucleotide polymorphisms (SNPs) in the quinolone resistance-determining region (QRDR) of gyrA. Mutations in the gyrA gene were compared by using mutant populations derived from wild-type Salmonella enterica serovar Enteritidis and its isogenic mutS:Tn10 mutator counterpart. Spontaneous mutations during nonselective growth were isolated by selection with either nalidixic acid, enrofloxacin, or ciprofloxacin. QRDR SNPs were identified in approximately 70% (512 of 695) of the isolates via colony hybridization with radiolabeled oligonucleotide probes. Notably, transition base substitution SNPs in the QRDR were dramatically increased in mutants derived from the mutS strain. Some, but not all, antibiotic-resistant mutants lacking QRDR SNPs were resistant to tetracycline and chloramphenicol, consistent with alterations in nonspecific efflux pumps or other membrane transport mechanisms. Changing the selection conditions shifted the mutation spectrum. Selection with ciprofloxacin was least likely to yield a mutant harboring either a QRDR SNP or chloramphenicol resistance. Selection with enrofloxacin was more likely to yield mutants containing Ser83[\rightarrow]Phe mutations, whereas selection with ciprofloxacin or nalidixic acid favored recovery of Asp87[\rightarrow]Gly mutants. Fluoroquinolone-resistant Salmonella strains isolated from veterinary or clinical settings frequently display a mutational spectrum with a preponderance of transition SNPs in the QRDR, the pattern found in vitro among mutS mutator mutants reported here. Both the preponderance of transition mutations and the varied mutation spectra reported for veterinary and clinical isolates suggest that bacterial mutators defective in methyl-directed mismatch repair may play a role in the emergence of quinolone and fluoroquinolone resistance in feral settings.


http://aac.asm.org/cgi/content/abstract/49/5/1720

The genotypic inhibitory quotient (GIQ) has been proposed as a way to integrate drug exposure and genotypic resistance to protease inhibitors and can be useful to enhance the predictivity of virologic response for boosted protease inhibitors. The aim of this study was to evaluate the predictivity of the GIQ in 116 protease inhibitor-experienced patients treated with lopinavir-
ritonavir. The overall decrease in human immunodeficiency virus type 1 (HIV-1) RNA from baseline to month 6 was a median of -1.50 log10 copies/ml and 40% of patients had plasma HIV-1 RNA below 400 copies/ml at month 6. The overall median lopinavir study-state Cmin concentration was 5,856 ng/ml. Using univariate linear regression analyses, both lopinavir GIQ and the number of baseline lopinavir mutations were highly associated with virologic response through 6 months. In the multivariate analysis, only lopinavir GIQ, baseline HIV RNA, and the number of prior protease inhibitors were significantly associated with response. When the analysis was limited to patients with more highly mutant viruses (three or more lopinavir mutations), only lopinavir GIQ remained significantly associated with virologic response. This study suggests that GIQ could be a better predictor of the virologic response than virological (genotype) or pharmacological (minimal plasma concentration) approaches used separately, especially among patients with at least three protease inhibitor resistance mutations. Therapeutic drug monitoring for patients treated by lopinavir-ritonavir would likely be most useful in patients with substantially resistant viruses.


http://aac.asm.org/cgi/content/abstract/47/2/594

Forty-nine protease inhibitor (PI)-experienced but amprenavir (APV)-naive patients experiencing virological failure were treated with ritonavir (RTV) (100 mg twice a day [b.i.d.]) plus APV (600 mg b.i.d.). Patients responded to therapy with a median viral load decrease of -1.32 log10 by week 12. The addition of low-dose RTV enhanced the minimal APV concentration in plasma (APV Cmin) up to 10-fold compared with that obtained with APV (1,200 mg b.i.d.) without RTV. Baseline PI resistance mutations (L10F/I/V, K20M/R, E35D, R41K, I54V, L63P, V82A/F/T/S, I84V) identified by univariate analysis and included in a genotypic score and APV Cmin at week 8 were predictive of the virological response at week 12. The response to APV plus RTV was significantly reduced in patients with six or more of the resistance mutations among the ones defined above. The genotypic inhibitory quotient, calculated as the ratio of the APV Cmin to the number of human immunodeficiency virus type 1 protease mutations, was a better predictor than the virological or pharmacological variables used alone. This genotypic inhibitory quotient could be used in therapeutic drug monitoring to define the concentrations in plasma needed to control replication of viruses with different levels of PI resistance, as measured by the number of PI resistance mutations.


http://aac.asm.org/cgi/content/abstract/48/4/1289

Rifampin is a major drug used in the treatment of tuberculosis infections, and increasing rifampin resistance represents a worldwide clinical problem. Resistance to rifampin is caused by mutations in the rpoB gene, encoding the {beta}-subunit of RNA polymerase. We examined the effect of three different rpoB mutations on the fitness of Mycobacterium tuberculosis. Rifampin-resistant mutants were isolated from a virulent clinical isolate of M. tuberculosis (strain Harlingen) in vitro at a mutation frequency of 2.3 x 10-8. Mutations in the rpoB gene were identified, and the growth rates of three defined mutants were measured by competition with the susceptible parent strain in laboratory medium and by single cultures in a macrophage cell line and in laboratory medium. All of the mutants showed a decreased growth rate in the three assays. The relative fitness of the mutants varied between 0.29 and 0.96 (that of the susceptible strain was set to 1.0) depending on
the specific mutant and assay system. Unexpectedly, the relative fitness ranking of the mutants differed between the different assays. In conclusion, rifampin resistance is associated with a cost that is conditional.


http://aac.asm.org/cgi/content/abstract/47/5/1760

We have developed a genetic system to monitor the activity of the hepatitis C virus (HCV) NS3 serine protease. This genetic system is based on the bacteriophage lambda regulatory circuit where the viral repressor cI is specifically cleaved to initiate the switch from lysogeny to lytic infection. An HCV protease-specific target, NS5A-5B, was inserted into the lambda phage cI repressor. The target specificity of the HCV NS5A-5B repressor was evaluated by coexpression of this repressor with a β-galactosidase (βgal)-HCV NS32-181/421-34 protease construct. Upon infection of *Escherichia coli* cells containing the two plasmids encoding the cI.HCV5AB-cro and the βgal-HCV NS32-181/421-34 protease constructs, lambda phage replicated up to 8,000-fold more efficiently than in cells that did not express the HCV NS32-181/421-34 protease. This simple, rapid, and highly specific assay can be used to monitor the activity of the HCV NS3 serine protease, and it has the potential to be used for screening specific inhibitors.


http://aac.asm.org/cgi/content/abstract/48/8/2888

Soil bacteria are among the most prodigious producers of antibiotics. The *Bacillus subtilis* LiaRS (formerly YvqCE) two-component system is one of several antibiotic-sensing systems that coordinate the genetic response to cell wall-active antibiotics. Upon the addition of vancomycin or bacitracin, LiaRS autoregulates the liaIHGFSR operon. We have characterized the promoter of the lia operon and defined the cis-acting sequences necessary for antibiotic-inducible gene expression. A survey for compounds that act as inducers of the lia promoter revealed that it responds strongly to a subset of cell wall-active antibiotics that interfere with the lipid II cycle in the cytoplasmic membrane (bacitracin, nisin, ramoplanin, and vancomycin). Chemicals that perturb the cytoplasmic membrane, such as organic solvents, are also weak inducers. Thus, the reporter derived from PliaI (the liaI promoter) provides a tool for the detection and classification of antimicrobial compounds.


http://aac.asm.org/cgi/content/abstract/47/5/1658

Tropheryma whippelii, the agent of Whipple's disease, grows fastidiously only in cell cultures without plaque production, and only three strains have been passaged. The formation of bacterial clumps in the supernatant precludes enumeration of viable bacteria and MIC determination. We evaluated the bacteriostatic effects of fluoroquinolones against two *T. whippelii* isolates by
measuring the inhibition of the DNA copy number increase by real-time quantitative PCR. The analysis of the T. whipplei genome database allowed the identification not only of the gyrA gene but also the parC gene encoding the alpha subunit of the natural fluoroquinolone targets DNA gyrase (GyrA) and topoisomerase IV (ParC), respectively. The parC gene was detected in actinobacteria for the first time. High ciprofloxacin MICs (4 and 8 μg/ml) were correlated with the presence in T. whipplei GyrA and ParC sequences with an alanine residue at positions 83 and 80 (Escherichia coli numbering), respectively. Alanines at these positions have previously been associated with increased fluoroquinolone resistance in E. coli and mycobacteria. However, the MIC of levofloxacin was low (0.25 μg/ml). The same T. whipplei GyrA and ParC sequences were found in two other cultured strains and in nine uncultured tissue samples from Whipple's disease patients, allowing one to speculate that T. whipplei is naturally relatively resistant to fluoroquinolones.


http://aac.asm.org/cgi/content/abstract/47/5/1719

A collection of Aspergillus fumigatus mutants highly resistant to itraconazole (RIT) at 100 μg/ml-1 were selected in vitro (following UV irradiation as a preliminary step) to investigate mechanisms of drug resistance in this clinically important pathogen. Eight of the RIT mutants were found to have a mutation at Gly54 (G54E, -K, or -R) in the azole target gene CYP51A. Primers designed for highly conserved regions of multidrug resistance (MDR) pumps were used in reverse transcriptase PCR amplification reactions to identify novel genes encoding potential MDR efflux pumps in A. fumigatus. Two genes, AfuMDR3 and AfuMDR4, showed prominent changes in expression levels in many RIT mutants and were characterized in more detail. Analysis of the deduced amino acid sequence encoded by AfuMDR3 revealed high similarity to major facilitator superfamily transporters, while AfuMDR4 was a typical member of the ATP-binding cassette superfamily. Real-time quantitative PCR with molecular beacon probes was used to assess expression levels of AfuMDR3 and AfuMDR4. Most RIT mutants showed either constitutive high-level expression of both genes or induction of expression upon exposure to itraconazole. Our results suggest that overexpression of one or both of these newly identified drug efflux pump genes of A. fumigatus and/or selection of drug target site mutations are linked to high-level itraconazole resistance and are mechanistic considerations for the emergence of clinical resistance to itraconazole.


http://aac.asm.org/cgi/content/abstract/46/3/925

A new natural TEM derivative, named TEM-87, was identified in a Proteus mirabilis isolate from an Italian hospital. Compared to TEM-1, TEM-87 contains the following mutations: E104K, R164C, and M182T. Kinetic analysis of TEM-87 revealed extended-spectrum activity against oxyimino cephalosporins (preferentially ceftazidime) and aztreonam. Expression of blaTEM-87 in Escherichia coli decreased the host susceptibility to these drugs.

http://aac.asm.org/cgi/content/abstract/48/7/2736

The nucleotide sequences of the pncA genes within 55 multidrug-resistant pyrazinamide-resistant Mycobacterium tuberculosis clinical isolates were determined. Fifty-three out of the 55 isolates were pyrazinamidase (PZase) negative. Four strains contained a wild-type pncA gene, and PZase activity was undetectable in two of these strains. Seven of the 18 identified pncA mutations found have not been described in previous studies.


http://aac.asm.org/cgi/content/abstract/49/2/488

To compare mutations in the DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) genes of Clostridium perfringens, which are associated with in vitro exposure to fluoroquinolones, resistant mutants were selected from eight strains by serial passage in the presence of increasing concentrations of norfloxacin, ciprofloxacin, gatifloxacin, or trovafloxacin. The nucleotide sequences of the entire gyrA, gyrB, parC, and parE genes of 42 mutants were determined. DNA gyrase was the primary target for each fluoroquinolone, and topoisomerase IV was the secondary target. Most mutations appeared in the quinolone resistance-determining regions of gyrA (resulting in changes of Asp-87 to Tyr or Gly-81 to Cys) and parC (resulting in changes of Asp-93 or Asp-88 to Tyr or Ser-89 to Ile); only two mutations were found in gyrB, and only two mutations were found in parE. More mutants with multiple gyrA and parC mutations were produced with gatifloxacin than with the other fluoroquinolones tested. Allelic diversity was observed among the resistant mutants, for which the drug MICs increased 2- to 256-fold. Both the structures of the drugs and their concentrations influenced the selection of mutants.


http://aac.asm.org/cgi/content/abstract/46/2/590

Mycoplasma gallisepticum enrofloxacin-resistant mutants were generated by stepwise selection in increasing concentrations of enrofloxacin. Alterations were found in the quinolone resistance-determining regions of the four target genes encoding DNA gyrase and topoisomerase IV from these mutants. This is the first description of such mutations in an animal mycoplasma species.


http://aac.asm.org/cgi/content/abstract/49/1/444

In this study the nucleotide sequence of the pncA gene from 59 Mycobacterium tuberculosis clinical isolates was analyzed. Mutations in the pncA gene were identified in 29 of 40
pyrazinamide-resistant isolates, and no pyrazinamidase activity was detected in 39 of them. Twelve mutations found in this work have not been described previously.


http://aac.asm.org/cgi/content/abstract/49/3/1017

Tigecycline is an expanded broad-spectrum antibacterial agent that is active against many clinically relevant species of bacterial pathogens, including Klebsiella pneumoniae. The majority of K. pneumoniae isolates are fully susceptible to tigecycline; however, a few strains that have decreased susceptibility have been isolated. One isolate, G340 (for which the tigecycline MIC is 4 {micro}g/ml and which displays a multidrug resistance [MDR] phenotype), was selected for analysis of the mechanism for this decreased susceptibility by use of transposon mutagenesis with IS903{phi}kan. A tigecycline-susceptible mutant of G340, GC7535, was obtained (tigecycline MIC, 0.25 {micro}g/ml). Analysis of the transposon insertion mapped it to ramA, a gene that was previously identified to be involved in MDR in K. pneumoniae. For GC7535, the disruption of ramA led to a 16-fold decrease in the MIC of tigecycline and also a suppression of MDR. Trans-complementation with plasmid-borne ramA restored the original parental phenotype of decreased susceptibility to tigecycline. Northern blot analysis revealed a constitutive overexpression of ramA that correlated with an increased expression of the AcrAB transporter in G340 compared to that in tigecycline-susceptible strains. Laboratory mutants of K. pneumoniae with decreased susceptibility to tigecycline could be selected at a frequency of approximately 4 x 10-8. These results suggest that ramA is associated with decreased tigecycline susceptibility in K. pneumoniae due to its role in the expression of the AcrAB multidrug efflux pump.


http://aac.asm.org/cgi/content/abstract/46/12/3823

Sixteen isolates of Enterococcus faecalis were recovered from retail poultry samples (seven chickens and nine turkeys) purchased from grocery stores in the greater Washington, D.C., area. PCR for known streptogramin resistance genes identified vat(E) in five E. faecalis isolates (three isolates from chickens and two isolates from turkeys). The vat(E) gene was transmissible on a ca. 70-kb plasmid, along with resistance to erythromycin, tetracycline, and streptomycin, by conjugation to E. faecalis and Enterococcus faecium recipient strains. DNA sequencing showed little variation between E. faecalis vat(E) genes from the chicken samples; however, one E. faecalis vat(E) gene from a turkey sample possessed 5 nucleotide changes that resulted in four amino acid substitutions. None of these substitutions in the vat(E) allele have previously been described. This is the first report of vat(E) in E. faecalis and its transferability to E. faecium, which indicates that E. faecalis can act as a reservoir for the dissemination of vat(E)-mediated streptogramin resistance to E. faecium.

We studied 20 Chlamydia pneumoniae isolates obtained from respiratory sites and atheroma tissue of patients from various geographic areas to determine the susceptibilities of these isolates to a new des-fluoroquinolone, garenoxacin, and to levofloxacin. In addition, we assessed the cultures with these isolates by PCR for the presence or absence of Mycoplasma sp. DNA. Both the MIC at which 90% of isolates are inhibited (MIC90) and the minimal bactericidal concentration at which 90% of isolates are killed (MBC90) for garenoxacin were 0.06 {micro}g/ml, and both the MIC90 and the MBC90 for levofloxacin were 2.0 {micro}g/ml. The activity of garenoxacin against C. pneumoniae was 32-fold greater than that of levofloxacin. Mycoplasma sp. DNA was detected by PCR in 17 of 20 cultures. Mycoplasma amplicons from five Mycoplasma DNA-positive C. pneumoniae cultures were sequenced and found to represent four Mycoplasma species. Our data demonstrate that C. pneumoniae cultures frequently contain Mycoplasma DNA and that its presence in C. pneumoniae cultures does not appear to affect the susceptibility results for the two fluoroquinolones that we tested.


An Italian nationwide survey was carried out to assess the prevalences and the antimicrobial susceptibilities of members of the family Enterobacteriaceae producing extended-spectrum (beta)-lactamases (ESBLs). Over a 6-month period, 8,015 isolates were obtained from hospitalized patients and screened for resistance to extended-spectrum cephalosporins and monobactams. On the basis of a synergistic effect between clavulenate and selected (beta)-lactams (ceftazidime, aztreonam, cefotaxime, cefepime, and ceftriaxone), 509 isolates were found to be ESBL positive (6.3%). Colony blot hybridization with blaTEM and blaSHV DNA probes allowed one to distinguish four different genotypes: TEM-positive, SHV-positive, TEM- and SHV-positive, and non-TEM, non-SHV ESBL types. MICs for each isolate (E-test) were obtained for widely used (beta)-lactams, combinations of (beta)-lactams with (beta)-lactamase inhibitors, aminoglycosides, and fluoroquinolones. Among ESBL-positive strains, Klebsiella pneumoniae, Proteus mirabilis, and Escherichia coli accounted for 73.6% of isolates. Overall, TEM-type ESBLs were more prevalent than SHV-type enzymes (234 versus 173), whereas the prevalence of strains producing both TEM- and SHV-type ESBLs was similar to that of isolates producing non-TEM, non-SHV enzymes (55 and 38, respectively). In vitro, all but one of the ESBL-producing isolates remained susceptible to imipenem. Susceptibility to other drugs varied: piperacillin-tazobactam, 91%; amoxicillin-clavulanic acid, 85%; cefoxitin, 78%; amikacin, 76%; ampicillin-sulbactam, 61%; ciprofloxacin, 58%; and gentamicin, 56%. Associated resistance to aminoglycosides and ciprofloxacin was observed most frequently among TEM-positive strains. Since therapeutic options for multiresistant Enterobacteriaceae are limited, combinations of (beta)-lactams and (beta)-lactamase inhibitors appear to represent an important alternative for treating infections caused by ESBL-producing Enterobacteriaceae.


The knowledge of the effects of antimicrobial agents on the normal vaginal microflora is limited.
The objective of the present study was to study the ecological impact of pivmecillinam on the normal vaginal microflora. In 20 healthy women, the estimated day of ovulation was determined during three subsequent menstrual cycles. Microbiological and clinical examinations were performed on the estimated day of ovulation and on day 3 in all cycles and also on day 7 after ovulation in cycles 1 and 2. Anaerobic and facultative anaerobic gram-positive rods, mainly species of lactobacilli and actinomycetes, dominated the microflora. One woman was colonized on the third day of administration with a resistant Escherichia coli strain, and Candida albicans was detected in one woman on days 3 and 7 in cycle 2. No other major changes in the normal microflora occurred during the study. Administration of pivmecillinam had a minor ecological impact on the normal vaginal microflora.


http://aac.asm.org/cgi/content/abstract/49/5/2015

Plasma-derived sequences of human immunodeficiency virus type 1 (HIV-1) protease from 1,162 patients (457 drug-naive patients and 705 patients receiving protease inhibitor [PI]-containing antiretroviral regimens) led to the identification and characterization of 17 novel protease mutations potentially associated with resistance to PIs. Fourteen mutations were positively associated with PIs and significantly correlated in pairs and/or clusters with known PI resistance mutations, suggesting their contribution to PI resistance. In particular, E34Q, K43T, and K55R, which were associated with lopinavir treatment, correlated with mutations associated with lopinavir resistance (E34Q with either L33F or F53L, or K43T with I54A) or clustered with multi-PI resistance mutations (K43T with V82A and I54V or V82A, V32I, and I47V, or K55R with V82A, I54V, and M46I). On the other hand, C95F, which was associated with treatment with saquinavir and indinavir, was highly expressed in clusters with either L90M and I93L or V82A and G48V. K45R and K20T, which were associated with nelfinavir treatment, were specifically associated with D30N and N88D and with L90M, respectively. Structural analysis showed that several correlated positions were within 8 Å of each other, confirming the role of the local environment for interactions among mutations. We also identified three protease mutations (T12A, L63Q, and H69N) whose frequencies significantly decreased in PI-treated patients compared with that in drug-naive patients. They never showed positive correlations with PI resistance mutations; if anything, H69N showed a negative correlation with the compensatory mutations M36I and L10I. These mutations may prevent the appearance of PI resistance mutations, thus increasing the genetic barrier to PI resistance. Overall, our study contributes to a better definition of protease mutational patterns that regulate PI resistance and strongly suggests that other (novel) mutations beyond those currently known to confer resistance should be taken into account to better predict resistance to antiretroviral drugs.


http://aac.asm.org/cgi/content/abstract/48/9/3260

Most antimicrobial peptides (AMPs) impair the viability of target bacteria by permeabilizing bacterial membranes. However, the proline-rich AMPs have been shown to kill susceptible organisms without causing significant membrane perturbation and may act by inhibiting the activity of bacterial targets. To gain initial insight into the events that follow interaction of a proline-rich peptide with bacterial cells, we used DNA macroarray technology to monitor transcriptional
alterations of Escherichia coli in response to challenge with a subinhibitory concentration of the proline-rich Bac7(1-35). Substantial changes in the expression levels of 70 bacterial genes from various functional categories were detected. Among these, 26 genes showed decreased expression, while 44 genes, including genes that are potentially involved in bacterial resistance to antimicrobials, showed increased expression. The generation of a transcriptional response under the experimental conditions used is consistent with the ability of Bac7(1-35) to interact with bacterial components and affect biological processes in this organism.


http://aac.asm.org/cgi/content/abstract/47/9/2732

The first outbreak of multidrug-resistant (MDR) typhoid fever in Vietnam was in 1993, and by 1995 nearly 90% of cases were MDR. Plasmid HCM1, sequenced in full, is an incHI1 plasmid from Salmonella enterica serovar Typhi strain CT18, isolated in Vietnam in 1993. Restriction analysis shows that pHCM1 shares a restriction fragment length polymorphism (RFLP) pattern with plasmids isolated from the first outbreak and 10 of 17 MDR plasmids isolated from sporadic cases occurring at the same time in Vietnam. A core region of pHCM1 has significant DNA sequence similarity to plasmid R27, isolated in 1961 from S. enterica in the United Kingdom. There are five regions of DNA in pHCM1 which are not present in R27. Two of these are putative acquisition regions; the largest is 34,955 kbp in length and includes sequences of several antibiotic resistance genes and several insertion sequences. The borders of this region are defined by two identical IS10 left elements, associated with an inversion of DNA or with a truncated Tn10 element. The second, smaller region is 14.751 kbp and carries a trimethoprim resistance gene dfr14A cassette associated with a class 1 integrase. In 1993 to 1994, restriction analysis revealed some variations in the structures of Salmonella serovar Typhi MDR plasmids which were mapped to the two putative acquisition regions and three smaller variable regions. In 1996 a single RFLP type, RFLP7, was found to carry the dfrA7 and sul-1 genes, which were not present on R27 or pHCM1. This plasmid type appears to have a selective advantage over other plasmids with the same resistance phenotype.


http://aac.asm.org/cgi/content/abstract/46/8/2582

Mutations associated with fluoroquinolone resistance in clinical isolates of Proteus mirabilis were determined by genetic analysis of the quinolone resistance-determining region (QRDR) of gyrA, gyrB, parC, and parE. This study included the P. mirabilis type strain ATCC 29906 and 29 clinical isolates with reduced susceptibility (MIC, 0.5 to 2 {micro}g/ml) or resistance (MIC, [>=]4 {micro}g/ml) to ciprofloxacin. Susceptibility profiles for ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofoxacin, moxifloxacin, and trovafloxacin were correlated with amino acid changes in the QRDRs. Decreased susceptibility and resistance were associated with double mutations involving both gyrA (S83R or -I) and parC (S80R or -I). Among these double mutants, MICs of ciprofloxacin varied from 1 to 16 {micro}g/ml, indicating that additional factors, such as drug efflux or porin changes, also contribute to the level of resistance. For ParE, a single conservative change of V364I was detected in seven strains. An unexpected result was the association of gyrB mutations with high-level resistance to fluoroquinolones in 12 of 20 ciprofloxacin-resistant isolates. Changes in GyrB included S464Y (six isolates), S464F (three
isolates), and E466D (two isolates). A three-nucleotide insertion, resulting in an additional lysine residue between K455 and A456, was detected in gyrB of one strain. Unlike any other bacterial species analyzed to date, mutation of gyrB appears to be a frequent event in the acquisition of fluoroquinolone resistance among clinical isolates of P. mirabilis.

http://aac.asm.org/cgi/content/abstract/46/4/1098

Klebsiella pneumoniae isolates from Taiwan medical centers (50 strains; 1998 to 2000) with a CTX-M resistance phenotype (ceftazidime susceptible and ceftriaxone or cefotaxime nonsusceptible) were selected for initial isoelectric focusing analysis. \{beta\}-Lactamases with pIs of 7.9 (n = 22) and 8.4 (n = 28) in addition to 5.4 and/or 7.6 were detected. DNA gene sequencing identified the \{beta\}-lactamases with pIs of 7.9 and 8.4 as CTX-M-14 and CTX-M-3, respectively. Molecular typing suggested inter- and intrahospital clonal dissemination of these Taiwanese CTX-M-producing Klebsiella strains.

http://aac.asm.org/cgi/content/abstract/46/12/3900

Laboratory strains of Mycosphaerella graminicola with decreased susceptibilities to theazole antifungal agent cyproconazole showed a multidrug resistance phenotype by exhibiting cross-resistance to an unrelated chemical, cycloheximide or rhodamine 6G, or both. Decreased azole susceptibility was found to be associated with either decreased or increased levels of accumulation of cyproconazole. No specific relationship could be observed between azole susceptibility and the expression of ATP-binding cassette (ABC) transporter genes MgAtr1 to MgAtr5 and the sterol P450 14{alpha}-demethylase gene, CYP51. ABC transporter MgAtr1 was identified as a determinant in azole susceptibility since heterologous expression of the protein reduced the azole susceptibility of Saccharomyces cerevisiae and disruption of MgAtr1 in one specific M. graminicola laboratory strain with constitutive MgAtr1 overexpression restored the level of susceptibility to cyproconazole to wild-type levels. However, the level of accumulation in the mutant with an MgAtr1 disruption did not revert to the wild-type level. We propose that variations in azole susceptibility in laboratory strains of M. graminicola are mediated by multiple mechanisms.

Arch Dermatol (1)

Context Beginning in 1957, patients have been described with localized alopecia characterized histopathologically by mucin deposition within hair follicles (follicular mucinosis [FM]). At least 2 distinct diagnostic entities have been proposed: one occurring in children and young adults without association with other diseases ("idiopathic" FM), the other occurring in elderly patients and associated with mycosis fungoides or Sezary syndrome ("lymphoma-associated" FM).

Objective To determine whether idiopathic and lymphoma-associated FM are distinct or related entities.

Design Case series.

Setting Department of Dermatology, University of Graz, Graz, Austria.

Patients Forty-four patients with FM were divided into 2 groups. Group 1 comprised 16 patients (mean age, 37.5 years) with no associated mycosis fungoides or Sezary syndrome; group 2 was made up of the other 28 (mean age, 52.2 years), who had clinicopathologic evidence of cutaneous T-cell lymphoma. Results Mean age was lower in patients with idiopathic FM, but a considerable overlapping among the 2 groups was present. Location on the head and neck region was common in both groups, but most patients with lymphoma-associated FM had lesions also on other body sites. In fact, solitary lesions at presentation were common in patients with idiopathic FM (11 [68.8%] of 16 patients), but uncommon in those with lymphoma-associated FM (2 [7.1%] of 28 patients). Histopathologic findings did not allow clear-cut differentiation of the 2 groups. Finally, a monoclonal rearrangement of the T-cell receptor (gamma) gene was demonstrated by polymerase chain reaction analysis in about 50% of tested cases from each group.

Conclusions Criteria previously reported to differentiate idiopathic from lymphoma-associated FM proved ineffective. In analogy to localized pagetoid reticulosis (Woringer-Kolopp disease), small-plaque parapsoriasis, and so-called solitary mycosis fungoides, idiopathic FM may represent a form of localized cutaneous T-cell lymphoma.

Arch Gen Psychiatry  (2)


Background Substantial evidence supports a role for dysfunction of the serotonin transporter in the pathogenesis of major depression. Several studies have found reciprocal interactions between the serotonergic system and both brain-derived neurotrophic factor and glutamate, which are known to modulate or affect hippocampal morphologic characteristics. Objective To examine the influence of a polymorphism (5-HTTLPR) in the promoter region of the serotonin transporter gene on hippocampal volumes in patients with major depression and healthy controls.

Design Baseline investigation of a prospective magnetic resonance imaging study with a 4-year follow-up period. Patients We examined 40 inpatients with major depression as well as 40 healthy controls matched for age, sex, and handedness. Main Outcome Measures Subjects underwent high-resolution magnetic resonance imaging. Furthermore, genotyping for the 5-HTTLPR biallelic polymorphism was performed, which consists of a 44-base pair insertion (L allele) or deletion (S allele). Results Patients with the L/L homozygous genotype had significantly smaller hippocampal gray matter (left hemisphere: P = .003; right hemisphere: P = .01) and white matter volumes (left hemisphere: P = .001; right hemisphere: P = .002) than controls with this genotype. No significant differences were found between patients and controls with the L/S or S/S genotype. Moreover, patients with the L/L genotype had significantly smaller hippocampal white matter volumes than those with the L/S or S/S genotype (P = .03). Conclusions These findings suggest that
homozygosity for the L allele is associated with decreased hippocampal volumes in patients with major depression but not in healthy controls. A possible explanation is that the interaction between the serotonergic system and neurotrophic factors as well as excitatory amino acid neurotransmission may affect hippocampal morphologic characteristics.


http://archpsyc.ama-assn.org/cgi/content/abstract/59/7/613

Background Evidence suggests that serotonin transporter gene promoter polymorphism (5HTTLPR)-dependent low transcriptional activity of the human serotonin transporter gene may be a genetic susceptibility factor for depression. We studied the behavioral responses to tryptophan depletion (TD) in healthy women with and without a first-degree family history of depression and examined the relationship to 5HTTLPR alleles. Methods Twenty-four healthy women with a negative family history of depression and 21 women with a positive family history of depression were genotyped for the polymorphism of the 5HTTLPR and then entered a double-blind, placebo-controlled, randomized crossover TD study. The effects of these interventions were assessed with measures of depression and plasma tryptophan levels. Results The TD induced a robust decrease of plasma tryptophan levels in all women irrespective of family history or 5HTTLPR genotypes. The s/s genotype of the 5HTTLPR was associated with an increased risk of developing depressive symptoms during TD irrespective of family history. In contrast, individuals with the l/l genotype did not develop depressive symptoms, irrespective of family history. Finally, s/l subjects without family history showed a mood response that was intermediate between the s/s and l/l subjects, while s/l subjects with a family history of depression showed the same depressiogenic effect of TD as seen in the s/s subjects. Conclusions The results of the present study suggest that the s-allele of the 5HTTLPR and a positive family history of depression are additive risk factors for the development of depression during TD.


http://archopht.ama-assn.org/cgi/content/abstract/120/11/1534

Objective To validate a real-time polymerase chain reaction (PCR) assay allowing rapid and sensitive detection and quantitation of 4 common infectious posterior uveitis pathogens. Methods A real-time PCR assay using previously validated primer sets for cytomegalovirus, herpes simplex virus, varicella-zoster virus, and Toxoplasma gondii was developed. A standard curve for quantitation of pathogen load was generated for each pathogen using SYBR Green I fluorescence detection. Ocular samples from patients with posterior uveitis and from negative control samples were assayed and compared with standards to identify pathogens and quantify infectious load. Results Sensitivity for detection of purified pathogen DNA by PCR was not reduced by application of the real-time method. Standard curves for the quantitation of pathogen
loads showed sensitivity to fewer than 10 organisms for all pathogens. The technique was applied to 2 clinical problems. First, sensitivities of existing monoplex and multiplex PCR were compared by real-time PCR. No significant difference in sensitivity was observed between multiplex and monoplex techniques. Second, pathogen loads of vitreous specimens from patients previously diagnosed as having infectious posterior uveitis were calculated. Pathogen loads were found to be generally higher for patients with disease caused by varicella-zoster virus than those caused by cytomegalovirus or herpes simplex virus. Conclusions Real-time PCR may be applied to infectious agents responsible for posterior uveitis. This technique will likely prove useful for the diagnosis of posterior uveitis as well as the linkage of pathogen to disease. Clinical Relevance Real-time PCR provides a rapid technique for quantitatively evaluating ocular samples for the presence of infectious pathogens.


http://archopht.ama-assn.org/cgi/content/abstract/121/7/1028

Objective To describe retinal and optic disc atrophy and a progressive decrease of visual function in 2 Japanese brothers. Both had a mutation in the CACNA1F gene, the causative gene of incomplete congenital stationary night blindness (CSNB). Methods We studied observational case reports and performed comprehensive ophthalmologic examinations including best-corrected visual acuity, biomicroscopy, ophthalmoscopy, fundus photography, and electroretinography. Genomic DNA was extracted from the peripheral blood, and all 48 exons of the CACNA1F gene were directly sequenced. Results The 2 brothers had retinal and optic disc atrophy and a progressive reduction of visual acuity with increasing age. Although these clinical features are not typical of previous patients with incomplete CSNB, both patients had an in-frame mutation with deletion and insertion in exon 4 of the CACNA1F gene. In both patients, the bright-flash, mixed rod-cone electroretinogram had a negative configuration, a characteristic of incomplete CSNB. However, the full-field scotopic and photopic electroretinograms were nonrecordable, indicating severe, diffuse retinal malfunction, which is not typical in incomplete CSNB. Conclusion These findings indicate that a mutation of the CACNA1F gene may be associated with retinal and optic disc atrophy with a progressive decline of visual function. Clinical Relevance In patients with retinal and optic disc atrophy associated with negative-type electroretinograms, a CACNA1F gene mutation should be considered.


http://archopht.ama-assn.org/cgi/content/abstract/122/10/1430

Objectives To establish that the protozoan Acanthamoeba is one of the causative organisms associated with non-contact lens-related keratitis in the Indian population and to develop a simple and sensitive diagnostic assay for clinical testing. Design DNA sequencing of nuclear 18S and 26S ribosomal DNA motifs was performed and compared with the reference Acanthamoeba strains, to establish the genetic identity of the putative amoeba isolates obtained from the corneal scrapings of non-contact lens-wearing patients with keratitis. Ribosomal DNA typing of clinical corneal scrapings from the patients with keratitis was performed by means of a simple agarose gel-based multiplex polymerase chain reaction assay, to detect the cases of Acanthamoeba keratitis. Results The ribosomal DNA analysis of 15 putative amoeba isolates obtained from the corneal scrapings of 14 patients with keratitis and 1 from the patients' environment established the isolates to be pathogenic forms of Acanthamoeba belonging to type T4 ribosomal DNA
genotype. Multiplex polymerase chain reaction assay was specific and sensitive enough to detect as low as 5 pg of Acanthamoeba DNA. Its utility as a reliable diagnostic assay was demonstrated directly with the use of 34 additional corneal scrapings. Conclusions Acanthamoeba is one of the causative organisms of keratitis in Indian patients with no history of contact lens usage. Moreover, the Acanthamoeba infection can be easily detected in the clinical samples by means of the simple multiplex polymerase chain reaction assay based on ribosomal DNA typing. Clinical Relevance This study suggests the need and means to determine the incidence and prevalence of Acanthamoeba keratitis in India and elsewhere. Moreover, the polymerase chain reaction assay would help in early and definitive diagnosis, leading to better prognosis of Acanthamoeba keratitis condition.


http://archopht.ama-assn.org/cgi/content/abstract/122/6/897

Objective Bornholm eye disease (BED) consists of X-linked high myopia, high cylinder, optic nerve hypoplasia, reduced electroretinographic flicker with abnormal photopic responses, and deuteranopia. The disease maps to chromosome Xq28 and is the first designated high-grade myopia locus (MYP1). We studied a second family from Minnesota with a similar X-linked phenotype, also of Danish descent. All affected males had protanopia instead of deuteranopia. Methods X chromosome genotyping, fine-point mapping, and haplotype analysis of the DNA from 22 Minnesota family individuals (8 affected males and 5 carrier females) and 6 members of the original family with BED were performed. Haplotype comparisons and mutation screening of the red-green cone pigment gene array were performed on DNA from both kindreds. Results Significant maximum logarithm of odds scores of 3.38 and 3.11 at {theta} = 0.0 were obtained with polymorphic microsatellite markers DXS8106 and DXYS154, respectively, in the Minnesota family. Haplotype analysis defined an interval of 34.4 cM at chromosome Xq27.3-Xq28. Affected males had a red-green pigment hybrid gene consistent with protanopia. We genotyped Xq27-28 polymorphic markers of the family with BED, and narrowed the critical interval to 6.8 cM. The haplotypes of the affected individuals were different from those of the Minnesota pedigree. Bornholm eye disease-affected individuals showed the presence of a green-red hybrid gene consistent with deuteranopia. Conclusions Because of the close geographic origin of the 2 families, we expected affected individuals to have the same haplotype in the vicinity of the same mutation. Mapping studies, however, suggested independent mutations of the same gene. The red-green and green-red hybrid genes are common X-linked color vision defects, and thus are unrelated to the high myopia and other eye abnormalities in these 2 families. Clinical Relevance X-linked high myopia with possible cone dysfunction has been mapped to chromosome Xq28 with intervals of 34.4 and 6.8 centimorgan for 2 families of Danish origin.

Arch Otolaryngol Head Neck Surg (2)


http://archotol.ama-assn.org/cgi/content/abstract/130/1/78
Objective To assess alcohol dehydrogenase 3 (ADH3) polymorphism at position Ile349Val as indicator of risk factor for upper aerodigestive tract (UADT) cancer to verify its association with UADT cancer in nonalcoholic or nonsmoking individuals. Design Cross-sectional study. Setting Primary care or referral center. Patients The study group consisted of 141 consecutive patients with newly diagnosed squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx admitted for surgical treatment. The comparison group consisted of 94 inpatients without cancer from the A. C. Camargo or other Sao Paulo (Brazil) hospital and 40 healthy individuals. Intervention All participants were interviewed and data were collected using a structured questionnaire. After written informed consent was obtained, 20 mL of blood was collected in heparinized tubes. Main Outcome Measures Odds ratio for ADH3 genotypes using logistic regression models. Results After adjustment for sex, age, tobacco use, and history of cancer in first-degree family relatives, a significantly higher odds ratio for UADT cancer was observed among individuals with AA genotype and low cumulative alcohol consumption (≤100 kg of ethanol) (odds ratio = 3.8 [95% confidence interval, 1.5-9.7]). A 4-fold increase in odds ratio for UADT cancer among individuals with AA genotype and low tobacco consumption (≤25 pack-years) was also found in the adjusted model. Conclusions These results suggest that genotype AA may be a risk factor for UADT cancer, especially in individuals with low alcohol or tobacco consumption. However, further epidemiological case-control or cohort studies, preferably prospective, are needed to establish the exact role of ADH3 polymorphism and its association with the development of UADT cancers.


http://archotol.ama-assn.org/cgi/content/abstract/131/2/147

Objectives To examine the association between cyclooxygenase-2 (COX-2) expression with epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS), and latent membrane protein 1 (LMP-1) expression and with COX-2 promoter methylation status in primary nasopharyngeal cancer (NPC) tumors and to determine COX-2 promoter methylation status in NPC cell lines. Design Retrospective study. Setting Patients with NPC were referred to the Department of Otolaryngology-Head and Neck Surgery for treatment. Patients Formalin-fixed, paraffin-embedded NPC specimens from 42 patients were obtained. Interventions Immunohistochemical expression of COX-2, EGFR, VEGF, iNOS, and LMP-1 was performed in 42 NPC samples. COX-2 promoter methylation status was studied in 20 separate specimens and in 4 NPC cell lines. Main Outcome Measures (1) COX-2, EGFR, VEGF, iNOS, and LMP-1 expression; and (2) COX-2 promoter methylation status. Results COX-2 was overexpressed in 79% of NPC specimens and was associated with EGFR status (P = .03) but not with LMP-1 or iNOS. In primary NPC tissue, methylation of the COX-2 promoter was seen in 4 of 7 COX-2-negative and 1 of 13 COX-2-positive immunohistochemical cases. COX-2 promoter methylation was found in the CNE-1 cell line. Conclusions Nasopharyngeal cancer may be a useful target for selective COX-2 inhibition. The absence of promoter methylation may be a necessary component of COX-2 overexpression, and promoter methylation may be one of the mechanisms that regulate COX-2 expression.

Objective To investigate whether interleukin 1\(\beta\) (IL-1\(\beta\)) exon 5 and IL-1 receptor antagonist (IL-1Ra) gene polymorphisms can be used as markers of susceptibility to febrile convulsions in children. Methods Children were divided into 2 groups: those with febrile convulsions (group 1; \(n = 51\)) and normal control subjects (group 2; \(n = 83\)). Polymorphisms for IL-1\(\beta\) exon 5 and IL-1Ra gene polymorphisms were detected by polymerase chain reaction. Genotypes and allelic frequencies for IL-1\(\beta\) exon 5 and IL-1Ra gene polymorphisms in both groups were compared. Results Genotype and allele frequencies for IL-1\(\beta\) exon 5 in both groups were not significantly different. Proportions of E1 homozygotes and E1/E2 heterozygotes for IL-1\(\beta\) exon 5 were 50 (98.1%) and 1 (1.9%), respectively, in group 1 and 82 (98.8%) and 1 (1.2%), respectively, in group 2. Frequencies of alleles E1 and E2 for IL-1\(\beta\) exon 5 were 101 (99.0%) and 1 (1.0%), respectively, in group 1 and 165 (99.4%) and 1 (0.6%), respectively, in group 2. Genotype proportions and allele frequencies for IL-1Ra between groups were significantly different. Proportions of genotypes I/I and I/II for IL-1Ra were 49 (96.1%) and 2 (3.9%) in group 1 and 69 (83.1%) and 14 (16.9%) in group 2. Frequencies of alleles I and II for IL-1Ra were 100 (98.0%) and 2 (2.0%) in group 1 and 152 (91.6%) and 14 (8.4%) in group 2. Conclusions The IL-1Ra allele I is associated with a higher susceptibility to febrile convulsion, which may become a useful marker for predicting the development of febrile convulsions. The IL-1\(\beta\) exon 5 gene polymorphisms are not a useful marker for predicting the susceptibility to febrile convulsions.

Arch Surg (2)


Hypothesis The cause of breast cancer is linked to many macroscopic events, including benign breast disease. In this study we asked whether molecular changes could discriminate fibroadenoma, which is one of the most common benign breast disease lesions associated or not with breast cancer. Design Retrospective cohort study. Setting Anticancer medical center. Subjects Archival tissues in 32 cases of fibroadenoma, diagnosed in the same breast as a breast carcinoma, are compared with a control group of 26 cases of fibroadenomas unaffected by breast cancer. Main Outcome Measures Histological features are characterized in all samples. The epithelial and stromal components are analyzed for a loss of heterozygosity and a microsatellite instability using a polymerase chain reaction-based method with 11 polymorphic microsatellite markers at 7 chromosomal regions frequently altered in breast cancer. The p53 gene mutations were also determined at exons 5 to 9. Results The frequency of complex fibroadenomas was similar in both groups (\(P = .42\)). Only in the case group did we observe proliferative lesions confined in fibroadenomas, including atypical ductal hyperplasia (2 cases), lobular neoplasia (3 cases), or low-grade ductal carcinoma in situ (2 cases). There is no significant morphological difference between the 2 groups. Neither microsatellite alterations nor p53 gene mutations are present in the fibroadenoma components. Loss of heterozygosity is found only in the epithelial

http://archsurg.ama-assn.org/cgi/content/abstract/139/7/712

Hypothesis Although genetic changes associated with the progression to Barrett esophagus and adenocarcinoma have been identified, changes in gene expression associated with gastroesophageal reflux disease have not been reported. We examined expression levels of several genes important in carcinogenesis and compared expression levels with alterations in esophageal acid exposure. Patients, Design, and Setting Prospective analysis of 61 patients initially seen with reflux symptoms at a private academic hospital. Interventions Paired esophageal biopsy specimens of squamous epithelium 3 cm above the squamocolumnar junction. All patients had 24-hour pH monitoring performed. Interventions Paired esophageal biopsy specimens of squamous epithelium 3 cm above the squamocolumnar junction. All patients had 24-hour pH monitoring performed. Main Outcome Measures Cyclooxygenase (COX) 1, COX-2, thymidylate synthase, human telomerase reverse transcriptase (hTERT), Bcl-2 protein, survivin protein, secreted protein acidic and rich in cysteine (SPARC), tetraspan (TSPAN), and caudal-type homeobox transcription factor 2 (CDX2) messenger RNA expression analysis was performed on snap-frozen, microdissected tissue using a quantitative reverse transcriptase-polymerase chain reaction method. Linear regression and the Pearson product moment correlation were used to relate gene expression to parameters of the 24-hour pH record. Results Expression levels of COX-2 correlated positively with the 24-hour pH score (r = 0.25, P =.05). There was no correlation between the expression of other tested genes and esophageal acid exposure. There was also no significant increase in COX-2 expression in patients with esophagitis or in those who used nonsteroidal anti-inflammatory drugs. Conclusions To our knowledge, these data provide among the first reported correlation of genetic changes and increased esophageal acid exposure in patients with gastroesophageal reflux symptoms. The changes in gene expression occur before any metaplastic changes in the tissue are apparent, and may in the future be useful in predicting which patients will progress through a metaplasia-dysplasia carcinoma sequence.
colonization by E histolytica and E dispar in HIV-infected persons and uninfected controls. Methods We assessed the prevalence of invasive amebiasis by case review of 951 HIV-infected persons and by serologic studies of 634 of the 951 HIV-infected persons, 429 uninfected controls with gastrointestinal symptoms, and 178 uninfected healthy controls using indirect hemagglutination antibody assay. We assessed the rate of intestinal colonization by E histolytica and E dispar by fecal antigen and polymerase chain reaction tests in 332 asymptomatic HIV-infected persons and 144 of the 178 uninfected healthy controls. Results Forty-nine (5.2%) of 951 HIV-infected persons had 51 episodes of invasive amebiasis. A high indirect hemagglutination antibody titer was detected in 39 (6.2%) of 634 HIV-infected persons compared with 10 (2.3%) of 429 uninfected controls with gastrointestinal symptoms and 0 of 178 uninfected healthy controls (P<.001). Stool specimens from 40 (12.1%) of 332 HIV-infected persons and 2 (1.4%) of 144 uninfected healthy controls were positive for E histolytica or E dispar antigen (P<.001). Ten (25.0%) of the 40 antigen-positive stool specimens from HIV-infected persons contained E histolytica. Conclusion Persons infected with HIV in Taiwan are at increased risk for invasive amebiasis and exhibit a relatively high frequency of elevated antibody titers and intestinal colonization with E histolytica.

Archives of Medical Research (1)


http://www.sciencedirect.com/science/article/B6VNM-4CFYMRS-3/2/07b50d9534a1d9cfa04bcd9e98be299

Background It is well documented that Giardia duodenalis undergoes surface antigenic variation both in vivo and in vitro. Proteins involved have been characterized and referred to as VSP (variable surface protein). Methods Two cloned cDNA inserts of 0.45 and 1.95 kb were obtained from G. duodenalis expression library and sequenced. Comparison sequence analyses were made against Genbank. PCR analysis was performed on G. duodenalis isolates to identify isolates bearing genes encoding such a peptide. Specific antiserum was prepared against 450-bp encoded peptide and tested by Western blot, immunofluorescence, and inhibition of adhesion of G. duodenalis to target cells. Results We cloned and characterized a G. duodenalis 450-bp DNA fragment; its DNA sequence analysis revealed that this fragment displayed 99% identity with vsp9B10A gene. Predicted amino acid sequence for this fragment also had significant (99%) identity to VSP9B10A. A second 1.95-kb insert, which encompassed the 450-bp cDNA fragment, was also isolated; its DNA and amino acid sequence displayed 99.5% identity with vsp9B10A gene and 99.2% with the corresponding inferred protein, respectively. This inferred protein contained 24 Cys-X-X-Cys motifs and long ORF of 642 amino acids. PCR analysis showed that DNA sequence encoding a fragment of this gene was present in P1, CIEA:0487:2-C-8 clone and in INP:180800-B2 G. duodenalis human isolates, while it was absent in sheep isolate of G. duodenalis INP:150593-J10. Conclusions Immunofluorescence analysis using antibodies raised against the peptide encoded by 450-bp fragment showed that expression of this epitope varies on trophozoite surface of the C-8 Mexican clone and is involved in parasite adhesion to target epithelial cells.

http://www.sciencedirect.com/science/article/B6T4J-4BWHNYN-2HT/2/3fc6f9d868205c80e5c0aa3d486efdb1

Rat cystatin S and rat cystatin C are members of family 2 (cystatin) of the cystatin superfamily. All members of the cystatin family inhibit cysteine proteinases to varying degree. The expression of these two inhibitors, which have a 48% similarity at the nucleotide level, was studied in the submandibular gland using reverse transcriptase-polymerase chain reaction (RT-PCR), Northern blot hybridization and in situ hybridization with digoxigenin-labelled DNA probes. Both inhibitors were expressed in the serous acinar cells of the submandibular gland. In accord with previous findings, cystatin S mRNA was induced by the [beta]-adrenergic agonist isoproterenol. The level of cystatin S mRNA, which was very low in the glands of untreated rats and was demonstrable by RT-PCR but not by Northern blot hybridization, was not altered by acute inflammation produced by turpentine. Neither the administration of isoproterenol nor acute inflammation had any effect on the level of cystatin C mRNA, indicating that [beta]-adrenoreceptors are not involved in the regulation of the cystatin C gene(s) in the submandibular gland. The data indicate that these two closely related genes, expressed in the same cells, are differently regulated. The consequence of this difference in gene regulation on the physiological and pathological roles of these inhibitors remains to be established.


Summary Apoptosis, also known as programmed cell death, is regulated by a number of inhibitory or stimulatory factors. In addition to the pro- and anti-apoptotic Bcl-2 family proteins, there is also a family of inhibitors of apoptosis protein (IAP). Survivin, a member of this IAP family, is selectively upregulated in most tumours. The objective of the present study was, therefore, to investigate the protein and mRNA expression of survivin, as well as the methylation status of the CpG sites in exon 1 of the survivin gene for 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal-pouch squamous-cell carcinomas. Immunohistochemical analysis for protein expression, RT-PCR for mRNA expression, and a PCR-based methylation assay were performed on 26 samples of hamster buccal pouches. The total study population was assigned into either one experimental group (15-week DMBA treatment; n = 13) or two control groups (untreated: n = 6; mineral-oil treated n = 7). Cytoplasmic staining of survivin protein and mRNA were detected in all of the hamster buccal-pouch tissue specimens treated with DMBA, whereas neither survivin protein nor survivin mRNA were noted for all of the untreated and mineral oil-treated hamster buccal-pouch tissue specimens. Furthermore, all the untreated and mineral-oil treated samples had a survivin-methylated allele, whereas the DMBA-treated cancerous tissues showed no evidence of survivin methylation. The results suggest that survivin may play an important role in DMBA-induced hamster buccal-pouch carcinomas, and that the gene expression may be modulated by an epigenetic mechanism.

http://www.sciencedirect.com/science/article/B6T4J-4BWF993-1TG/2/b49a89ac26ac6e3fb41e88d1f9dd4b97

The fate of the progeny of human oral gingival keratinocytes was mapped in stratified epithelial tissues in vitro by following the expression of a marker gene in genetically related clones. Oral epithelial progenitor cells were genetically marked at high efficiency by transducing them with a retrovirus vector that carried the gene for a histochemically detectable product, Escherichia coli [beta]-galactosidase ([beta]-gal). These cells were then grown in submerged cultures and on collagen rafts at the air-liquid interface to demonstrate the distribution of genetically marked cells in a differentiating tissue in vitro. The dynamics of transduced cells showed that clonally related cells were arranged in discrete units of labelled cells and these clusters were defined as 'clonal proliferation units'. The size and configuration of these units were related to the proliferative potential and differentiating capacity of the cell that was initially transduced. This model demonstrates the relation between clonally related cells and tissue architecture for oral keratinocytes in vitro.


Retinoids alter the patterning of murine odontogenesis in vivo and stimulate epithelial proliferation. Because odontogenesis is dependent on proliferation of mandibular epithelium, the effects of retinol on the patterning of odontogenic epithelium were studied. These experiments control for developmental stage, applied retinoid concentration and duration of exposure. Explants exposed for 24 h to 0.1 [mu]g/ml of retinol exhibited enhanced odontogenesis. Day-9 mandibles exposed to retinol at 1-5 [mu]g/ml had altered epithelial patterns consistent with those in previous in vivo experiments, including supernumerary epithelial buds in regions associated with supernumerary incisors in vivo. These changes were associated with a dose-dependent increase in epithelial proliferation and a prolonged expression of epidermal growth factor (EGF) mRNA. Altered expression of EGF mRNA may be responsible for the disrupted pattern of the dental lamina. This is the first report of a retinoid-induced alteration in EGF mRNA expression.


http://www.sciencedirect.com/science/article/B6T4J-4BYT00G-2F/2/32a953f6446ce52f04e9d5bba93a16d4

Reverse transcription and cDNA amplification (polymerase chain reaction) of total RNA preparations were used to characterize the expression of EGF mRNA in the mandibular arch of day 9-17 mouse embryos. EGF mRNA was present in mandibles at day 9 and 10 but not at days 11-17. Separate RNA preparations from epithelium and mesenchyme at day 10 revealed EGF mRNA in both components.

http://www.sciencedirect.com/science/article/B6T4J-3VXN1WT-B/2/c9c5c849a5fee25452d5e7b16ed0397c

Mycoplasma fermentans and other mycoplasma species may be associated with human immunodeficiency virus infection. Little is known about the ecology of this micro-organism and its natural habitat. A polymerase chain reaction (PCR)-based assay was used to detect \textit{M. fermentans} in whole saliva. The hypothesis was tested that \textit{M. fermentans} is present on the mucosal surfaces of the mouth and oropharynx. Whole saliva was collected from 110 adults. The 206-bp amplification product of DNA purified from these samples was detected in ethidium bromide-stained 6\% polyacrylamide gels in 49 (44.5\%) samples tested. All samples were confirmed by Southern blotting with a probe based on an internal sequence of the expected amplification product. The data suggest that this organism is often found in saliva and on oropharyngeal mucosal surfaces. Saliva may play a part in its transmission between individuals. Saliva sampling may be helpful in further studies of the ecology and distribution of the micro-organism in human populations.


http://www.sciencedirect.com/science/article/B6T4J-3X10T7S-9/2/a2022779c14a796e6e4e150ee4ee8fa22f

Periodontal ligament (PDL) cells have osteoblast-like features and are capable of differentiating into osteogenic cells. As human osteoblasts express oestrogen receptor mRNA, it is possible that PDL cells do so also, but findings have been conflicting. To determine whether they do express oestrogen receptor mRNA, the reverse transcriptase-polymerase chain reaction was performed with two different primers. Cells were obtained from a healthy periodontal ligament of premolar extracted for orthodontic reasons. The human breast adenocarcinoma cell-line MCF7 was used as a positive control. Expression of oestrogen receptor mRNA was detected in PDL cells with one of the primers but with less intensity than in MCF7 cells. Southern hybridization confirmed these results. These findings suggest that PDL cells express oestrogen receptor mRNA at low levels.


http://www.sciencedirect.com/science/article/B6T4J-42NRCGC-7/2/b3f3bcb949ad787d2cc98a0edc5dece

A recent preliminary (unpublished) study showed that phosphodiesterase (PDE) 3A and 3B are expressed in rat submandibular glands. Here, PDE3 activity was detected in homogenates of rat submandibular gland acinar epithelial (SMIE) cells, but not rat A5 (epithelial duct) cells. Most of the PDE3 activity in SMIE cells was recovered in the particulate fraction. Only PDE3B mRNA was detected by reverse transcription-polymerase chain reaction in RNA from SMIE cells. The nucleotide sequence of the fragment was identical to the sequence of rat PDE3B. The PDE3 specific inhibitor, OPC3689 (10 and 50 [\mu]M), inhibited the growth of SMIE cells (19 and 63\%), but not A5 cells. As the submandibular gland contains many types of cells, these results indicate that PDE3B may regulate a cAMP pool that is important in submandibular gland acinar epithelial
cell function.


Summary
Changes in intracellular Ca2+ concentration ([Ca2+]i) induced by agonists were simultaneously monitored in rat submandibular acini and ducts using a Ca2+ imaging system. Substance P (SP) elicited marked increases in [Ca2+]i in acini but not in ducts. Carbachol (CCh) increased [Ca2+]i in both acini and ducts, but the maximal level was higher in acini than in ducts. In contrast, epinephrine (Epi) also induced an increase in [Ca2+]i in acini and ducts, but to a greater extent in ducts than in acini. Isoproterenol (ISO) caused a small but significant increase in [Ca2+]i in ducts but not acini. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis using total RNA extracted from highly purified acinar and ductal cells showed that substance P receptor mRNA was present in acini at higher levels than in ducts. In contrast, [alpha]1a-adrenoceptor mRNA was more strongly expressed in ducts than in acini. The muscarinic receptors (M3 and M5) and [beta]-adrenoceptors ([beta]1 and [beta]2) were expressed at equivalent levels in both cell types. These results confirm that acini and ducts exhibit significant differences in agonist-induced Ca2+ responses. Furthermore, substance P- and epinephrine-induced Ca2+ responses were consistent with receptor mRNA expression in acini and ducts, but carbachol- and isoproterenol-induced [Ca2+]i increases were not.


The expression of mRNA for amylase was examined using the reverse transcriptase-polymerase chain reaction (RT-PCR). An amylase product was strongly detected in parotid and pancreas, but less strongly in liver. The degree of identity between the PCR products was assessed by restriction-enzyme mapping using two restriction enzymes, EcoRI and Scal, and DNA sequencing. The PCR product from pancreas was cut by both EcoRI and Scal, while the products from parotid and liver were cut by EcoRI but not by Scal. The sequence of the parotid product was 90.4% homologous to that of the pancreas, and 100% homologous to that of the liver. These results indicate that the same amylase mRNA may be expressed in parotid and liver. In addition, the expression of amylase mRNAs in other rat tissues was investigated using RT-PCR, and the sensitivity of each PCR product to Scal was tested. A weak single band was detected in submandibular gland, sublingual gland and stomach. Scal digestion cut the stomach product into two fragments, but had no effect on the submandibular and sublingual products. Thus, it may be possible to classify amylase isoenzymes into pancreatic and parotid types based on the sensitivity of their PCR products to Scal.

The discriminative power of the arbitrarily primed polymerase chain reaction (AP-PCR) in differentiating between Streptococcus mutans and Strep. sobrinus species, serotypes and clones was investigated. Mutans streptococcal isolates (127) obtained from 65 individuals (1-10 isolates per individual) were AP-PCR typed separately with two random primers, OPA-05 and OPA-13. Bacterial cell lysates were used as a template in PCR reactions, which made AP-PCR easy and fast to perform. Eighty-one isolates from 19 individuals were also ribotyped to compare the discriminative ability of ribotyping and AP-PCR techniques. AP-PCR performed with the two primers differentiated between Strep. mutans and Strep. sobrinus isolates, but neither primer detected serotype-specific amplification products. OPA-05 distinguished two main AP-PCR patterns among Strep. mutans isolates and one main pattern among Strep. sobrinus isolates, whereas OPA-13 found one main AP-PCR pattern among Strep. mutans isolates and two main patterns among Strep. sobrinus isolates. Ribotyping and AP-PCR revealed 40 and 33 different types among 81 selected isolates, respectively. Both techniques detected intra-individual heterogeneity in 16 out of 19 participants. The results indicate that AP-PCR has good discriminative ability in differentiating between mutans streptococcal clones and that the technique is suitable for epidemiological studies on mutans streptococci.


http://www.sciencedirect.com/science/article/B6T4J-47TWXJP-5/2/91db1375dd04686a7f6f25946692930

Background: The exact pathomechanism of inflammation progress and fibrosis in chronic obstructive sialadenitis is unknown. The aim of the present study was to assess whether there is an association between transforming growth factor beta (TGF-β) and fibrogenic process of chronic sialadenitis. Methods: Tissue samples of 12 patients with chronic sialadenitis and 4 normal tissue samples of the submandibular gland were examined immunohistochemically for identification of TGF-β. TGF-β1 messenger RNA (mRNA) expression was analysed semiquantitatively using reverse transcription polymerase chain reaction and gel electrophoresis to correlate its expression levels with stages of the disease. Results: TGF-β1 positive cells could be found in the secretory duct system of all examined samples. However, an intense TGF-β immunoreactivity was observed in inflamed salivary glands. With progress of disease TGF-β1 mRNA expression increases significantly. Conclusion: Expression of TGF-β in chronic sialadenitis and its apparent increase in advanced stages of the disease, suggests that this growth factor may play a role in glandular fibrosis.


http://www.sciencedirect.com/science/article/B6T4J-4135WX1-6/2/a368ff50b41f84ec854f0dcc2dec869f

Primary cultures of dental papilla-derived cells have a limited lifespan in vitro and can be maintained only up to passage 7-9 before showing senescence, but in vitro investigations often require a large number of cells showing phenotypic characteristics of the original tissue. To overcome this shortcoming, second-passage cells established from calf molar tooth germs by enzymatic pretreatment of the dental papilla were transfected by electroporation with pSV3neo, coding for the oncogene simian virus 40 large t antigen and a neomycin-resistance gene. Under selection by G418 (neomycin), four cell clones were isolated by single cell dilution at passage 15. Integration of simian virus 40 large t antigen and expression of the gene products were determined in cell clones by polymerase chain reaction (PCR) and immunohistochemistry. Four
transfected cell lines (clones B, C, D and no. 12) were maintained in culture for over 1.5 years. For cell characterization, gene expression of procollagen \([\alpha1](I)\) and osteocalcin was evaluated by reverse transcriptase (RT)-PCR with cDNA obtained from the established cell lines at passage 20. Expression of collagen type I, osteocalcin and dentine phosphoprotein was evaluated immunohistochemically at passage 20 and after 1.5 years of continuous cell culture. Gene expression and the expression of mineralized tissue-specific proteins was demonstrated with RT-PCR and immunohistochemistry within all four immortalized cell lines. Expression of dentine phosphoprotein was observed in three simian virus 40 large t antigen-transfected cell lines, suggesting the immortalization of odontoblast-like cells in vitro. Thus, transfection of bovine dental papilla-derived cells resulted in immortal cell lines exhibiting phenotypic characteristics of the original tissue.


http://www.sciencedirect.com/science/article/B6T4J-3T4D5S4-6/2/12c2a5d507c888904b36b93d48caaa59

With immunocytochemistry numerous nerve fibres containing neuropeptide Y (NPY) were found in human molar pulp tissue, often around small blood vessels. Reverse transcriptase-polymerase chain reaction, using specific primers, detected mRNA of the human NPY Y1 receptor in the human pulp tissue. Thus, both NPY-containing nerve fibres and NPY Y1 receptor mRNA are present in human tooth pulp, possibly regulating vascular tone and pain perception.


http://www.sciencedirect.com/science/article/B6T4J-3VHV8M0-2/2/b39243ff7737e474394e18b7c3ff742c0

Numerous nerve fibres containing calcitonin gene-related peptide (CGRP) were found by immunocytochemistry in human molar pulp. These nerves were often seen around small blood vessels and as free endings without vascular contact. In the trigeminal ganglion a large number of CGRP-immunoreactive nerve-cell bodies, mostly of small to medium size, was encountered. Reverse transcriptase-polymerase chain reaction, using specific sense and antisense primers, detected mRNA expression of the human CGRP1 receptor in the pulp tissue and the trigeminal ganglion. Thus, both CGRP-containing nerve fibres and CGRP1 receptor mRNA are present in human tooth pulp, where they may be involved in the regulation of vascular tone and other local reactions to injury.


http://www.sciencedirect.com/science/article/B6T4J-4BWHP0R-2JC/2/879205bb4f7f5b5562406be70c54881

Cultured dental follicle cells from rat mandibular molars transcribe colony-stimulating factor-1 (CSF-1) mRNA as determined by reverse-transcription polymerase chain reaction. In turn, the CSF-1 mRNA appears to be translated, as seen by immunoperoxidase staining. Interleukin
(IL-1[alpha]) stimulates increased transcription of the CSF-1 gene in a concentration- and time-dependent manner. Moreover, CSF-1 itself has an autocrine effect on transcription of the CSF-1 gene. Because others have shown that in vivo injection of CSF-1 accelerates tooth eruption and because the dental follicle is required for eruption to occur, this study demonstrates the possible relation between CSF-1 and the follicle; namely, the source of CSF-1 for tooth eruption might be the dental follicle. In turn, regulation of gene expression for CSF-1 by IL-1[alpha] and CSF-1 may play a part in signalling the onset of tooth eruption.


http://www.sciencedirect.com/science/article/B6T4J-485Y675-5/2/3653830a8e469857c11510f843469e5f

Tooth eruption requires the presence of the dental follicle, a loose connective tissue sac that surrounds each unerupted tooth. The follicle appears to regulate many of the cellular and molecular events of eruption, including the formation of osteoclasts needed to resorb alveolar bone to form an eruption pathway. To that end, the expression of the tumour necrosis factor-[alpha] (TNF-[alpha]) gene was examined in the dental follicle as a possible regulator of osteoclastogenesis. TNF-[alpha] was expressed slightly in the dental follicle of the first mandibular molar of the rat beginning at day 3 postnatally, but maximal expression was seen at day 9, a time that correlates with a slight burst of osteoclast formation seen at day 10 postnatally. In vitro, TNF-[alpha] was not expressed constitutively in the follicle cells but incubating them with interleukin 1[alpha] resulted in a strong expression of TNF-[alpha] after only 0.5 h. TNF-[alpha] itself enhanced monocyte chemotactic protein 1 (MCP-1) and vascular endothelial growth factor (VEGF) gene expression. It also slightly decreased the expression of osteoprotegerin after 3-h incubation but this returned to the control level at 6 h. MCP-1 and VEGF could aid in recruiting mononuclear cells (osteoclast precursors) to the dental follicle. In addition to the potential role of TNF-[alpha] in tooth eruption, this study suggests that the periodontal ligament derived from the dental follicle might have the capacity to synthesize TNF-[alpha], and thereby contribute to the destructive events of periodontitis.


http://www.sciencedirect.com/science/article/B6T4J-3RJR056-4/2/b552f26f1c89d9c066f63a4e73cccbe7

The prevalence and cellular distribution of human herpesvirus 7 (HHV-7) in archival labial salivary glands was analysed for virus-specific DNA sequences by polymerase chain reaction (PCR) and in situ hybridization signals. In addition, the cellular expression of HHV-7-encoded protein was detected by immunohistochemical staining with a virus-specific monoclonal antibody. Eleven of 20 samples were positive for the HHV-7 DNA sequence by PCR. Eighteen of 20 tissues analysed by in situ hybridization showed signals in ductal, serous and mucous cells. Some nuclei of these cells and also the myoepithelial population were positive. In immunolocalization studies, all 20 salivary glands consistently showed HHV-7-expressed protein in the cytoplasm of ductal cuboidal and columnar cells. The protein was also found in the cytoplasm of mucous and serous acinar cells that were immunopositive for HHV-7. The observations are consistent with the suggestion that the labial salivary gland is a site for virus replication, potential persistence and a source of infective HHV-7 in saliva.

http://atvb.ahajournals.org/cgi/content/abstract/22/4/611

The mouse is useful in studies of vascular biology because of its well-defined genetics and because the mouse genome can be manipulated. However, because only small amounts of mRNA can be extracted from blood vessels, the quantification of gene expression in individual mice is difficult. Endothelial NO synthase (eNOS) plays a major role in the regulation of vascular tone and growth. In addition, there appear to be sex differences in the production of NO under basal conditions in mouse aortas. The goals of this study were to develop a real-time polymerase chain reaction (PCR) method to quantify eNOS mRNA in blood vessels from mice and to examine eNOS mRNA levels in vessels from male and female mice. Blood vessels were isolated from C57BL/6 mice. Total RNA from individual mice was isolated and reverse-transcribed. The number of molecules of eNOS mRNA (after reverse transcription) was determined against cDNA standards, with 18S rRNA used as a control for RNA input and reverse-transcription efficiency. When expressed as copy numbers per nanogram of total RNA or as the ratio of eNOS to 18S rRNA, eNOS mRNA was lower in the aortas of female mice than in those of male mice at 7 to 9 months of age. In contrast, no difference in eNOS mRNA was found in the aortas of 2-month-old mice. In addition, eNOS mRNA levels were similar in the carotid, cerebral, and coronary arteries. These findings provide the first quantitative measurements of eNOS mRNA by using real-time PCR in the vessels of mice and suggest age- and sex-related differences in the basal levels of eNOS mRNA in mice. In addition, the eNOS region that was used for real-time PCR was amplified and sequenced for monkeys and other species. With modifications, this region may be used to design real-time PCR for eNOS in other species.


http://atvb.ahajournals.org/cgi/content/abstract/24/7/1253

Objective-- Determining the role of specific muscarinic (M) receptor subtypes mediating responses to acetylcholine (ACh) has been limited by the specificity of pharmacological agents. Deletion of the gene for M5 receptors abolished response to ACh in cerebral blood vessels but did not affect dilation of coronary arteries. The goal of this study was to determine the M receptors mediating responses to ACh in coronary circulation using mice deficient in M2 or M3 receptors (M2-/-, M3-/-, respectively). Methods and Results-- Coronary arteries from respective wild-type, M2-/-, or M3-/- mice were isolated, cannulated, and pressurized. Diameter was measured with video microscopy. After preconstriction with U46619, ACh produced dose-dependent dilation of coronary arteries that was similar in wild-type and M2-/- mice. In contrast, dilation of coronary arteries from M3-/- mice to ACh was reduced by ~80% compared with wild type. The residual response to ACh was atropine insensitive. Relaxation of coronary arteries to other stimuli was similar in M2-/- and M3-/- mice. Similar results were obtained in aorta rings.
Conclusion-- These findings provide the first direct evidence that relaxation to ACh in coronary circulation is mediated predominantly by activation of M3 receptors. This study examined the M receptor subtype (M2 versus M3 receptors) involved in the response of coronary circulation to ACh using mice deficient in the genes for M2 and M3 receptors. M3 receptor activation and not M2 receptors primarily mediates responses to ACh in the coronary circulation.


Peroxisome proliferator activated receptor (PPAR) alpha is a member of the nuclear receptor superfamily that regulates key proteins involved in fatty acid oxidation, extracellular lipid metabolism, hemostasis, and inflammation. A L162V polymorphism at the PPARA locus has been associated with alterations in lipid and apolipoprotein concentrations. We studied the association among lipids, lipoproteins, and apolipoproteins and the presence of the L162V polymorphism in 2373 participants (1128 men and 1244 women) in the Framingham Offspring Study. The frequency of the less common allele (V162) was 0.069. The V162 allele was associated with increased serum concentrations of total and LDL cholesterol in men (P=0.0012 and P=0.0004, respectively) and apolipoprotein B in men (P=0.009) and women (P=0.03 after adjustment for age, body mass index, smoking, and use of β-blockers, diuretics or estrogens). Apolipoprotein (apo) C-III concentrations were higher in carriers of the V162 allele. The association of the L162V polymorphism on LDL cholesterol concentration was greatest in those who also carried the E2 allele at the APOE locus and the G allele at the APOC3 3238C>G polymorphism. This suggests that alterations in triglyceride-rich lipoprotein metabolism may be involved in the generation of the increase LDL cholesterol observed with the L162V PPARA polymorphism.

Atherosclerosis (31)


Objective: Several studies show that the inflammatory component in atherosclerosis may contribute to increased risk for cardiovascular disease (CVD). Interleukin-6 (IL-6) is a key pro-inflammatory and immune-stimulatory cytokine of presumed importance for CVD and the metabolic syndrome. Methods and results: In this case-control study, 1179 surviving myocardial infarction (MI) cases and 1528 healthy controls were genotyped for three IL-6 promoter SNPs, and serum concentrations of IL-6 and C-reactive protein (CRP) were measured. In men, MI risk assessed as odds ratios (OR) was higher with increasing IL-6 levels, with the highest compared to the lowest IL-6 quartiles giving an OR of 2.7 [95% CI 1.7-4.4]. The ORs were independent from the effects of elevated CRP which were associated with modest MI risks (OR=1.6 [95% CI 1.0-2.5]). Also, synergistic interactions between high IL-6 levels and hypercholesterolaemia further increased MI risk estimates. The -174C allele was associated with lower serum-insulin levels.
among male controls but did not significantly influence MI risk or IL-6 levels. Conclusions: Elevated IL-6 levels are important risk markers for MI in men, the risk being further enhanced through synergistic interaction with hypercholesterolaemia. The data provide no clear evidence that polymorphisms in the IL-6 promotor region play a significant role in the pathogenesis of MI, and it remains to be further evaluated whether or not the -174C allele is of relevance for insulin resistance.


http://www.sciencedirect.com/science/article/B6T12-44X0BBJ-9/2/8c1a42cf4e1dc543ecf949009ce93b54

Serum levels of Lp(a) lipoprotein are under genetic control and a high level is a risk factor for atherosclerotic disease. We have examined the aorta of LPA transgenic mice and their non-transgenic litter mates who had all been given a regular, not lipid fortified diet. When sacrificed, the animals had an average age of 66 weeks. Lipid lesions were observed in the aorta of 13 out of 18 LPA transgenic mice and in five out of 21 non-transgenic animals. The difference is statistically significant. We conclude that LPA transgenic mice develop lipid lesions in aorta more frequently than non-transgenic animals, even on a diet with a low fat content. LPA transgenic mice on a normal diet could be a useful animal model for the study of spontaneous human atherosclerosis, its treatment and prevention.


http://www.sciencedirect.com/science/article/B6T12-48V895N-4/2/85af3dd8194fa0e91c0968d903b27593

Hepatic lipase (HL) is an important determinant of high-density lipoprotein (HDL) concentrations. A common C-to-T substitution at position -514 of the promoter region of the HL gene has been shown to be associated with HL activity and HDL cholesterol (HDL-C) levels. The current study examines the influence of this polymorphism on both levels and serial changes of HDL-C from childhood to adulthood in a community-based sample of 707 white and 291 black unrelated individuals aged 4-38 years using a repeated measures analysis. The frequency of the -514T allele was lower in whites than in blacks (0.228 vs. 0.545, PP=0.003) in white males with values in the order of T/T>T/C>C/C. Although a similar trend was seen, the genotype effect was not significant in white females and blacks. Further, the slopes of the age trajectories of HDL-C were similar in three genotype groups in blacks and whites. A sex-genotype interaction effect (P=0.043) on longitudinal profiles of HDL-C levels was found in whites, but not in blacks. White males showed a stronger genotype effect (3.6 mg/dl, P=0.003) than white females (0.5 mg/dl, P=0.601). Thus, the -514T variant of the HL gene is consistently associated with higher levels of HDL-C longitudinally since childhood, but not with rate of change over time. These results suggest that the HL gene may play an important role in the regulation of HDL-C levels from childhood to adulthood, especially in white males.

Apolipoprotein D (APOD, gene; apoD, protein) is a plasma high-density lipoprotein (HDL)--associated glycoprotein, with a putative role in the cholesterol (CHOL) transport pathway. An apoD protein polymorphism has been previously reported by us. The cathodically shifted pattern seen on isoelectric focusing gels, controlled by the APOD*2 allele, was found to be unique to populations of African ancestry. To characterize the molecular basis of the protein polymorphism and to identify new mutations, we used a combination of SSCP, DHPLC and DNA sequencing techniques to screen the entire coding region of the APOD gene. We identified three distinct missense mutations, including Phe36Val, Tyr108Cys, and Thr158Lys with frequencies ranging from 2.1 to 2.8% in 722 African blacks from Nigeria. In addition, a common 8 bp deletion polymorphism was observed in intron 1 with a carrier frequency of 30.1%. The missense mutation, Thr158Lys correlated with the APOD*2 allele of the protein polymorphism. None of the 454 Caucasians screened for these polymorphisms showed any variation. We also determined the effect of these polymorphisms on plasma lipid levels in the African black population by generalized linear model (GLM). The Val36 allele was associated with significantly decreased HDL3-C (P=0.027) and apoA-I (P=0.030) levels among females. The Lys158 allele was associated with significantly increased Lp(a) (P=0.018) and triglyceride (P=0.017) levels, among females and males, respectively. In addition, males heterozygous for both intron 1 and codon 108 polymorphisms showed significantly increased HDL-C (P=0.011), HDL3-C (P=0.041), HDL2-C (P=0.009), apoA-I (P=0.005) and decreased LDL-C (P=0.025) levels. The results of our study show that the APOD gene harbors several polymorphisms, which are unique to African populations. Further study of these polymorphisms may help to characterize the role of apoD in lipid metabolism, and in cardiovascular disease among African populations.


http://www.sciencedirect.com/science/article/B6T12-4DH2JC2-1/2/e4e297458b6c0b7103547511ad74cc85

Chronic Chlamydia pneumoniae infection and autoimmunity to heat shock protein 60 (Hsp60) have both been documented to be associated with atherosclerosis. Herein, we studied the effects of C. pneumoniae infection and a diet with a low-cholesterol supplement on the development of autoantibodies to mouse Hsp60 and early lipid lesions in the aortic valve of C57BL/6JBom mice. In addition, pulmonary infection was investigated. C57BL/6JBom mice were given one to three C. pneumoniae inoculations and fed either a regular diet or a diet enriched with 0.2% cholesterol. Autoantibody responses against mouse Hsp60 developed in both diet groups when the mice were infected with C. pneumoniae and in uninfected mice fed a cholesterol-enriched diet. C. pneumoniae infections increased subendothelial foam cell accumulation in mice on a 0.2% cholesterol-enriched diet (p = 0.022), without apparent hypercholesterolemia. These in vivo data suggest that autoantibodies against mouse Hsp60 develop as a consequence of cholesterol feeding and repeated C. pneumoniae infections. Further, infectious burden increased early lipid lesions in C57BL/6JBom mice fed a cholesterol-enriched diet.


http://www.sciencedirect.com/science/article/B6T12-442R2PP-
Macrophage infiltration, inflammatory processes and oxidatively modified low density lipoprotein (LDL) are known contributing factors in the formation of the atherosclerotic plaque. To determine whether a direct link might exist between these factors, we examined the effect of oxidized LDL upon proinflammatory cytokine production in adherent human peripheral blood mononuclear leukocytes. Oxidized LDL, as well as a combination of cholesterol and 25-hydroxycholesterol, induced tumor necrosis factor-[alpha] (TNF[alpha]) and interleukin-[beta] (IL-1[beta]) mRNA as measured by quantitative real time PCR, by a maximum of two- to fourfold following a 24-h incubation. Analysis of cell culture supernatants revealed a concomitant stimulation of TNF[alpha] and IL-1[beta] secreted protein as determined by ELISA. Treatment of human peripheral blood mononuclear leukocytes with oxidized LDL or the combination of cholesterol and 25-hydroxycholesterol caused activation of p38[alpha] as determined by the ability of immunoprecipitated p38 to phosphorylate an ATF-2 fusion protein, a surrogate substrate of p38[alpha]. VK-19911 (Pyridine, 4-[4-(4-fluorophenyl)-1-(4-piperidinyl)-1H-imidazol-5-yl]-dihydrochloride), a specific inhibitor of p38[alpha], prevented the induction of TNF[alpha] and IL-1[beta] by oxidized LDL in a dose-dependent manner. Activated p38[alpha] is known to be involved in the stabilization of cyclooxygenase-2 mRNA in response to stimuli such as lipopolysaccharide; however, in the setting of oxidized LDL-induced p38[alpha] activation, COX-2 mRNA levels were not affected. Taken together, the data imply a potential role for p38[alpha] activation in lipid-associated inflammatory processes.


http://www.sciencedirect.com/science/article/B6T12-442R2PP-6/2/81daffedaeca21d36d4e29902b658423

Atherosclerosis is associated with arterial deposition of low density lipoprotein (LDL) and lipoprotein(a), Lp(a). Both lipoproteins have been detected in atherosclerotic vessels; however, while LDL has been shown to be only blood-derived, it is not clear whether Lp(a) is also produced within the vessel wall. In the present investigation we studied gene expression of apo(a) and apoB in human blood vessels. Aorta, carotid arteries and liver specimens from 29 adult and pediatric autopsy cases were studied by RT-PCR and Southern blot analysis with primers and probes specific for apo(a), apoB and GAPDH (a control housekeeping gene). The mRNA of apo(a), but not apoB, was found within the vessel wall in both adult atherosclerotic arterial vessels and in pediatric non atherosclerotic vessels. Neither apo(a) nor apoB mRNA was detected in femoral veins. To verify the nature of the detected transcripts, we cloned the 162 base pair (bp) RT-PCR product derived from the arterial wall total RNA. Nucleotide sequencing revealed 100% homology with the apo(a) gene. Thus, while LDL in atherosclerotic arteries is exclusively blood-derived, the accumulation of Lp(a) within the artery may be due in part to in situ production of apo(a) within the vessel wall.


http://www.sciencedirect.com/science/article/B6T12-47WDJYS-3/2/d113e15447cd5ba620c4503dcffa7662

The fatty acid transport proteins (FATPs) have been implicated in facilitated cellular uptake of
non-esterified fatty acids (NEFAs), thus having the potential to regulate local and systemic NEFA concentrations and metabolism. Hypothesising that genetic variation within the FATP genes may affect lipid metabolism, we investigated a G/A substitution at position 48 in intron 8 of the fatty acid transport-1 (FATP1) gene with respect to associations with fasting and post-prandial plasma lipid and lipoprotein variables in 628 healthy 50-year-old Swedish men and 426 Swedish women, aged 37-65 years. A subset of 105 men with the apoE3/E3 genotype underwent an oral fat tolerance test. Although fasting plasma TG concentrations were not different, male A/A individuals had significantly higher post-prandial TG concentrations and VLDL1 (Sf 60-400 apoB100)-to-VLDL2 (Sf 20-60 apoB100) ratio compared to male G/A and G/G individuals. A/A individuals apparently failed to suppress plasma NEFA concentrations during the oral fat tolerance test. Furthermore, fasting plasma concentrations of the largest, most buoyant LDL subfraction (LDL-I) were significantly lower in carriers of the A allele in the male cohort. Electromobility shift assays and reporter gene studies indicated that binding of nuclear factors and effect on transcriptional activity differ between the intron 8 alleles. These findings suggest that through regulation of NEFA trafficking, FATP1 might play a role in post-prandial lipid metabolism and development of cardiovascular disease.


http://www.sciencedirect.com/science/article/B6T12-442R2PP-F/2/3985d2132adb636dccca0533e6f69636

Linkage and association of the apo AI-CIII-IV gene region to familial combined hyperlipidemia (FCHL) was reported previously, based on the presence of genetic variants in the apo CIII and apo AI gene. No data were available yet on the contribution of the apo A-IV locus. Two DNA variants in exon 3 of the apo A-IV gene, A (Thr)347T (Ser) and [CTGT]3-4 were characterized by sequencing the coding region of the apo A-IV gene and were analyzed in our Dutch FCHL cohort (30 probands, 159 affected relative, 317 unaffected relatives and 218 spouses). The genotype frequency of the A347T variant was different in probands and spouses. In probands no 2/2 carriers were found, resulting in a significant decreased frequency of the 2-allele (P3-4 variant between the groups. Homozygous 4/4 carriers in spouses had a more favorable lipid profile (LDL-cholesterol and apo B, P347T with other markers in the gene cluster, and the absence of linkage disequilibrium with [CTGT]3-4 marker and the MspI-AI marker in the apo A-I promoter showed that these two apo A-IV variants reside on different haplotypes from the apo A-I and apo C-III markers. This was illustrated by extensive haplotype analysis. The present data on the contribution of DNA variants in the apo A-IV gene support our previous observations that the apo AI-CIII-AIV gene cluster has a complex genetic contribution to FCHL both by conferring susceptibility and protection.


http://www.sciencedirect.com/science/article/B6T12-3SV50H5-B/2/8fc2389f14c60928d9c2c99f44cc5f5e

Familial combined hyperlipidemia (FCHL) is a frequent cause of premature coronary artery disease. Affected family members are characterized by different combinations of elevated cholesterol and/or triglyceride levels. A reduction in lipoprotein lipase (LPL) activity has been observed in a subgroup of FCHL patients. Recently, we have demonstrated an increased frequency of mutations in the LPL gene in Dutch FCHL patients compared to normolipidemic
controls. In the present study, we have applied a pedigree-based maximum likelihood method to study the effect of LPL mutations on the phenotypic expression of FCHL in families. In 40 FCHL probandi, three different previously reported mutations in the LPL gene were identified resulting in amino acid changes, D9N, N291S, and S447X. The D9N mutation in exon 2 appeared to be in strong linkage disequilibrium with a T->G substitution at position -93 in the promoter region of the LPL gene. We present data that the -93T->G/D9N haplotype is associated with significantly higher levels of LDL and VLDL cholesterol, and VLDL triglycerides. Interestingly, the effect was only observed in male carriers. In line with our previous observations, these results further sustain that the LPL gene is a susceptibility gene for dyslipidemia which explains part of the variability in the phenotype observed among FCHL family members.


http://www.sciencedirect.com/science/article/B6T12-40TXYD4-9/2/65676fc41b1921eaf38b4b4b52235aee

Familial combined hyperlipidemia (FCHL) is a heritable lipid disorder characterized by multiple lipoprotein phenotypes within a single family. Previously, we have shown an increased incidence of mutations in the LPL gene which was associated with elevated levels of very low density lipoprotein (VLDL) and decreased levels of high density lipoprotein among the families studied. Now, we report the results of our study on the hepatic lipase gene. We found the HL V73M variant to be present in four FCHL families. By means of a pedigree-based maximum log-likelihood method we analyzed the effect of this variant on the lipid levels in these families. Carriers of the HL V73M variant revealed significantly higher levels of total cholesterol (PP<0.01). These findings show that the HL V73M mutant explains another part of the variability in the phenotype observed among FCHL family members, compared with mutations in the LPL gene. Family analysis shows that in these FCHL families, carriers of mutations in the LPL or HL genes have an increased risk for FCHL compared with their non-carrier relatives.


http://www.sciencedirect.com/science/article/B6T12-3XV28WS-7/2/24c3df383110fd61bc3255c23ecbba27

Atherosclerotic plaques contain a significant number of macrophage foam cells and are associated with an inflammatory state. Inflammation induces the secretion from monocytes and other cells of cytokines, reactive oxygen species, proteinases and proteinase inhibitors among many other molecular species. AAT is prominent among the serine proteinase inhibitors and is an important regulator of leukocyte elastase and proteinase-3. It has been shown that the stable AAT-proteinase complex can upregulate AAT biosynthesis, and we have shown that the shorter, carboxyl terminal peptide (C-36) resulting from proteinase cleavage of AAT polymerizes, and in its fibrillar form alters cellular metabolism. To test for a possible link between the inflammation-generated C-36 peptide and cellular processes associated with atherogenesis, we have studied the effects of the fibrillar form of this peptide at varying concentrations on human monocytes in culture. We have found that fibrillar C-36 at concentrations of greater than or equal to 5 [mu]mol/l in monocyte cultures for 24 h significantly increases LDL binding and uptake, upregulates LDL receptors, induces cytokine production and glutathione reductase activity, and upregulates AAT synthesis. The expression of CD36 protein, LDL Scavenger receptor, is also upregulated by fibrillar C-36 and native LDL in the presence of C-36-activated monocytes is more oxidized than
with unactivated control monocytes. The majority of monocytes cultured for 24 h in the presence of C-36 fibrils were transformed morphologically into macrophages. These data establish a direct molecular link, mediated by C-36 peptide of AAT, between inflammation and the oxidation and accumulation of lipid in monocyte-derived macrophages. This may be important for an understanding of the events conducing to atherogenesis.


http://www.sciencedirect.com/science/article/B6T12-3YHWPPD-H/2/faa1d9b783a27004d652d5cf7d1f6e98

Heterozygous familial hypercholesterolemia (FH) is one of the most common potentially fatal single-gene diseases leading to premature coronary artery disease, but the majority of heterozygous FH patients have not been diagnosed. FH is due to mutations in the gene coding for the low-density lipoprotein (LDL) receptor, and molecular genetic diagnosis may facilitate identification of more FH subjects. The Danish spectrum of 29 different mutations, five of which account for almost half of heterozygous FH, is intermediate between that of countries such as South Africa, where three mutations cause 95% of heterozygous FH in the Afrikaners, and Germany or England, where there are many more mutations. In clinical practice, a strategy for the genetic diagnosis of FH, tailored to the mutational spectrum of patients likely to be seen at the particular hospital/region of the country, will be more efficient than screening of the whole LDL receptor gene by techniques such as single-strand conformation polymorphism (SSCP) analysis in every heterozygous FH candidate. In Aarhus, Denmark, we have chosen to examine all heterozygous FH candidates for the five most common LDL receptor gene mutations (W23X, W66G, W556S, 313+1G->A, 1846-1G->A) and the apoB-3500 mutation by rapid restriction fragment analysis. Negative samples are examined for other mutations by SSCP analysis followed by DNA sequencing of the exon indicated by SSCP to contain a mutation. If no point mutation or small insertion/deletion is detected, Southern blot or Long PCR analysis is performed to look for the presence of large gene rearrangements. In conclusion, our data suggest that an efficient molecular diagnostic strategy depends on the composition of common and rare mutations in a population.


http://www.sciencedirect.com/science/article/B6T12-3YVDR05-6/2/df0c95ebd96964bba8dce7840f8798d5

HMG-CoA reductase inhibitor drugs or 'statins' have been shown to effectively reduce plasma total cholesterol (CHOL), CHOL associated with low-density-lipoprotein (LDL), and triglycerides (TG). In addition, slight elevations in HDL-CHOL are also typically observed. Poloxamer 407 (P-407), a nonionic surfactant, effectively elevates both plasma CHOL and especially TG in a dose-controlled fashion and results in formation of atherosclerotic lesions in the aortas of C57BL/6 mice without the requirement of dietary cholic acid [1 and 2]. The purpose of the present study was to assess whether a typical statin, namely atorvastatin (Lipitor(R)) would significantly reduce P-407-induced hypercholesterolemia and hypertriglyceridemia as well as cause regression of atherosclerotic lesions resulting from administration of P-407 to C57BL/6 mice. C57BL/6 mice in the present study were treated with either normal saline (C, controls), 0.5 g/kg of P-407 (P), or a high-fat, high-cholesterol, cholate-containing diet (HF) for 120 days. Mice in all groups were then equally and randomly divided and treated with either atorvastatin or saline for an additional 120
days. Beginning at Day 121 and using mice in groups P and HF as an example, one-fourth of the mice in each group received 20 mg/kg per day of atorvastatin with either concomitant HF feeding or P-407 administration ('progression' treatment groups), one-fourth received 20 mg/kg per day of atorvastatin following cessation of HF feeding or P-407 administration, one-fourth received saline (placebo) with either simultaneous HF feeding or P-407 administration ('progression' placebo groups), and one-fourth received saline (placebo) following cessation of HF feeding or P-407 administration. Total plasma CHOL was significantly (P<0.05) elevated compared to plasma TG of C mice. With discontinuation of P-407 administration, total plasma TG declined rapidly in P mice with atorvastatin-treated mice typically demonstrating lower plasma TG concentrations relative to saline-treated P mice. Aortas of mice treated with 20 mg/kg per day of atorvastatin in both groups P and HF, whether maintained on the HF-diet or treated with P-407 from Day 120 to 240 or whether each treatment was terminated at Day 120, revealed no presence of atherosclerotic lesions relative to saline-treated mice and were indistinguishable from aortas retrieved from C mice. Atorvastatin at a dose of 20 mg/kg per day not only significantly reduced the plasma CHOL and TG concentrations, but also resulted in regression of atherosclerotic lesions induced in C57BL/6 mice by administration of P-407 or ingestion of a HF-diet containing cholic acid.


http://www.sciencedirect.com/science/article/B6T12-48NJ842-B/2/4e4db13e86e4dbe06c1ce07bfdf72

Oxidized low-density lipoprotein (oxLDL) exhibits many atherogenic effects, including the promotion of monocyte recruitment to the arterial endothelium and the induction of scavenger receptor expression. However, while atherosclerosis involves chronic inflammation within the arterial intima, it is unclear whether oxLDL alone provides a direct inflammatory stimulus for monocyte-macrophages. Furthermore, oxLDL is not a single, well-defined entity, but has structural and physical properties which vary according to the degree of oxidation. We tested the hypothesis that the biological effects of oxLDL will vary according to its degree of oxidation and that some species of oxLDL will have atherogenic properties, while other species may be responsible for its inflammatory activity. The atherogenic and inflammatory properties of LDL oxidized to predetermined degrees (mild, moderate and extensive oxidation) were investigated in a single system using human monocyte-derived macrophages. Expression of CD36 mRNA was up-regulated by mildly- and moderately-oxLDL, but not highly-oxLDL. The expression of the transcription factor, proliferator-activated receptor-[gamma] (PPAR[gamma]), which has been proposed to positively regulate the expression of CD36, was increased to the greatest degree by highly-oxLDL. However, the DNA binding activity of PPAR[gamma] was increased only by mildly- and moderately-oxLDL. None of the oxLDL species appeared to be pro-inflammatory towards monocytes, either directly or indirectly through mediators derived from lymphocytes, regardless of the degree of oxidation.


http://www.sciencedirect.com/science/article/B6T12-49W36T3-1/2/00381201887ac42eb9eb3f6637d2c0da

Objective: In the past decade, elevated homocysteine concentration has achieved widespread recognition as an independent risk factor in the development of atherosclerosis. 3-
Deazaadenosine (c3Ado) is a potent inhibitor and substrate for S-adenosylhomocysteine hydrolase and therefore may reduce homocysteine concentrations. The current study investigated the effect of c3Ado on serum homocysteine, atherosclerotic lesions, and the expression of adhesion molecules in apoE-knockout mice. Methods and results: Animals were placed on an atherogenic diet with or without c3Ado for 12 and 24 weeks. Frozen cross-sections of the aortic sinus and the proximal aorta were analyzed by computer-aided planimetry for fatty plaque formation. Macrophages, VCAM-1 and ICAM-1 were quantified by immunohistochemistry and oligo-cell reverse transcription polymerase chain reaction after laser microdissection. Application of c3Ado resulted in significant reduction of homocysteine levels by 35.9 and 45.3% after 12 and 24 weeks, respectively (P3Ado (PPPConclusion: Our results demonstrate that c3Ado induces a marked reduction of homocysteine concentrations which might explain in part the anti-atherogenic effect of the drug.


Hyperhomocysteinemia is an independent risk factor of cardiovascular disease and associated with insulin resistance, although their causal relationship remains unclear. A previous report has shown that high concentration of homocysteine damages mitochondrial gene expression, function and structure. As we found recently, the mitochondrial DNA (mtDNA) contents are inversely correlated with insulin resistance parameters. Thus there is possibility that plasma total homocysteine (tHcy) level is somewhat correlated with mtDNA content. Sixty healthy women (mean age 40.3+/−20.9 yr, range 18-78 yr) were recruited to investigate the correlation of plasma tHcy level and mtDNA content in peripheral blood. A significant negative correlation was found between plasma tHcy levels and mtDNA content (r=-0.507, Pr=0.407), W/H ratio (r=0.370), total cholesterol (r=0.338), LDL-cholesterol (r=0.317) and insulin resistance (HOMA-IR score) (r=0.261); and a negative correlation with folate (r=-0.273). MtDNA content showed negative correlations with age (r=-0.407), BMI (r=-0.440), W/H ratio (r=-0.659), SBP (r=-0.350), total cholesterol (r=-0.340), triglyceride (r=-0.376), LDL-cholesterol (r=-0.349), fasting plasma insulin (r=-0.483), and insulin resistance (HOMA-IR score) (r=-0.423); and a positive correlation with folate (r=0.299). In this study, there was a significant inverse correlation between plasma tHcy level and mtDNA content. Further study will be warranted to elucidate the mechanism by which two factors are associated.


Background: An insertion/deletion (I/D) polymorphism in the gene encoding angiotensin-converting enzyme (ACE) has been associated with serum ACE levels. The association between the ACE I/D polymorphism and coronary heart disease is unclear. Electron-beam-computed tomography (EBT) is a technique to non-invasively quantify the amount of coronary calcification. We investigated the association between the ACE I/D polymorphism and coronary calcification. Methods and results: The Rotterdam Coronary Calcification Study is a population-based study in subjects aged 55 years and over. EBT scanning was performed in 2013 participants. Coronary calcification was quantified according to the Agatston score. The ACE I/D polymorphism was available for 1976 subjects. Geometric mean calcium scores in men with the
II, ID and DD genotype were 167, 207 and 219, respectively. However, the difference in calcium score (p = 0.19 for ID versus II; p = 0.15 for DD versus II) and the trend (ptrend = 0.17) were not significant. Calcium scores in women with the II, ID and DD genotype were 44, 42 and 36, respectively. There were no significant differences in calcium score (p = 0.78 for ID versus II; p = 0.29 for DD versus II), neither was the trend (ptrend = 0.27). After we stratified on cardiovascular risk factors, no associations were present.

Conclusion: The present study failed to show an association between the ACE I/D polymorphism and coronary calcification in the general population. Also, no significant associations were present between the ACE I/D polymorphism and coronary calcification in strata of cardiovascular risk factors.


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http://www.sciencedirect.com/science/article/B6T12-45S9MBX-1/2/0d10b82a221d96bdc9ef1eccda07ce3

Matrix metalloproteinase 3 (MMP3) is expressed in human coronary atherosclerotic lesions and is known to be involved in degradation of the plaque and to be co-localized with calcium and fibrin deposits in advanced lesions, indicating a possible role of MMP3 in arterial calcification. The MMP3 gene promoter polymorphism leads to low promoter activity 6A6A, intermediate promoter activity 5A6A and high promoter activity 5A5A genotypes. To determine whether these genotypes predict the extent of atherosclerosis we investigated their association with different types of coronary lesions in an autopsy series of 300 middle-aged white Finnish men (aged 35-69 years) from the Helsinki Sudden Death Study (HSDS). Areas of the coronary wall covered with different atherosclerotic lesions were measured and MMP3 genotypes were determined by PCR and minisequencing. In men >=53 years the mean area of calcified lesion in the most severely affected coronary artery was significantly associated with the MMP3 genotype (P=0.029). Subjects with high promoter activity genotypes had on average larger calcified lesion areas than those with the low-activity genotype. The MMP3 genotype (P=0.025) persisted as an independent predictor of mean calcified lesion area after stepwise adjustment for age, BMI, hypertension, diabetes, number of affected vessels and smoking. These data provide evidence that the proposed effect of MMP3 in the process of atherogenesis may be modified by the MMP3 genotype.


Objective: Matrix metalloproteinases 3 and 9 (MMP3 and MMP9) are present in atherosclerotic plaques and co-operate in the degradation of the fibrous cap of the atheroma, leading to fissuring
and ultimately to acute coronary thrombosis. The functional genetic polymorphisms in the promoters of MMP3 and MMP9, which lead to low- and high-transcription activity genotypes, have been shown to be associated with myocardial infarction and angiographically measured atherosclerosis individually, whereas their effects in combination are not yet known. In order to assess the two disease loci simultaneously, we investigated the association of combined low and high promoter activity genotypes with different types of coronary lesions in an autopsy cohort of 300 Caucasian males aged 33-69 years (Helsinki Sudden Death Study). Methods: Genotyping at these loci was performed by PCR, restriction enzyme digestion and minisequencing, and areas of the coronary wall covered with atherosclerotic lesions were measured using computer-assisted morphometry. Results: In analysis of covariance (ANCOVA) with age, body mass index, hypertension, diabetes, smoking and alcohol consumption as covariates, a significant interaction between the MMP3 and MMP9 genotypes was observed on area of complicated lesions (P = 0.012). Men with high promoter activity genotypes for both loci had, on average, more than two times larger area of complicated lesions (250%) compared with those men who had low promoter activity genotypes (P = 0.008), but these loci showed no association with myocardial infarction. Conclusions: The joint action of two susceptibility loci, rather than single MMP genes alone, and the particular combination of MMP3 and MMP9 genotypes present at these loci may contribute to heterogeneity in the presentation of atherosclerosis.


http://www.sciencedirect.com/science/article/B6T12-45PRTMD-3/2/41bf9a054e52f3941f040b5d56baab92

We attempted to detect chlamydial antigens in canine atherosclerotic lesions from seven dogs by immunohistochemical technique using anti-Chlamydia psittaci (C. psittaci) polyclonal and anti-C. pneumoniae monoclonal antibodies. Immunopositive signals to both antibodies were recognized in the atherosclerotic lesions of the aortas, coronary and splenic arteries of all dogs. Positive signals were found in the foamy cytoplasm of infiltrated macrophages and extracellular matrices in the lesions. In some lesions, cytoplasm of the endothelial cells and smooth muscle cells was also immunopositive against both antibodies. By electron microscopy, chlamydial microorganisms were found in the cytoplasm of endothelial cells and smooth muscle cells. Using polymerase chain reaction (PCR), detection of C. pneumoniae DNAs were performed in the spleen, heart (coronary arteries) and kidney in one of the seven dogs. Positive 314 bp PCR products were obtained in all samples of the dog. These results confirmed the presence of viable Chlamydiae in atheromas and supported the conclusion that the organism may be an active factor in the pathogenesis of canine, as well as human atherosclerosis.


http://www.sciencedirect.com/science/article/B6T12-4DM2RWD-3/2/5cc2df5bf648f270f11de2e11a575f784

Objectives: Macrophage migration inhibitory factor 1[alpha] (MIF), a cytokine with immunoregulatory functions has been suggested to be involved in atherosclerotic plaque development. However, little is known about MIF-inducing conditions in the atherosclerotic process and the association of MIF with plaque instability. Methods and results: Forty-two carotid endarterectomy samples from 36 patients and 4 aortic samples from young accident victims (as healthy controls) were analyzed for MIF staining, MIF expressing tissues in the atherosclerotic
plaque are mainly mononuclear cells (MNCs), but also endothelial cells of intimal microvessels (MVECs). The magnitude and the intensity of their MIF expression was associated with the progression of plaques from early lesions (Stary I-III) to complicated plaque stages (Stary IV-VIII). In highly inflammatory and neovascularized regions of the plaques the colocalization of MIF expressing MNCs with CD40-L and angiotensin II (Ang II)-producing MNCs could be established. This finding supports the notion that CD40-L fusion protein and Ang II are able to induce MIF production in the monocytic cell line THP-1. Furthermore hypoxia ([less-than or equal to]1% O2) as a further proinflammatory and especially proangiogenetic factor was able to stimulate MIF secretion by THP-1, human monocytes and HUVECs. Hyperglycemia and insulin remained without effect. Conclusion: MIF is expressed in advanced atherosclerotic lesions in close correlation with signs of instability, such as mononuclear cell inflammation and neointimal microvessel formation. Furthermore, the colocalization of MIF with Ang II-producing MNCs and CD40-L+ cells in these plaques and the finding that proathero- and -angiogenic mediators such as CD40-L, Ang II and hypoxia are able to stimulate MIF expression in vitro suggest an important role of MIF in the modulation of atherosclerotic plaque stability.


http://www.sciencedirect.com/science/article/B6T12-44HXDHV-13/2/035df8283bdfce3b5d8b20a2544be08f

Chlamydia pneumoniae infection generally starts in the respiratory tract and probably disseminates systemically in the blood stream within alveolar macrophages. We investigated the prevalence of C. pneumoniae DNA in peripheral blood mononuclear cells (PBMC) in patients with acute ischaemic heart disease. Samples of blood were obtained from 93 consecutive patients with acute ischaemic heart disease and from 42 healthy subjects, for detection of C. pneumoniae DNA in PBMC by polymerase chain reaction (PCR) and for serology. C. pneumoniae DNA in PBMC was detected in 25.8% (24/93) of the patients with acute ischaemic heart disease and in 4.8% (2/42) of the healthy subjects (P=0.008). C. pneumoniae IgG was found in 76.3% of patients and in 45.2% of healthy subjects (P=0.0008) while C. pneumoniae IgA was found in 59.1% and in 33.3%, respectively (P=0.01). No correlation was found between anti-C. pneumoniae antibody titers and positive PCR results. The detection of C. pneumoniae DNA in PBMC may aid in selecting patients who may benefit from antibiotic treatment; however, to support this contention, longitudinal studies on patients treated with antibiotics would also be necessary.


http://www.sciencedirect.com/science/article/B6T12-4D5KT9K-1/2/bba14efc20409ef97308d64fe6969177

Paraoxanase (PON 1), a high-density lipoprotein-associated enzyme, exerts an antiatherogenic effect by protecting low-density lipoproteins (LDL) against oxidation. A common polymorphism at codon 192(Q/R) of the PON 1 gene has been shown to be associated with an adverse lipoprotein profile and increased coronary artery disease (CAD) risk. However, these observations are based mostly on case-control studies involving relatively older adults. This study examined the frequency and phenotypic (lipoprotein variables) effect of the Q192R variant in a community-based sample of 1786 black and white young adults (mean age: 32.5 years; 69% white, 44%
In addition, the genotypic effect of this polymorphism on ultrasonographically measured carotid artery intima-media thickness (IMT), a surrogate measure of CAD risk, was examined in a subsample of 436 young adults (mean age: 32.6 years; 70% white, 42% male). The frequency of the variant allele (R192) was higher in blacks than in whites (0.668 versus 0.297, \( \text{P} = 0.041 \)), whereas the opposite was true in blacks (\( \text{P} = 0.008 \)). Neither the Q nor the R allele was associated with LDL cholesterol and triglycerides in both races. The genotypic effect on the carotid IMT adjusted for the covariates including lipoprotein variables was not apparent in whites or blacks. However, among whites, the carotid IMT was lower in carriers (QR + R) versus non-carriers (QQ) of the variant allele among females (\( \text{P} = 0.008 \)) and non-smokers (\( \text{P} = 0.026 \)). In addition, the variant allele negated the adverse positive relationship between the carotid IMT and triglycerides among whites (\( \text{P} = 0.212 \) for carriers versus \( \text{P} < 0.001 \) for non-carriers). These results indicate a differential effect of the Q192R variant on HDL cholesterol in whites versus blacks and a beneficial interaction effect of the variant allele with individual's sex, smoking status or triglyceride levels on the carotid IMT among whites.


http://www.sciencedirect.com/science/article/B6T12-4806D7K-2/2/797b256e667f1f8b4ab3db3864f8a2ec

The cholesteryl ester transfer protein (CETP) gene has been implicated in the variation of HDL levels but most studies have focused on only one or a few genetic variations. In order to properly understand the role of CETP in determining phenotype, it is necessary to examine the entire gene and all its common polymorphisms. The coding regions, adjacent introns, and proximal 5' and 3' regions were resequenced from an ethnically diverse population. Novel and previously known polymorphisms were then characterized and associations with HDL and CETP mass levels determined. The polymorphism most highly associated with CETP was 629 bp upstream of the transcription start site while the polymorphism most highly associated with HDL was a VNTR 1946 bp upstream of the transcription start site. Genetic variation in the CETP gene is associated with protective HDL levels. The ethnic diversity of some SNPs and complex interplay among them dictate careful analysis of the whole gene prior to conclusions about the role of individual polymorphisms.


http://www.sciencedirect.com/science/article/B6T12-3VXK4-8/2/c5209d13494c1dc6f03495e52cb395

We compared biochemical and molecular methods for the identification of heterozygous carriers of mutations in the cystathionine [beta]-synthase (CBS) gene. Eleven relatives of seven unrelated patients with homocystinuria due to homozygous CBS deficiency and controls were studied with respect to total homocysteine concentrations before and after methionine loading. In addition, we determined CBS activity in cultured skin fibroblasts and tested for the presence of five known mutations by a PCR-based method in these seven patients, their relatives and controls. The results demonstrate that measurement of homocysteine after methionine loading and assay of CBS enzyme activity in cultured fibroblasts identify most but not all heterozygotes. There was significant correlation between homocysteine concentrations and CBS activities only after methionine loading (\( r = 0.12, 0.48, 0.48 \) and 0.50 at 0, 4, 6 and 8 h, respectively). Among the homozygous patients, molecular approaches identified five T833C and two G919A mutations out of 14 independent alleles, confirming the studies of others that these represent the two most
prevalent mutations. In addition, we found that three of six heterozygotes with the T833C allele had post-methionine loading homocysteine levels which overlapped with controls and of the other three, one (as well as an obligate heterozygote who did not carry any of the five mutant alleles tested) had CBS activity comparable to that of controls. These findings demonstrate that genotyping is useful as an adjunctive method for the diagnosis of the heterozygous carrier state of CBS deficiency.


http://www.sciencedirect.com/science/article/B6T12-435MFHN-X/2/1f952e6758459ba39b1a831b27430

Platelets are thought to contribute to development of restenosis following percutaneous coronary interventions. The glycoprotein la/IIa complex is a major platelet collagen receptor, its surface expression being influenced by two, linked single nucleotide polymorphisms (C807T and G873A) in the glycoprotein la gene. T807 is associated with increased expression of this integrin receptor. We assessed whether T807 is associated with an increased risk of restenosis in 1769 consecutive patients treated with coronary stenting. 6-month follow-up angiograms were available in 82.4% of the patients. C807T genotype distribution was CC in 35.8%, CT in 47.6% and TT in 16.6% of the patients. Restenosis (diameter stenosis [ges]50% at follow-up angiography) occurred in 32.9% of CC, 31.5% of CT and 32.1% of TT patients (P = 0.87). The rate of major adverse cardiac events (death, myocardial infarction or need of reintervention) within 1 yr was 21.6% for CC, 21.7% for CT and 21.2% for TT patients (P = 0.98). Thus, carriage of the GP Ia T807 allele is not associated with an increased risk of restenosis or unfavorable late outcome following coronary artery stenting.


http://www.sciencedirect.com/science/article/B6T12-45HD82P-3/2/297f48d69ad0d3d1cd2c02504609e968

Low density lipoprotein receptor deficient (LDLR-KO) and apolipoprotein E deficient (apo E-KO) mice both develop hyperlipidemia and atherosclerosis by different mechanisms. The aim of the present study was to compare the effects of simvastatin on cholesterol levels, endothelial dysfunction, and aortic lesions in these two models of experimental atherosclerosis. Male LDLR-KO mice fed a high cholesterol (HC; 1%) diet developed atherosclerosis at 8 months of age with hypercholesterolemia. The addition of simvastatin (300 mg/kg daily) to the HC diet for 2 more months lowered total cholesterol levels by ~57% and reduced aortic plaque area by ~15% compared with the LDLR-KO mice continued on HC diet alone, PP<0.05. In contrast, in age-matched male apo E-KO mice fed a normal diet, the same treatment of simvastatin elevated serum total cholesterol by ~35%, increased aortic plaque area by ~15%, and had no effect on endothelial function. These results suggest that the therapeutic effects of simvastatin may depend on the presence of a functional apolipoprotein E.

Tranilast (N(3,4-dimethoxycinnamoyl)anthranilic acid), an agent which in cell culture inhibits transforming growth factor-[beta] (TGF-[beta]) secretion and antagonises the effects of TGF-[beta] and platelet-derived growth factor (PDGF) on cell migration and proliferation, has been reported to reduce the incidence of restenosis after angioplasty in angiographically validated human clinical trials. We investigated in a rat model of balloon angioplasty whether tranilast's effects in vivo could be attributed to inhibition of expression of TGF-[beta] and/or its receptor types. Using a standardised reverse transcriptase-polymerase chain reaction (RT-PCR) assay, we examined the effects of three doses of tranilast (25, 50 and 100 mg/kg) on the expression of two TGF-[beta] isoforms, the types I and II TGF-[beta] receptors and two putative TGF-[beta] responses, induction of integrins [alpha]v and [beta]3 mRNA, 2 h after oral administration and 26 h after vessel injury. Tranilast attenuated in a dose-dependent and reversible manner the injury-induced increases in mRNA levels encoding TGF-[beta]1, TGF-[beta]3, two type I TGF-[beta] receptors ALK-5 and ALK-2, and the type II receptor T[beta]RII. At the highest dose mRNA levels encoding TGF-[beta]1 and T[beta]RII were attenuated to levels approaching or below those observed in uninjured vessels. Messenger RNAs encoding TGF-[beta]3, ALK-5 and ALK-2 were all attenuated by between 70 and 74% (all [P][alpha]v and [beta]3 observed after vessel injury, by 90 and 72%, respectively. We also investigated, in cultured smooth muscle cells derived from injured carotid arteries, the extent to which tranilast (300 mg/l) attenuated any increases in expression of type I and type II receptors stimulated by PDGF-BB and TGF-[beta]1, growth factors implicated in smooth muscle cell migration and proliferation in injured vessels. Increases in mRNA levels of the type I receptors ALK-5 and ALK-2 induced by PDGF-BB and TGF-[beta]1 were almost completely prevented by tranilast. Tranilast also prevented the PDGF-BB induced increases in T[beta]RII but only partially inhibited the TGF-[beta]1 induced upregulation of T[beta]RII. We conclude that tranilast can inhibit transcriptional mechanisms associated with the upregulation of TGF-[beta] and its receptor types in balloon catheter injured vessels. It is possible that these mechanisms contribute to its ability to reduce the frequency of restenosis after angioplasty.


Inflammation and innate immunity may play a role in the pathogenesis of atherosclerosis. The Asp299Gly polymorphism in the toll-like receptor 4 (TLR4) gene reduces responsiveness to lipopolysaccharide and has been associated with reduced incidence and slower progression of carotid atherosclerosis. We analyzed this polymorphism in relation to susceptibility to and severity of coronary artery disease. Methods: 1400 participants (mean age: 63 years, 31% female) in the Southampton Atherosclerosis Study were genotyped for the TLR4 Asp299Gly polymorphism using the tetra-primer PCR method. Results: There was no significant difference between the frequencies of the Asp/Gly or Gly/Gly genotypes combined, compared to the Asp/Asp genotype, in patients with 0, 1, 2 or 3 coronary arteries with >50% stenosis ([chi]3d.f.2=0.4, P=0.94). No associations were observed between genotype groups and cardiac risk factors (P>0.05). Conclusion: The findings of this study do not support the hypothesis that the TLR4 Asp299Gly polymorphism influences predisposition to and progression of coronary artery disease.
Objectives: Mutations in connexin26 (GJB2) are one of the most frequent causes of prelingual hearing impairment. Several different types of one-base deletions in exon2 were the most common type of GJB2 mutation regardless of ethnicity, including 35delG in American-European populations, 235delC in Japanese population and 167delT in Ashkenazi Jewish population. Various types of one-base substitutions were also considered to be causative mutations of GJB2 associated hearing impairment. This article describes a rapid and high-throughput screening procedure for the detection of one-base deletion/substitution in GJB2 with less invasive sampling procedure in the implication for the clinical application. Methods: 53 hearing-impaired children and 50 healthy controls were admitted to take part in this study program. DNA samples obtained from buccal swab were used to amplify the exon2 of GJB2, and single run with an automated sequencer was used to identify the one-base deletion. Single-base substitutions were also screened by primer-extension procedure with dye terminators. The presence of both types of mutations was confirmed by direct sequence of the GJB2 exon2. Results: Two of 50 controls (4%) included one-base deletion in GJB2 as heterozygote. 14 of 53 hearing impaired cases (26.4%) contained deletion in GJB2 either as homozygote (five cases) or heterozygote (nine cases) form. Sequencing analysis of whole exon2 of GJB2 identified all these deletions as 235delC. Primer-extension analysis revealed additional mutations with single base substitutions in three cases with compound heterozygote with 235delC. Conclusions: Rapid screening procedure of GJB2 can be potentially useful for the identification of prelingual deafness.

Stimulation of nicotinic acetylcholine receptors (nAChR) excites peripheral sensory nerve fibres, but also exert antinociceptive effects. The differences in these nAChR-mediated effects could be related to the expression of different nAChR subtypes located on nociceptive neurons. In the present study, we focused on the recently described [alpha]10-nAChR subunit, and on [alpha]4 and [alpha]7 subunits, which are the most abundant subunits in the central nervous system. In nociceptive neurons from thoracic and lumbar dorsal root ganglia (DRG), nAChR subunits were found at transcriptional (RT-PCR), translational (immunohistochemistry) and functional levels. Cultured DRG neurons express mRNA for the subunits [alpha]2-7 and [alpha]10. The [alpha]-subunit proteins 4, 7 and 10 were colocalised in virtually all nociceptive neurons that were identified by immunoreactivity for the vanilloid receptor TRPV-1. These findings were
corroborated by current recordings and calcium measurements, which revealed excitatory inward currents and calcium responses in capsaicin sensitive neurons.


http://www.sciencedirect.com/science/article/B6VT5-41WBD7V-1/2/65020520773ee4851ea39c7d8cd2af31

In studies of the central and peripheral autonomic nervous system, it has become increasingly important to be able to investigate mRNA expression patterns within specific neuronal populations. Traditionally, the identification of mRNA species in discrete populations of cells has relied upon in situ hybridization. An alternative, relatively simple procedure is 'multiplex' reverse transcription-polymerase chain reaction (RT-PCR), conducted on single neurons after their in vitro isolation. Multiplex single-cell RT-PCR can be used to examine the expression of multiple genes within individual cells, and can be combined with electrophysiological, pharmacological and anatomical (retrograde labelling) studies. This review focuses on a number of key aspects of this approach, methodology, and both the advantages and the limitations of the technique. We also provide specific examples of work performed in our laboratory, examining the expression of alpha2-adrenergic receptors in catecholaminergic cells of the rat brainstem and adrenal medulla. The application of single-cell RT-PCR to future studies of the autonomic nervous system will hopefully provide information on how physiological and pathological conditions affect gene expression in autonomic neurones.


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The aim of this study was to investigate the expression of the [alpha]2-adrenergic receptors in the adrenal medulla, and to examine the mechanism by which clonidine and related drugs inhibit acetylcholine (ACh)-induced whole-cell currents in adrenal chromaffin cells. Reverse transcription-polymerase chain reaction (RT-PCR) performed on punches of rat adrenal medulla demonstrated expression of mRNA for the [alpha]2A-, [alpha]2B- and [alpha]2C-adrenergic receptors. Similar experiments conducted with tissue punches obtained from the adrenal cortex did not reveal expression of these receptor subtypes. Whole-cell currents were recorded in isolated chromaffin cells using the perforated-patch configuration. ACh (50 [mu]M) evoked inward currents with a peak amplitude of 117.8+/-9.3 pA (n=45; Vhol=-60 mV). The currents were inhibited in a dose-dependent manner (0.5-50 [mu]M) by clonidine, UK 14,304 and rilmenidine (agonists of [alpha]2/imidazoline receptors), as well as by SKF 86466 and efaroxan (antagonists). Adrenaline and noradrenaline (50-100 [mu]M) had no significant effect. Thus, although the adrenal medulla expresses mRNA for the [alpha]2-adrenergic receptors, the lack of agonist-antagonist specificity observed in our whole-cell recordings (in the absence of intracellular dialysis) provides additional evidence against the possibility that these inhibitory effects are mediated by classical [alpha]2 or imidazoline receptor interactions.
A number of studies suggest melanocortin (MC) system involvement in nociceptive modulation. Although the mechanism through which this occurs is still unknown, experimental evidence would suggest a primary role of MC4 receptors. To further investigate the implication of this MC receptor subtype in chronic pain, we have studied the effects of several MC antagonists on spinal nerve ligation-induced nociceptive behavior in rats. The intrathecal injection of synthetic antagonists with different selectivity to MC4 receptor and of an endogenous antagonist (Agouti related protein; AgRP) reduced mechanical allodynia in neuropathic rats, as measured by von Frey hair test. Treatments produced an anti-allodynic effect at the dose of 1.5 nmol (25-30% maximum possible effect, MPE, P < 0.05). To further investigate the possible physiological role of AgRP in pain modulation we studied its expression in both sham and neuropathic rat spinal cord and dorsal root ganglia (DRG) by quantitative real time PCR and immunohistochemistry. AgRP was present in both spinal cord and DRG, and its expression, was unchanged in neuropathic animals. In conclusion MC4 receptor antagonists with different selectivity profile, induce anti-allodynic effects in one of the most relevant neuropathic pain model. In addition the expression of AgRP in spinal cord and DRG suggests an endogenous tonic inhibitory control on MC system activity. In pathological conditions this steady control could be insufficient to cope with an over activated MC system leading to increase in nociception. These data suggest that targeting MC4 with synthetic antagonists could restore the balance and hence reduce nociception.

Previous studies in humans have demonstrated a high co-morbidity between alcoholism and smoking. This co-morbidity between alcohol and nicotine dependence can be attributed, in part, to common genetic factors. In rodents, behavioral and physiological responses to alcohol and nicotine also appear to share common genetic influences. In this report, the genetic correlation between free-choice oral nicotine and oral alcohol consumption was evaluated using an ascending two-bottle choice paradigm in C57BL/6 X C3H/HeJ F2 intercross mice. For all concentrations of nicotine (25, 50, and 100 [mu]g/ml) and alcohol (3, 6, and 10%) tested, nicotine consumption was significantly correlated with alcohol consumption. Nicotine consumption at the highest nicotine concentration tested (100 [mu]g/ml) showed low, but significant, correlations with the number of [3H]-cytisine binding sites in the hippocampus (r = 0.307) and the number of [125I]-[alpha]-bungarotoxin binding sites in the cortex (r = -0.328). No significant correlations between alcohol consumption and the number of either [3H]-cytisine or [125I]-[alpha]-bungarotoxin binding sites was observed. A polymorphism in the nicotinic receptor [alpha]4 subunit gene, Chrna4, showed a trend with nicotine consumption and a significant association with alcohol consumption in female but not male mice. These results indicate that common genetic factors influence nicotine and alcohol consumption in mice. However, neither individual differences in the expression of [3H]-cytisine or [125I]-[alpha]-bungarotoxin binding nicotinic receptors nor the polymorphism in Chrna4 likely contribute to the genetic overlap that influences the consumption...
of both of these drugs of abuse in C57BL/6 X C3H/HeJ F2 mice.


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The differential sensitivity following the administration of [Delta]9-THC to 3 mouse strains, C57BL/6, DBA/2 and ICR mice, indicated that some of the neurobehavioral changes may be attributable to genetic differences. The objective of this study was to determine the extent to which the cannabinoid (CB1) receptor is involved in the observed behavioral changes following [Delta]9-THC administration. This objective was addressed by experiments using: (1) DNA-PCR and reverse PCR; (2) systemic administration of [Delta]9-THC, and; (3) intracerebral microinjection of [Delta]9-THC. The site specificity of action of [Delta]9-THC in the brain was determined using stereotaxic surgical approaches. The intracerebral microinjection of [Delta]9-THC into the nucleus accumbens was found to induce catalepsy, while injection of [Delta]9-THC into the central nucleus of amygdala resulted in the production of an anxiogenic-like response. Although the DNA-PCR data indicated that the CB1 gene appeared to be identical and intronless in all 3 mouse strains, the reverse PCR data showed two additional distinct CB1 mRNAs in the C57BL/6 mouse which also differed in pain sensitivity and rectal temperature changes following the administration of [Delta]9-THC. It is suggested that the diverse neurobehavioral alterations induced by [Delta]9-THC may not be mediated solely by the CB1 receptors in the brain and that the CB1 genes may not be uniform in the mouse strains.

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http://www.sciencedirect.com/science/article/B6WBK-48KF92V-5/2/14cca1f733688b025d7da776d5fd31519

We developed the real-time PCR quantification of endothelin-A (ET-A) and endothelin-B (ET-B) receptor genes and present their relative expression levels in various adult tissues and during development in mouse using the 2(-Delta Delta C(T)) method. ET-A and ET-B receptors were detected in all tissues examined. Gene expression of ET-A and ET-B receptors increases during the later stages of embryonic development in lung, heart, liver, kidney, and skin and reaches a maximum on the first one or two days after birth. The results, in agreement with our data on endothelin (ET) ligands, suggest that the ET system may be involved in the emergence and maintenance of functions vital after birth in these organs. These findings were corroborated through observation of the correlation between the gene expression and (poly)peptide production of the ET system in normal skin before and after parturition.

We report the characterization of a cell system employing Chinese hamster ovary (CHO) cells and CHO cells transfected with the scavenger receptor class A (CHO-SRA) using extracellularly produced reactive oxygen species (ROS) in order to study the endocytic function of the scavenger receptor. The oxidative environment was produced using tert-butyl hydroperoxide (TBH) and characterized by flow cytometry and cell viability. Once an adequate oxidative environment was established, binding and internalization studies of radiolabeled acetylated LDL particles (125I-labeled Ac-LDL) with CHO-SRA cells were carried out. RT-PCR analysis using total RNAs from CHO-SRA cells revealed that oxidative stress does not alter the expression of the scavenger receptor. However, internalization of 125I-labeled Ac-LDL through this receptor carried out by these cells was completely abolished under extracellularly oxidative conditions. Together, these results support the idea that an oxidative stress produced extracellularly, inhibiting the endocytosis of the scavenger receptor, could help to understand and explain the mechanisms by which several physiologically important ligands are accumulated in the extracellular space with its consequent cell damage.


Using RNA interference (RNAi), we specifically down-regulate protein expression in differentiated human skeletal myotube cultures. Serum stimulation of myotubes increases glucose uptake. Using a sensitive photolabeling technique, we demonstrate that this increase in glucose uptake is accompanied by increased cell-surface content of glucose transporter (GLUT) 1. Using RNAi, we specifically reduce GLUT1 mRNA and protein expression, leading to inhibition of serum-mediated increase in glucose transport. Thus, we demonstrate the utility of RNAi in a primary human differentiated cell system, and apply this methodology to demonstrate that serum-mediated increase in glucose transport in human skeletal muscle cells is dependent on GLUT1.


Cell apoptosis operates as an organizing mechanism in biology in addition to removing effete cells. We have recently proposed that during bone remodeling, osteocyte apoptosis steers osteonal alignment in relation to mechanical loading of the whole bone [J. Biomech. 36 (2003) 1453]. Here we present evidence that osteocyte apoptosis in cell culture is modulated by shear stress. Under static culture conditions, serum starved osteocytes exposed phosphatidylserine (PS) on their cell membrane 6 x more often than periosteal fibroblasts and 3 x more often than osteoblasts. Treatment with shear stress reduced the number of osteocytes that exposed PS by 90%, but did not affect the other cell types. Fluid shear stress of increasing magnitude, dose-dependently stimulated Bcl-2 mRNA expression in human bone cells, while shear stress did not
change Bax expression. These data suggest that disuse promotes osteocyte apoptosis, while mechanical stimulation by fluid shear stress promotes osteocyte survival, by modulating the Bcl-2/Bax expression ratio.

http://www.sciencedirect.com/science/article/B6WBK-48J43CC-7/2/f6ef6abad09b9d1034cc8323f73a3998

Cyclooxygenase (COX) is the key enzyme in the production of prostaglandins, which are essential for the response of bone to mechanical loading. We determined which COX-isoform, COX-1 or COX-2, determines loading-induced prostaglandin production in primary bone cells in vitro. Mouse and human bone cells reacted to 1 h of pulsating fluid flow (PFF, 0.6 +/- 0.3 Pa at 5 Hz) with an increased prostaglandin E2 production, which continued 24 h after cessation of PFF. Inhibition of COX-2 activity with NS-398 abolished the stimulating effect of PFF both at 1 h and at 24 h post-incubation, while inhibition of COX-1 by SC-560 affected neither the early nor the late response to flow. PFF rapidly stimulated COX-2 mRNA expression at 1 h but did not affect COX-1 mRNA expression. COX-2 mRNA expression was still significantly enhanced 24 h after cessation of PFF. We conclude that COX-2 is the mechanosensitive form of COX that determines the response of bone tissue to mechanical loading.

http://www.sciencedirect.com/science/article/B6WBK-4B0PR53-13/2/a3e703b64108916ea20bdfe6ae4bc84

Free oxygen radicals contribute to gastric mucosal damage induced by acetylic-salicylic acid (ASA). Vitamin C has been shown to reduce gastric toxicity of ASA in humans. We intended to assess the role of heme oxygenase-1 (HO-1) in this process by application of these substances to AGS and KATO III cells. HO-1 expression was monitored by real-time RT-PCR, Western blot, and HO activity measurement. HO-1 mRNA was significantly elevated by either ASA or vitamin C in gastric epithelial cells, combination of both substances further increased expression. HO-1 protein and enzyme activity rose in cells exposed to vitamin C alone or combined with ASA, but not after stimulation with ASA alone. In contrast to endothelia, in which ASA simultaneously induces HO-1 mRNA and protein expression, gastric epithelial cells require vitamin C to translate HO-1 mRNA into active protein, which then may exert gastroprotection by its antioxidant and vasodilative properties.


Familial lecithin:cholesterol acyltransferase (LCAT) deficiency is a hereditary disorder with clinical manifestations including corneal opacity, premature atherosclerosis and renal failure. In this
study, we analyzed the molecular base underlying a case of Japanese LCAT deficiency, in which both LCAT mass and activity of the proband were nearly absent. DNA blot hybridization analysis showed no gross rearrangement in the LCAT gene of the proband. The nucleotide sequence analysis of the cloned LCAT gene demonstrated only an extra nucleotide "C" insertion at the first exon, when compared to the sequence of wild type. This single base insertion caused a shift of the following reading frame, probably resulting in a truncated abnormal LCAT polypeptide that consist of only 16 amino acids. The direct sequence analysis of PCR-amplified DNA showed only the same insertion, indicating that the LCAT-deficient proband is a homozygote for the mutant allele. These results indicate that the clinical and biochemical feature of the patient is mainly caused by a complete deficiency of the enzyme based on a homozygous abnormality of LCAT gene.


http://www.sciencedirect.com/science/article/B6WBK-4CCNSW8-7/2/76eaa4392f1b86742e0a3fffd720aa5b

Several putative polyadenylation sequences and an adenylate plus timidylate rich element (ARE) are present at the 3' end of the rat advanced glycation end products receptor (RAGE) gene. Two transcripts are generated by the use of alternative polyadenylation sequences, one containing the ARE sequence in its 3'-untranslated region (3'-UTR). Transfections of CHO-k1 or NRK cells with constructs expressing the 3'-UTRs of the transcripts fused to a green fluorescence protein mRNA show that the ARE sequence has a negative effect on protein expression correlating with a decrease in the amount of mRNA, as shown in CHO-k1 transfected cells. When transfected cells were incubated in the presence of Actinomycin D the amount of fluorescence decreased in cells transfected with the ARE sequence, indicating that this sequence induces lower mRNA stability. Thus, alternative polyadenylation signals and an ARE sequence provide a novel mechanism for the regulation of the rat RAGE gene expression.


http://www.sciencedirect.com/science/article/B6WBK-4B3JS9Y-J/2/3674f88234c4b3cd0d18bdf297093d92

Overexpression of the [gamma]2 chain of laminin-5 has been linked to tumor invasion and an unfavorable prognostic value, but the role of this adhesion molecule in cancer progression remains unclear. Because dog models of human cancers provide the opportunity of clarifying the relation between laminin-5 and tumor malignancy we have isolated and characterized the cDNA of dog [gamma]2 chain. Comparative analysis of the nucleotide sequence revealed high identity between the dog and the human [gamma]2, including the intermolecular molecule binding sites and the regulatory promoter sequences. Moreover, expression of a recombinant human [gamma]2 chain in dog keratinocytes results in assembly and secretion of hybrid laminin-5 molecules, which underscore the functional relevance of the [gamma]2 conserved domains. We have also determined the syntenic location of the dog laminin-5 loci on CFA7. Our study provides a basis for therapeutical approaches of epithelial cancers of [gamma]2 using dogs as large animal models.
The human cytochrome CYP2A13, which is mainly expressed in the respiratory tract, has been shown to be highly efficient in vitro in the metabolism of tobacco-smoke carcinogens and procarcinogens such as 4-methylnitroso-1-(3-pyridyl)-1-butanone (NNK). In order to investigate the extent of CYP2A13 genetic polymorphism in a French Caucasian population of 102 individuals, a screening for sequence variations in the 5'-untranslated and protein encoding regions of its gene was performed using a polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) strategy. Six polymorphisms in the coding region were identified, including two rare missense mutations (C474G or Asp158Glu, G967T or Val323Leu) and one nonsense mutation (Arg101Stop). This deleterious mutation, the most frequent (5%) in our population, presumably encodes a severely truncated protein. The influence of the nonsense mutation in lung cancer susceptibility was examined by PCR-SSCP using peripheral blood DNA from 204 cases of lung cancer and 201 controls. The CYP2A13*7 allele, which harbours the C301T mutation, was present in 2.0% of controls and 3.4% of cases. However, multivariate analysis showed an elevated risk for small cell lung cancer in subjects heterozygous for the null allele (odds ratio OR=9.9; 95% confidence interval CI=1.9-52.2). This increased risk was not linked to other histological types of lung cancer.


In order to clone the third subtype of human alpha-2 adrenergic receptor, polymerase chain reaction (PCR) was employed. Primer pairs corresponding to the conserved sequences in transmembrane domains III and VI were used to selectively amplify the adrenergic receptor genes of the alpha-2 type from human genomic DNA. Sequence data obtained from a clone PCRA2, 885 bps in length, revealed the presence of significant (80%) homology with the known alpha-2A and 2B subtypes of human in the transmembrane domains, whereas distinct variations were noted in the third cytoplasmic loop. When compared with a recently reported alpha-2 receptor gene of rat, essentially identical sequences were found in the transmembrane domains III-VI, except one amino acid in domain VI. As high as 65% homology was also found in the third cytoplasmic loop. We suggest this amplified fragment represents the partial gene of the third subtype of alpha-2 adrenergic receptor in human.

Two genes encoding thermostable xylanases, named xyn10A and xyn11A, from an alkalophilic Bacillus firmus were cloned and expressed in Escherichia coli. The E. coli harboring either gene showed clear zone with Congo red clearance assay on xylan plate. The Xyn10A and Xyn11A have molecular weights of 45 and 23 kDa, respectively, and both show activities on xylan-zymogram. The xyn10A encodes 396 amino acid residues and is very similar to an alkalophilic xylanase A from alkalophilic Bacillus halodurans. The Xyn11A contains 210 amino acid residues and only one amino acid different from an endo-[beta]-1,4-xylanase from B. halodurans. From alignment of the amino acid sequences with other xylanases, Xyn10A and Xyn11A belong to family 10 and 11 glycosyl hydrolases, respectively. Both show activities over the pH range of 4-11 at 37 [deg]C and over 80% activities at 70 [deg]C. Interestingly both still retain over 70% activities after 16 h preincubation at 62 [deg]C.


The ATM protein, which is mutated in the inherited disease ataxia telangiectasia (AT), is a key regulator of the cells' DNA damage response. AT cells also exhibit constitutive activation of transcriptional regulators such as p53, E2F, AP1, and NF[kappa]B. Inactivation of ATM may therefore alter the cells' transcriptional profile. ATM expression in HeLa cells was silenced with siRNA expressed from a plasmid based vector, generating a stable cell line, HeLaATM601. HeLaATM601 cells displayed minimal levels of ATM protein and had a 10-fold increase in sensitivity to ionizing radiation. DNA microarray analysis demonstrated that 35 genes were upregulated and five genes were downregulated in HeLaATM601 cells. Genes upregulated in the absence of ATM included interferon-response proteins, cell cycle regulators, integral membrane proteins, and adhesion and extracellular matrix proteins. Using real-time PCR, these genes were also upregulated in cells derived from AT patients. Inactivation of the ATM protein therefore has a significant impact on the transcriptional profile of the cell.


Inflammation, metastasis and ischemia are processes that require lymphocyte or leukocyte cell recognition and adherence to endothelial counter receptors such as ICAM-1. Mapping the sites of interaction of ICAM-1 with LFA-1, the receptor for ICAM-1 on lymphocytes, may lead to the design of novel inhibitors of inflammation or metastasis. To this end, recombinant soluble ICAM-1 cDNA was engineered into the baculovirus expression system, which is capable of expressing large amounts of proteins. These constructs were designed to contain a protein leader sequence...
so that the transfected insect cells would secrete the recombinant polypeptide into the culture media for ease of isolation. We engineered four constructs of ICAM-1 into the baculovirus system and obtained relatively high expression of two soluble forms of ICAM-1, a two domain and a five domain form. These truncated proteins were isolated and shown to promote adherence of HL-60 cells and Molt-4 cells. These recombinant soluble proteins also inhibited cell adherence to purified intact ICAM-1 isolated from K562 cells.


http://www.sciencedirect.com/science/article/B6WBK-4D5NSXM-5/2/9b49c6e5bb3e4431cbbc6bedddaa1e60

Domain II of the hepatitis C virus internal ribosome entry site is a major RNA structure involved in the viral mRNA translation. It comprises four different structural domains. We performed in vitro selection against the apical loop of the domain II and we identified RNA aptamers folding as an imperfect hairpin with an internal loop of interacting with the apical loop of the domain II. This RNA-RNA interaction creates apical loop-internal loop complex. The aptamer binds the target with an apparent Kd of 35 nM. In this study, the main structural elements of the target and the aptamer involved in the formation of the complex are characterized by mutation, deletion, and RNase probing analysis. We demonstrate that a complementary loop flanked by G,C rich upper and lower stems are crucial for such RNA-RNA interactions.


http://www.sciencedirect.com/science/article/B6WBK-4FB91BN-M/2/ccf5b647379397dd64549d4a7decb5fd

During reverse transcription of the human immunodeficiency virus type 1 (HIV-1), a 90- or 99-nucleotide long DNA flap is formed at the centre of the viral cDNA. The presence of a central DNA flap in lentiviral vectors improves transduction efficiency significantly. We analysed the stimulation of lentiviral vector transduction by a DNA flap present at ectopic positions in the viral cDNA. A HIV-1 vector containing the cPPT/CTS fragment immediately downstream of the 5'-LTR performed as well as the wild-type cPPT-vector. Cloning of the cPPT/CTS fragment in front of the 3'-LTR resulted as well in a vector with higher transduction efficiency than a vector without central flap. These results demonstrate that the position of the DNA flap is not essential for its function in the context of HIV-1-derived lentiviral vectors. This may have consequences for vector design and our understanding of the functioning of the HIV-1 DNA flap.


http://www.sciencedirect.com/science/article/B6WBK-490GY7F-18/2/e411cf7e545072b231bbc833f3923e09

Schistosoma mansoni, a human parasite that constitutes a major health problem in developing
countries, escapes from host defenses and survives in the human bloodstream. Here, we report the cloning of a S. mansoni ATP-diphosphohydrolase ortholog (SmATPDase1). Southern blots indicated that in S. mansoni it is a single-copy gene. RT-PCR revealed that SmATPDase1 is expressed in five stages of the parasite life cycle, namely cercaria, schistosomula, adults, eggs, and miracidia. Using confocal microscopy, SmATPDase1 protein was immunolocalized on the external surface in all stages, except eggs, being conspicuously present in adults. ATPDase, which is present on the outer surface of endothelial cells lining human blood vessels, has been implicated in thromboregulation by promoting ADP hydrolysis and inhibition of platelet aggregation. The presence of an ATPDase ortholog on the surface of S. mansoni suggests that the enzyme might play a role in the escape from host defenses that would involve platelet activation.


http://www.sciencedirect.com/science/article/B6WBK-49M059Y-3/2/666e08b7069c6435077c52315105736e

Recent works have shown that mechanical loading can alter the metabolic activity of chondrocytes cultured in 3D scaffolds. In this study we determined whether the stage of development of engineered cartilaginous constructs (expanded adult human articular chondrocytes/Polyactive foams) regulates the effect of dynamic compression on glycosaminoglycan (GAG) metabolism. Construct maturation depended on the culture time (3-14 days) and the donor (4 individuals). When dynamic compression was subsequently applied for 3 days, changes in GAG synthesized, accumulated, and released were significantly positively correlated to the GAG content of the constructs prior to loading, and resulted in stimulation of GAG formation only in the most developed tissues. Conversely, none of these changes were correlated with the expression of collagen type II mRNA, indicating that the response of chondrocytes to dynamic compression does not depend directly upon the stage of cell differentiation, but rather on the extracellular matrix surrounding the cells.


http://www.sciencedirect.com/science/article/B6WBK-4700Y3D-5/2/44b9b8fa761814e7010dd7a949e6756a

Histamine H1, H2, and H3 receptors are expressed by osteoblastic MC3T3-E1 (E1) cells derived from mouse calvaria. Expression of the osteoclast differentiation factor (ODF)/receptor activator of nuclear factor-[kappa]B ligand (RANKL) transcript was induced in E1 cells and bone marrow stromal cells (ST2). Histamine markedly increased the steady-state level of ODF/RANKL mRNA in a dose-dependent manner. The effect of histamine on expression of ODF/RANKL mRNA by E1 cells was transient, with a peak at 6 h. Western blot analysis revealed that histamine increased production of ODF/RANKL protein by E1 cells at 12 h. In cocultures of E1 cells and mouse bone marrow cells, histamine stimulated osteoclastogenesis in the presence of 1,25-dihydroxyvitamin D3 and this effect was blocked by preincubation with neutralizing antibody against ODF/RANKL. These results suggest that histamine regulates osteoclastogenesis, at least in part, through induction of ODF/RANKL expression by osteoblasts and bone marrow stromal cells.

http://www.sciencedirect.com/science/article/B6WBK-4698SG2-J/2/792a8ad3d4d2b10b76d73e1b112e72fc


http://www.sciencedirect.com/science/article/B6WBK-471W6P2-6/2/6278c8519a71177edf06e62aa7af6cc

The human pregnane X receptor (hPXR) plays a key role in the regulation of both drug metabolism and efflux by inducing the expression of CYP3A4 and MDR1 gene. Using reverse transcription-polymerase chain reaction (RT-PCR) analysis, we identified seven novel splicing variants of hPXR in tissue from a single human liver. The expression of hPXR-related transcripts in the liver samples of 15 Caucasian individuals was subsequently determined by RT-PCR assays. The pattern of expression levels of these transcripts varied among liver samples. These results suggest that the hPXR is expressed as several different transcripts in liver tissues, apparently due to alternative as well as defective gene splicing. Furthermore, because this study provides the possibility of interindividual differences in hPXR transcript profiles, these alternative splicings for hPXR may largely contribute to the interindividual variability in CYP3A4 and P-glycoprotein induction.


http://www.sciencedirect.com/science/article/B6WBK-485P765-B/2/d1189ab774d2ff86f64c3ef1ae31c4f

Sarco/endoplasmic reticulum-type calcium transport ATPases (SERCA enzymes) pump calcium ions from the cytosol into the endoplasmic reticulum. We report that in addition to the ubiquitously expressed SERCA2b isoform, a new splice variant of SERCA2 can be detected (SERCA2c) that arises from the inclusion of a short intronic sequence located between exons 20 and 21 of the SERCA2a isoform. Sequence analysis revealed classical splice donor and acceptor sites, as well as a branch-point site. Due to the presence in the new exon of an in-frame stop codon that is preceded by a 17 bp coding sequence, this mRNA potentially codes for a protein with a truncated C-terminus containing a short unique C-terminal peptide stretch. SERCA2c message was detected in epithelial, mesenchymal, and hematopoietic cell lines, as well as in primary human monocytes. Moreover, we found that during monocytic differentiation total SERCA2 ATPase expression is induced on the protein and mRNA level and that the novel SERCA2c messenger is also up-regulated during this process. These data indicate that the alternative splicing pattern of the 3' region of the SERCA2 primary transcript is more complex than that previously thought and that this enzyme may be involved in the process of monocyte differentiation.

Semicarbazide-sensitive amine oxidase (SSAO) activity in plasma is increased in diabetes, and in particular, in diabetic patients with vascular complications. It has been speculated that SSAO is involved in the development of such complications due to the production of cytotoxic compounds. In this work, we have induced diabetes in a previously described mouse-model, overexpressing SSAO in smooth muscle cells. SSAO activity was estimated as well as expression of the endogenous mouse gene and human transgene using real-time PCR. Diabetes induced an increase in SSAO activity in serum, kidney, and adipose tissue of transgenic animals. An inverse correlation between SSAO activity and mouse SSAO mRNA levels was observed in transgenic animals with diabetes. These results further support the suggestion of a negative feedback control of the SSAO gene expression. The increased SSAO activity in diabetes is most likely dependent on post-transcriptional modifications or activation of existing inactive enzyme molecules.


This study was conducted to identify plasma membrane Ca2+-transporting ATPases present in rat kidney. Characterization of the cDNAs of the plasma membrane Ca2+-ATPases revealed a family of proteins with regions of highly conserved amino acid sequence. To examine the extent of the diversity of rat renal plasma membrane Ca2+-ATPases, we used the polymerase chain reaction to detect additional gene products in rat kidney mRNA that shared these conserved regions. Sequences corresponding to three previously known rat plasma membrane Ca2+-ATPases were obtained. In addition, we found sequence corresponding to a new putative plasma membrane Ca2+-ATPase. Our results demonstrate that the rat kidney contains at least four different plasma membrane Ca2+-ATPases and the complexity of this multigene family is greater than previously thought.


A cDNA of the complete coding region of rat IL-10 was cloned and sequenced using RNA isolated from a cultured population of thoracic duct T-lymphocytes obtained from Trichinella spiralis infected animals. The OX8- OX22-T-helper cells were stimulated in vitro with Concanavalin A for 24 hours prior to harvest. Reverse transcription of cellular RNA was primed with oligo-dT followed by amplification of IL-10 specific cDNA by polymerase chain reaction with synthetic oligo nucleotide primers chosen from two highly conserved regions of mouse and human IL-10. The sequence of the coding region of the amplified, cloned rat IL-10 cDNA is 90% identical to the mouse and 82% identical to the human IL-10 cDNA coding regions.
Functional cDNA clones for human NK-2 receptor were isolated from human lung RNA using a polymerase chain reaction (PCR) based method (RACE-PCR). In this method the cDNA was isolated as 5' end and 3'-end fragments; the entire cDNA was obtained by RNA-PCR. The sequence derived was 398 amino acids in length encoding an open-reading frame that was highly homologous to both the bovine and rat NK-2 receptor. The entire human cDNA sequence was cloned into a mammalian expression vector and mRNA was synthesised by transcription. Applications of tachykinins caused membrane current responses in Xenopus oocytes injected with the synthesised mRNA. The most potent of the three tachykinin peptides tested was neurokinin A. We have screened a human cosmid library and isolated a clone which contains the entire NK-2 receptor gene. The gene contains five exons and we have determined the complete sequence of the exons and the intron-exon junctions.

We hypothesized that host antiviral genes induced by type I interferons might affect the natural course of severe acute respiratory syndrome (SARS). We analyzed single nucleotide polymorphisms (SNPs) of 2',5'-oligoadenylate synthetase 1 (OAS-1), myxovirus resistance-A (MxA), and double-stranded RNA-dependent protein kinase in 44 Vietnamese SARS patients with 103 controls. The G-allele of non-synonymous A/G SNP in exon 3 of OAS-1 gene showed association with SARS (p = 0.0090). The G-allele in exon 3 of OAS-1 and the one in exon 6 were in strong linkage disequilibrium and both of them were associated with SARS infection. The GG genotype and G-allele of G/T SNP at position -88 in the MxA gene promoter were found more frequently in hypoxemic group than in non-hypoxemic group of SARS (p = 0.0195). Our findings suggest that polymorphisms of two IFN-inducible genes OAS-1 and MxA might affect susceptibility to the disease and progression of SARS at each level.

We designed a polymerase chain reaction-based strategy to obtain information about the origin and distribution of a newly discovered proliferating cell nuclear antigen (PCNA) homolog. Carrot genomic segments were amplified using degenerate primers for two conserved regions of known PCNA homologs. The genes encoding the larger PCNA as well as typical PCNA contained introns. Thus, unlike processed PCNA pseudogenes in mammals, the larger homolog is not generated through reverse transcription of a typical PCNA mRNA. Moreover, introns of the larger
PCNA homolog were positioned at the characteristic sites in plant PCNA genes. Attempts to amplify cDNA for an additional PCNA homolog from mammalian cells have been unsuccessful. These results suggest that the larger PCNA homolog was generated, presumably through gene duplication, after the divergence of the and.


http://www.sciencedirect.com/science/article/B6WBK-46VBH5P-G/2/c3201088f2e17936204101be36bf188e

We have characterized the gene for human phosphodiesterase 8B, PDE8B, and cloned the full-length cDNA for human PDE8B (PDE8B1) and two splice variants (PDE8B2 and PDE8B3). The PDE8B gene is mapped to the long arm of chromosome 5 (5q13) and is composed of 22 exons spanning over ~200 kb. The donor and acceptor splice site sequences match the consensus sequences for the exon-intron boundaries of most eukaryotic genes. PDE8B1 encodes an 885 amino acid enzyme, containing an N-terminal REC domain, a PAS domain, and a C-terminal catalytic domain. PDE8B2 and PDE8B3 both have deletion in the PAS domain and encode 838 and 788 amino acid proteins, respectively. RT-PCR analysis revealed that while PDE8B1 is the most abundant variant in thyroid gland, PDE8B3, but not PDE8B1, is the most abundant form in brain. These findings suggest that selective usage of exons produces three different PDE8B variants that exhibit a tissue-specific expression pattern.


http://www.sciencedirect.com/science/article/B6WBK-4B721J3-1/2/6190db4f4600d4c3f6a5687d5ddfb05

The objective of the present study was to fabricate and use a bovine liver complementary DNA (cDNA) microarray to profile genome-wide gene expressions in the liver of cow throughout pregnancy. A cDNA library was prepared from liver total RNA collected from cows during estrous cycle and pregnancy, and from fetuses at different stages of pregnancy. The sequenced clones were compiled and annotated by basic local alignment search tool (BLASTn) and spotted onto glass slides. The annotated liver array represented 2675 genes. Of which, 1442 were known genes while 617 sequences had matches with sequences found in expressed sequence tags databases. In addition, 616 unknown sequences were found and these sequences may possibly be identified as candidates for novel bovine genes. For gene expression profiling studies, total RNA from livers of cows slaughtered on days 19, 27-28, 49-58, 150, and 245 of pregnancy (test RNAs) was separately reverse transcribed and labeled with either cyanine 5-fluorescent dye (Cy5) or Cy3. The test samples were individually compared with liver total RNA collected from nonpregnant cycling cows (control RNA) after reverse transcription and labeling with the opposite dye following a two-color hybridization method. After scanning, image acquisition, and normalization, genes that showed either more than 1.5-fold (test/control) induction or repression were selected for further analyses. Hierarchical clustering algorithm showed a clear induction of most liver genes on days 27-28 of pregnancy. Self-organizing maps algorithm identified groups of genes whose differential expression patterns were similar across pregnancy. In conclusion, we described fabrication of a bovine liver cDNA microarray, and demonstrate, for the first time, differential expression patterns of a large number of coregulated liver genes in parallel throughout pregnancy in the bovine.

http://www.sciencedirect.com/science/article/B6WBK-488VWWG-5/2/24d210f2e9ba4b62a7db97d3c3b870f1

Features characteristic to rheumatoid arthritis (RA) including synovial overgrowth and joint destruction are experimentally produced by augmenting c-fos gene expression. We show that cyclin dependent kinase inhibitor p21waf1/cip1, that inhibits cell proliferation, is down-regulated in conjunction with up-regulation of c-fos in the lymphocytes of patients with RA. As to the mechanism of down-regulation of p21waf1/cip1 gene expression, transfection studies in U937 cells showed that c-fos down-regulated phosphorylation and dimerization of signal transducers and activators of transcription (STAT) 1, thereby inhibiting interferon [gamma]-induced transactivation of p21waf1/cip1. Phosphorylation of STAT1 was indeed decreased in the lymphocytes of patients with RA. Thus, under overexpression of c-fos gene, c-Fos inactivates STAT1 to down-regulate p21waf1/cip1 gene expression in the lymphocytes of patients with RA, and in this way may enhance proliferation of lymphocytes.


http://www.sciencedirect.com/science/article/B6WBK-4FM3VXK-B/2/0a4cb51dc13edfbca43f8e184ec3a090

Adrenomedullin (AM) is postulated to exert organ-protective effects. It is expressed in the renal glomeruli, but its roles in the glomerular podocytes have been poorly elucidated. In the present study, we investigated the expression and regulation of AM in recently established conditionally immortalized mouse podocyte cell line in vitro and podocyte injury model in vivo. The cultured differentiated podocytes expressed AM mRNA and secreted measurable amount of AM. AM secretion from the podocytes was increased by H2O2, hypoxia, puromycin aminonucleoside (PAN), albumin overload, and TNF-[alpha]. Real-time RT-PCR analysis revealed that AM mRNA expression in the podocytes was enhanced by PAN and TNF-[alpha], both of which were suppressed by mitochondrial antioxidants. Furthermore, AM expression was upregulated in the glomerular podocytes of PAN nephrosis rats. These results indicated that AM expression in the podocytes was upregulated by stimuli or condition relevant to podocyte injury, suggesting its potential role in podocyte pathophysiology.


http://www.sciencedirect.com/science/article/B6WBK-490GY7F-16/2/d46c4540e7cf8b170b334dd1178b023c

CRTH2, the second receptor for prostaglandin D2 (PGD2), is thought to play a role in allergic inflammations through the induction of chemotactic migration and/or the activation of Th2, eosinophils, and basophils, in humans. We previously identified the mouse CRTH2 homolog of human CRTH2 and suggest that animal models would provide a clear understanding on the precise function of CRTH2 in allergic disorders. To this end we have confirmed that mouse
CRTH2 is similar in gene structure to human CRTH2 and revealed that mouse CRTH2 is predominantly expressed in the eosinophils derived from IL-5-transgenic mice. Moreover, mouse CRTH2 harbors the ability to bind PGD2 with high affinity and intracellular Ca2+ mobilization in a Gi-dependent manner and chemotactic responses in several transfected cell lines. The results demonstrated here indicate that mouse CRTH2 is the functional ortholog of human CRTH2 and paves the way for future analysis of the in vivo functions of CRTH2.


http://www.sciencedirect.com/science/article/B6WBK-4938JB7-4/2/facc18d926da26d0590f8c8be0cb1be4

The aim of this study was to gain insight into early events in the lung epithelial cells following acute Cd exposure. We adopted the polymerase chain reaction (PCR)-based subtraction technique and found several genes that were upregulated in immortalized rat lung type 2 epithelial cells (SV40T2). The upregulation of those genes was confirmed by Northern blot analysis and categorized into three groups (highly, moderately, and weakly inducible genes). Heme oxygenase-1 (HO-1), HSP 72, hepatic steroid hydroxylase/CYP1A2, and Cd-inducible gene 1 (cdig1, a new gene, Accession Nos. AB086233 and AB086234) were highly inducible genes, testosterone-repressed prostate message 2 mRNA was moderately inducible, and collagen-binding protein and cdig2 (another new gene, Accession No. AB086193) mRNAs were weakly inducible. The expression of cdig1 increased linearly with time up to 9 h, while that of HO-1 reached the maximum value at 4 h in response to 10 [mu]M Cd.


We have isolated and sequenced overlapping cDNA clones for rat KC*. The 0.93 kb cDNA has a single open reading frame of 288 nucleotides, and substantial sequence identity with the platelet-factor 4 family members mouse KC, hamster gro, and human gro. Using cloned cDNA as a probe, expression of KC mRNA in lavaged rat alveolar macrophages (AMs) increased after lipopolysaccharide (LPS) treatment. We also studied expression in vitro by a rat fetal lung fibroblast cell line, RFL-6. Expression of KC mRNA in RFL-6 cells increased after treatment with interleukin 1 or with conditioned medium from rat AMs treated with LPS.


http://www.sciencedirect.com/science/article/B6WBK-47F797V-4/2/da8d7b50cc805da77bdf93c34fb80d

NPHS1 encodes nephrin, the core protein of the interpodocyte slit diaphragm of the kidney glomerulus. NPHS1 is the causative gene for congenital nephrotic syndrome of the Finnish type
(CNF) with massive, treatment resistant proteinuria. We report here the establishment of a novel nephrin-like gene, NLG1 encoding filtrin, a protein with substantial homology to human nephrin. Filtrin is a type I transmembrane protein consisting of 708 amino acids. Together with the recently cloned NEPH1, NLG1 establishes a new nephrin-like subgroup of genes belonging to the immunoglobulin superfamily of cell adhesion molecules. The RNA dot blot experiment revealed that the NLG1 mRNA expression is widely distributed but most prominently observed in the pancreas and lymph nodes. The expression of NLG1 mRNA in kidney glomeruli was verified with RT-PCR. Further immunoblotting studies with antifiltrin antibody showed a specific band at 107 kDa in the human and rat glomeruli. In immunofluorescence microscopy specific staining of glomeruli but also proximal and distal parts of the nephron was seen in human kidney cortex. Due to its structural similarity and sequence homology as well as partially consistent expression pattern with nephrin we propose that filtrin belongs to a functionally important complex of proteins of the glomerular filtration barrier.


http://www.sciencedirect.com/science/article/B6WBK-4D2XF00-8/2/0550cd6b5f55efb262894e9cf9016896

The formation of DNA methylation patterns is one of the epigenetic events that underlie mammalian development. The Sphk1 CpG island is a target for tissue-dependent DNA methylation as well as a template for generating multiple subtypes. The number of mammalian non-coding RNA genes is rapidly expanding. In this study, we found endogenous antisense transcripts, Khps1 subtypes with different sizes (600-20,000 nt). A subtype, Khps1a, was a 1290-bp, non-coding, 5'-capped and 3'-polyadenylated RNA that originated from the CpG island and overlapped with a tissue-dependent differentially methylated region (T-DMR) of Sphk1. Intriguingly, overexpression of two fragments of Khps1 caused demethylation of CG sites in the T-DMR. Furthermore, this RNA-directed demethylation was associated with DNA methylation at three CC(A/T)GG sites in the T-DMR. The link between the RNA-directed CG demethylation and non-CG methylation provides a novel mechanism of epigenetic regulation and potential tool for epigenetic manipulation of mammalian cells.


http://www.sciencedirect.com/science/article/B6WBK-4F60JXD-6/2/11e2a99391f0eea705d4623cf0aa2edc

Toll-like receptors (TLRs) have been identified recently as crucial signaling receptors mediating the innate immune recognition. Though induction of TLR2 or TLR4 by 12-O-tetradecanoyl phorbol 13-acetate (TPA) in leukemia cells has been reported, however, the mechanism by which TPA up-regulates TLR2 or TLR4 remains poorly understood. In this study, we investigated the effect of TPA on induction of TLR2 in U937 cells. TPA markedly induced TLR2 mRNA and protein expressions. TLR2 expression in response to TPA was attenuated by pretreatments with GF109203X and Go6976 (inhibitors of protein kinase C (PKC)) and PD98059 (an inhibitor of extracellular signal-regulated kinases (ERKs)), but not SB203580 (an inhibitor of p38s) and SP600125 (an inhibitor of c-Jun N-terminal kinases), suggesting involvement of PKC and ERKs in this response. Moreover, TPA-induced PKC activation was linked to generation of reactive oxygen species, which were dispensable for TLR2 expression in U937 cells. Pretreatments with GF109203X blocked TPA-induced phosphorylation of ERKs, suggesting activation of ERKs by
PKC. In addition, TPA induced nuclear factor-[kappa]B (NF-[kappa]B) activation, which was shown by increased nuclear translocation of p65 NF-[kappa]B and degradation of I[kappa]B-[alpha], a NF-[kappa]B inhibitory protein. Importantly, TPA-induced TLR2 expression was inhibited by blockage of NF-[kappa]B activation using NF-[kappa]B inhibitors, including MG132 and BAY11-7085. Specifically, TPA-induced nuclear translocation of NF-[kappa]B was effectively attenuated by GF109203X and PD98059, suggesting PKC and ERK regulation of NF-[kappa]B nuclear localization in response to TPA. Together, these results suggest that TPA-induced TLR2 expression in U937 cells may be at least in part mediated through activation of PKC and ERKs as well as NF-[kappa]B transcription factor, and that cross-talk between PKC or ERKs and NF-[kappa]B may exist.


http://www.sciencedirect.com/science/article/B6WBK-4CXDD97-9/2/f9454b2ac1363bd2b03900e9f22ed448

Induction of human [beta]-defensin 2 (HBD-2) by interleukin-1[beta] (IL-1[beta]) in epithelial cells has been reported. However, the mechanism by which IL-1[beta] up-regulates HBD-2 remains poorly understood. In this study, we investigated the effect of IL-1[beta] on induction of HBD-2 in A549 cells. IL-1[beta] markedly increased HBD-2 mRNA expression in concentration- and time-dependent manners. HBD-2 mRNA expression in response to IL-1[beta] was attenuated by pretreatment of GF109203X, Go6976, and staurosporine [inhibitors of protein kinase C (PKC)], SB203580 [an inhibitor of p38 mitogen-activated protein kinase (MAPK)], SP600125 [an inhibitor of c-Jun N-terminal kinase (JNK)], and LY294002 [an inhibitor of phosphatidylinositol-3-kinase (PI3K)], but not PD98059 [an inhibitor of extracellular signal-regulated kinase (ERK)], suggesting involvement of PKC, p38 MAPK, JNK, and PI3K in this response. Interestingly, IL-1[beta] induced nuclear factor-[kappa]B (NF-[kappa]B) activation in A549 cells, which was shown by increased nuclear translocation of p65 NF-[kappa]B and degradation of I[kappa]B-[alpha]. Importantly, IL-1[beta]-induced nuclear translocation of p65 NF-[kappa]B was effectively attenuated by GF109203X and PD98059, suggesting PKC and ERK regulation of NF-[kappa]B nuclear localization in response to TPA. Together, these results suggest that IL-1[beta] induces HBD-2 mRNA expression in A549 cells through activation of NF-[kappa]B transcription factor as well as activation of signaling proteins of PKC, p38 MAPK, JNK, and PI3K, but not ERK.


http://www.sciencedirect.com/science/article/B6WBK-48D378G-6/2/be107447b9504293534b048a03fbbc121

Lipoprotein lipase (LPL), a key enzyme for triglyceride hydrolysis, is an insulin-dependent enzyme and mainly synthesized in white adipose tissue (WAT) and skeletal muscles (SM). To explore how pioglitazone, an enhancer of insulin action, affects LPL synthesis, we examined the effect of pioglitazone on LPL mRNA levels in WAT or SM of brown adipose tissue (BAT)-deficient mice, which develop insulin resistance and hypertriglyceridemia. Both LPL mRNA of WAT and SM were halved in BAT-deficient mice. Pioglitazone increased LPL mRNA in WAT by 8-fold, which was
substantially associated with a 4-fold increase of peroxisome proliferator activated receptor (PPAR)-[gamma] mRNA (r=0.97, p<0.0001), whereas pioglitazone did not affect LPL mRNA in SM. These results suggest that pioglitazone exclusively increases LPL production in WAT via stimulation of PPAR-[gamma] synthesis. Since pioglitazone does not affect LPL production in SM, this would contribute to prevent the development of insulin resistance due to lipotoxicity.


http://www.sciencedirect.com/science/article/B6WBK-4D75K9P-9/2/b93897dc39588c4860a822ad7b5edb26

We cloned cyclin B1, B2, and B3 cDNAs from the eel testis. Northern blot analysis indicated that these cyclin B mRNAs were expressed and increased from day 3 onward after the hormonal induction of spermatogenesis, and that cyclin B3 was most dominantly expressed during spermatogenesis. In situ hybridization showed that cyclin B1 and B2 were present from the spermatogonium stage to the spermatocyte stage. On the other hand, cyclin B3 mRNA was present only in spermatogonia. Although mouse cyclin B3 is expressed specifically in the early meiotic prophase, these results indicate that eel cyclin B3 expression is limited during spermatogenesis to spermatogonia, but is not present in spermatocytes. These facts together suggest that eel cyclin B3 is specifically involved in spermatogonial proliferation (mitosis), but not in meiosis.


http://www.sciencedirect.com/science/article/B6WBK-49M6MN8-D/2/861e69b4f66bcbde73a6ab9817f6e2c6d

Encephalomyocarditis (EMC) virus induces insulin-dependent diabetes and myocarditis in several strains of mice. The T-cell receptor (TCR) V[beta] genes of infiltrating T cells in the pancreas and myocardium of BALB/C mice infected with EMC virus D-variant (EMC-D virus) were analyzed. Using a nested two-step polymerase chain reaction (PCR), TCR V[beta] cDNAs were cloned and sequenced. Two and four kinds of TCR V[beta] clones were obtained from T cells infiltrating into the pancreas and myocardium of BALB/C mice infected with EMC-D virus, respectively. The infiltrating lymphocytes in the diabetic mice expressed V[beta] 8.1, 8.2, and 8.3 genes predominantly. Previously, the use of V[beta] 8.2 has been reported in autoimmune diseases such as murine experimental allergic encephalomyelitis (EAE) and non-obese diabetic (NOD) mouse. This study suggests that mice infected with EMC virus are a useful animal model for autoimmune diseases such as insulin-dependent diabetes.


http://www.sciencedirect.com/science/article/B6WBK-4C0V4YB-5/2/f10acc345a710cb8a3c2f3133f7a679c
Congenital heart disease (CHD) is a major clinical manifestation of Down syndrome (DS). We recently showed that chimeric mice containing a human chromosome 21 (Chr 21) exhibited phenotypic traits of DS, including CHD. Our previous study showed that myosin light chain-2a (mlc2a) expression was reduced in the hearts of chimeric mice and DS patients. We found that phosphatidylethanolamine binding protein (PEBP) was also downregulated in Chr 21 chimeras in this study. As mlc2a is involved in heart morphogenesis, and PEBP controls the proliferation and differentiation of different cell types, these genes are candidates for involvement in DS-CHD. The DS-CHD candidate region has been suggested to span between PFKL and D21S3, which is the STS marker near the ETS2 loci. To identify gene(s) or a gene cluster on Chr 21 responsible for the downregulation of mlc2a and PEBP, we fragmented Chr 21 at the EST2 loci, by telomere-directed chromosome truncation in homologous recombination-proficient chicken DT40 cells. The modified Chr 21 was transferred to mouse ES cells by microcell-mediated chromosome transfer (MMCT), via CHO cells. We used ES cell lines retaining the Chr 21 truncated at the ETS2 locus (Chr 21E) to produce chimeric mice and compared overall protein expression patterns in hearts of the chimeras containing the intact and the fragmented Chr 21 by two-dimensional electrophoresis. While mouse mlc2a and PEBP expression was downregulated in the chimeras containing the intact Chr 21, the expression was not affected in the Chr 21E chimeras. Therefore, we suggest that Chr 21 gene(s) distal from the ETS2 locus reduce mouse mlc2a and PEBP expression in DS model mice and DS. Thus, this chromosome engineering technology is a useful tool for identification or mapping of genes that contribute to the DS phenotypes.


http://www.sciencedirect.com/science/article/B6WBK-4BT1R50-2/2/195abf9e88b3ec269a52e23772f06120

Ginsenosides, the major active ingredients of ginseng, have a variety of biomedical efficacies such as anti-aging, anti-oxidation, and anti-inflammatory activities. To understand the effects of compound K (20-O-[beta]-glucopyranosyl-20(S)-protopanaxadiol), one of the major metabolites of ginsenosides, on the skin, we assessed the expression levels of about 100 transcripts in compound K-treated HaCaT cells using cDNA microarray analysis. One gene up-regulated by compound K was hyaluronan synthase 2 (HAS2). Semi-quantitative RT-PCR showed that compound K increased HAS2 mRNA in time- and dose-dependent manners. ELISA and immunocytochemistry using hyaluronan (HA)-binding protein showed that compound K effectively increased HA production in HaCaT cells. Finally, treatment of compound K on hairless mouse skin increased the amount of HA in the epidermis and papillary dermis. Our study suggests that topical application of compound K might prevent or improve the deteriorations, such as xerosis and wrinkles, partly ascribed to the age-dependent decrease of the HA content in human skin.


http://www.sciencedirect.com/science/article/B6WBK-4DMXDC6-1YB/2/3237e7a92382be373c228a58305c0351

In this study we have used a reverse transcription polymerase chain reaction (RT-PCR) to demonstrate that adult primary human osteoblasts and SaOS-2, a human osteosarcoma-derived cell line with osteoblastic properties, express cellular retinol-binding protein I (CRBP I), cellular retinoic acid-binding protein II (CRABP II), and very low levels of CRABP I. We also show that
CRABP II is expressed in the adult liver, which does not express CRABP I. The results suggest that CRABP II is the important isoform in the adult bone as well as in the adult liver. Since the 9-cis retinoic acid receptor (RXR) [alpha] previously has been shown to be expressed predominantly in the liver, CRABP II might be involved in the transport of 9-cis retinoic acid to its nuclear receptor.


http://www.sciencedirect.com/science/article/B6WBK-45WHRBK-N/2/d64d980f70c3e61fe9d78cd014220b95


http://www.sciencedirect.com/science/article/B6WBK-4DXRY44-201/2/6dbc663f6ac2683e3fda5b21fc3b13e

Recombination in a bovine papillomavirus shuttle vector carrying direct repeats of Moloney murine leukemia virus LTR sequence was examined. Differently from similar vectors carrying direct repeats of SV40 polyA addition signal or neomycin resistance gene, the vector exhibited no homologous recombination between the repeats. Instead, illegitimate recombination took place. There were two major types of recombination products from the restriction cleavage pattern. The plasmids in independent cellular clones belonging to the same recombination type shared the identical crossover point. Thus, in this plasmid, illegitimate recombination occurred at preferential sites involving exactly the same sequences.


http://www.sciencedirect.com/science/article/B6WBK-4DYVHVW-F6/2/ace038692e9b2fe7f2f73ba2a45466f0

An acidic variant of serum amyloid A (SAA) identified previously by isoelectrofocusing in a family of Turkish origin has been characterized at the genomic level. DNA sequence analysis revealed that individuals expressing the variant pI6.1/pI5.7 isoforms (the mother and three of four children) were heterozygous at the SAA1 gene locus. Their SAA1 gene sequences contained an adenine, as well as the usual guanine, at the position corresponding to the second base of codon 72. The presence of both bases predicts two SAA1 protein sequences, one having aspartic acid and the other glycine at position 72. While the Gly-72 SAA1 (+/- Arg-1) sequence represents the normal pI6.5/pI6.0 isoforms, the Asp-72 SAA1 (+/- Arg-1) sequence corresponds to the variant pI6.1/pI5.7 isoforms.

Gastric cancer remains the second leading cause of cancer deaths worldwide. Patients usually present late with local invasion or metastatic diseases. The present study investigated the expression level of liver-intestine cadherin (LI-cadherin) by RT-PCR and its correlation with clinicopathological data in 71 pairs of tumor and non-cancerous gastric mucosa. Protein expression level of LI-cadherin was determined by Western blotting and immunohistochemistry. The mRNA of LI-cadherin was highly expressed in tumor as compared to non-cancerous mucosa. Lymph node metastasis was significantly associated with the expression of LI-cadherin (p=0.038). On multivariate analysis, T staging and LI-cadherin expression were found to be independent factors associated with lymph node metastasis.


The gene organization of human TAPL (TAP-like, ABCB9) was determined. The TAPL gene consists of 12 exons including the first non-coding exon on human chromosome 12q23.34. Three alternative splicing variants of the 12th exon have been identified by 3'RACE using RNA from human cell lines and isolated lymphocytes. As expected from the similarity of the amino acid sequences of TAP1, TAP2, and TAPL, the intron insertion points in these three genes are essentially the same. However, the TAP2 and TAPL genes are closely related, since each has common non-coding exon and splicing isoforms. The novel splicing variants of TAPL termed 12B and 12C have shorter carboxyl terminal amino acid sequences than 12A, reportedly a conserved isoform in rodents and human. The proximal promoter region of the TAPL gene lacks a canonical TATA-box but contains several GC-box elements. The 60 bp upstream sequence containing two GC-boxes from the human TAPL transcriptional start site confers basal promoter activity.


A technique based on RNA-PCR was successfully employed for the detection of guanylate cyclase-A (GC-A) mRNA in the rat retina. Three sets of primers designed from the published cDNA sequence of rat brain guanylate cyclase-A (GC-A) produced amplification products of expected sizes from the retina as well as brain. Analysis of retinal PCR products yielded a 970 bp sequence, which showed 100% homology to the cDNA sequence of GC-A (2343-3312 bp region). Northern blot analysis was not very sensitive for the detection of GC-A mRNA in the retina. The results indicate that the mRNA for GC-A (or a closely related form) is probably expressed in the retina, but at a lower level than that found in the brain.

Lai, J.-Y., N. D. Borson, et al. (2004). "Mitosis increases levels of secretory leukocyte protease inhibitor in
Chronic wounds are a major health care burden. Multiple factors produced by healing wounds play important roles in efficient and orderly wound healing. Secretory leukocyte protease inhibitor (SLPI) is constitutively expressed in epithelial cells, and its expression is increased by inflammation. SLPI has antimicrobial activities and improves wound healing. We hypothesized that SLPI expression correlates with keratinocyte growth rate and is increased by epidermal growth factor (EGF). Keratinocytes were isolated from neonatal foreskin. Subconfluent and confluent culture conditions were used. SLPI-specific primers were designed for use in quantitative-competitive reverse-transcription polymerase chain reaction assays to detect and quantify SLPI mRNA expression levels. SLPI levels were increased 2-fold in subconfluent cultures and 1.6-fold in confluent cultures to which EGF had been added. Confluent cultures also demonstrated 7-fold (growth factor depleted) and 6-fold (EGF) increase in SLPI RNA copy number, compared to subconfluent cultures. The results indicate that EGF increases SLPI expression.


Southern hybridization suggests that the zebrafish genome contains multiple zinc-finger genes related to the putative mouse developmental genes, Krox-20 and Krox-24. The polymerase chain reaction was employed to amplify and clone the zinc-finger regions of genes related to Krox-20, from two fish species and, for comparison, mouse, hamster and fox. DNA sequence analyses suggest that the genes cloned include the guppy homologue of Krox-20 and the zebrafish homologue of Krox-24, and that these genes diverged prior to the separation of the lineages leading to teleosts and to mammals.

Lang, T., K. Ikejima, et al. (2004). "Leptin facilitates proliferation of hepatic stellate cells through up-regulation of platelet-derived growth factor receptor." Biochemical and Biophysical Research Communications 323(3): 1091.

In the present study, we investigated the effect of leptin on proliferation of hepatic stellate cells (HSCs) in vitro. Proliferation of 3-day cultured rat HSCs was assessed by incorporation of 5-bromo-2'-deoxyuridine (BrdU) into the nuclei. The percentages of BrdU-positive cells were increased in the presence of PDGF-BB (5 ng/ml) for 8 h as expected. Co-incubation with leptin (10-100 nM) potentiates this PDGF-dependent increase in BrdU positive cells in a dose-dependent manner. Messenger RNA for PDGF receptor [alpha] and [beta] subunits was increased almost 2- to 3-fold by incubation with leptin for 6 h. Further, pre-incubation with leptin for 6 h enhanced PDGF-induced increases in phospho-p44/42 MAP kinase and phospho-Akt levels in a dose-dependent manner. In the same condition, however, leptin per se did not increase phospho-STAT 3 and phospho-p44/42 MAP kinase levels. Instead, leptin increased phospho-Akt levels in HSCs within 30 min, suggesting that the phosphatidylinositol 3 kinase
(PI3K)/Akt pathway is involved in the mechanism by which leptin accelerates the proliferation of HSCs. In conclusion, the present study clearly indicated that leptin potentiates PDGF-dependent proliferative responses of HSCs in vitro.


http://www.sciencedirect.com/science/article/B6WBK-47T8BCF-5/2/b557cb91e00d036f8d86a81cbabb65d2

In most mammalian species, serum amyloid A isoform 3 (SAA3) appears to be the predominant SAA isoform expressed extrahepatically. However, human SAA3 gene expression has not been detected previously and, therefore, this gene was referred to as a pseudogene. We report for the first time the transcriptional expression of human SAA3. Human SAA3 gene expression was detected by RT-PCR after stimulation of mammary gland epithelial cells with either prolactin (PRL) or lipopolysaccharide (LPS). The full-length 655 bp cDNA sequence for this mammary-associated serum amyloid A3 (M-SAA3) was obtained using 5' and 3' rapid amplification of cDNA ends (RACE). The human M-SAA3 transcript would conceptually translate into a 42 residue mature protein, which is smaller than other mammalian SAA3 isoforms that are typically 104-113 amino acids in length. This study defines the cDNA sequence for human SAA3 and also demonstrates the upregulation of M-SAA3 expression in response to the lactational hormone PRL or to an acute phase stimulant such as LPS.


http://www.sciencedirect.com/science/article/B6WBK-496FN2B-1/2/ab0ac686f66ab205f8e54c6c15a29c00

By means of computational methods, we identified an uncharacterized human transcript, Chromosome 1 open reading frame 36 (C1orf36), that is expressed in the retina and that maps to 1q32.3. The cDNA contains an open reading frame of 585 bp that encodes a 195-aminoacid protein with a predicted mass of 22.7 kDa. An alternatively spliced transcript in a retinoblastoma cell line, encoding for a truncated peptide, was also identified. PCR experiments performed using human cDNA from several sources indicate that C1orf36 has a preferential expression in the retina. Accordingly, in situ hybridization experiments, performed using as probe a murine C1orf36 cDNA fragment, detected a hybridization signal on mouse retinal adult sections. The C1orf36 protein shares homology with putative proteins in Mus musculus and Fugu rubripes, suggesting evolutionary conservation of its function. Additional sequence analysis of the C1orf36 gene product predicts its subcellular mitochondrial localization and the presence of both evolutionary conserved phosphorylation sites and regions adopting a coiled-coil conformation. We also defined the genomic structure of the gene. This enabled us to perform a mutational analysis of the C1orf36 coding region of about 300 patients affected by retinitis pigmentosa. No pathological mutations were detected in this analysis.

In order to gain insight into the expression profiles of the earthworm midgut, we analyzed 1106 expressed sequence tags (ESTs) derived from the earthworm midgut cDNA library. Among the 1106 ESTs analyzed, 557 (50.4%) ESTs showed significant similarity to known genes and represented 229 unique genes of which 166 ESTs were singletons and 63 ESTs manifest as two or more ESTs. While 552 ESTs (49.9%) were sequenced only once, 230 ESTs (20.8%) appeared two to five times and 324 ESTs (29.3%) were sequenced more than five times. Considering this redundancy of expression, it is likely that the gene expression profile of the earthworm midgut would be polarized. The expression of globin-related proteins, including ferritin and linker chain, and fibrinolytic enzymes appeared to account for 10.1% and 4.7% of the total ESTs analyzed in this study, respectively. This suggests that the prime functions of the midgut in the earthworm would be associated with protein hydrolysis as well as globin formation. Among the recognized protein-coding genes, the gene category involved in protein synthesis appeared to be the largest one accounting for 15.6% of the expression in the midgut, followed by gene categories associated with energy (11.2%), homeostasis (10.8%), metabolism (3.6%), cytoskeleton (2.5%), and protein fate (1.4%). With regard to functional aspects, the most abundantly expressed genes were associated with respiratory pigment (10.1%), cellular respiration (8.6%), and fibrin hydrolysis (4.7%). In addition, we were able to identify novel ESTs in the earthworm, which were related to the innate immune system, including destabilase, a possible antagonist of transglutaminase.


Dipeptidyl peptidase-IV (DPP-IV) regulates metabolism by degrading incretins involved in nutritional regulation. Metformin and pioglitazone improve insulin sensitivity whereas glyburide promotes insulin secretion. Zucker diabetic rats were treated with these antidiabetic agents for 2 weeks and DPP-IV activity and expression were determined. Serum DPP-IV activity increased whereas tissue activity decreased as the rats aged. Treatment of rats with metformin, pioglitazone, and glyburide did not alter DPP-IV mRNA expression in liver or kidney. Metformin and pioglitazone significantly (P r = 0.92) and glucagon-like peptide-1 levels (r = -0.49). Metformin, pioglitazone, and glyburide had no effect on serum DPP-IV activity in vitro, indicating these are not competitive DPP-IV inhibitors. We propose the in vivo inhibitory effects observed with metformin and pioglitazone on serum DPP-IV activity results from reduced DPP-IV secretion.


The molecular mechanism regulating spermatogenesis at different developmental stages remains largely unknown. In a vitamin A-deficiency (VAD) rat model, five distinct histologically defined, stage-synchronized testes: (i) resting spermatagonia and preleptotene spermatocytes at Day 0 of
post-vitamin A treatment (PVA); (ii) early pachytene spermatocytes at Day 7 PVA; (iii) late pachytene at Day 15 PVA; (iv) round spermatids at Day 25 PVA; and (v) elongated spermatids at Day 35 PVA were used to study gene expression profiles by mRNA differential display. Twenty-four differentially expressed cDNA fragments were identified and cloned; oligonucleotide sequence analyses indicated that there are 12 novel gene sequences, half of which share no apparent match in current GenBank/EMBL databases. Other 12 VAD clones share sequence homology to membrane channel and transport, transcription and translation, cell cycle and morphogenesis, inducer and transducer, surface or secreted glycoproteins or enzymes, and other miscellaneous molecules. Semi-quantitative RT-PCR analyses against different stages of VAD testes demonstrated: (i) restricted expression of VAD1.2 and 1.3 (novel) on Day 25 PVA when round spermatids form; (ii) escalating pattern of VAD12 (Cx43) in Sertoli cells; and (iii) relative constant levels of VAD4 (A5D3), VAD26.1 (ribonuclease), and VAD27 (GRP8) in spermatogenesis.


http://www.sciencedirect.com/science/article/B6WBK-48WJNMK-1/2/85c77e197817e162dbb9ef68f818f3e65c

The present study examined the existence of the adenosine A1, A2A, and A2B receptors and the effect of receptor activation on cAMP accumulation and protein phosphorylation in primary rat skeletal muscle cells. Presence of mRNA and protein for all three receptors was demonstrated in both cultured and adult rat skeletal muscle. NECA (10-9-10-4 M) increased the cAMP concentration in cultured muscle cells with an EC50 of (95% confidence interval)=15 (5.9-25.1) [μM], whereas CGS 21680 (10-9-10-4 M) had no effect on cAMP accumulation. Concentrations of [R]-PIA below 10-6 M had no effect on cAMP accumulation induced by either isoproterenol or forskolin. NECA resulted in phosphorylation of CREB with an EC50 of (95% confidence interval)=1.7 (0.40-7.02) [μM], whereas ERK1/2 and p38 phosphorylation was unchanged. The results show that, although the A1, A2A, and A2B receptors are all present in skeletal muscle cells, the effect of adenosine on adenylyl cyclase activation and phosphorylation of CREB is mainly mediated via the adenosine A2B receptor.


http://www.sciencedirect.com/science/article/B6WBK-45NSFW1-12/2/dca757c5427b47c9a4c66bb108ab9b


http://www.sciencedirect.com/science/article/B6WBK-47GHMY9-1/2/fe94cc9467728600e11046e8ae57b9f

Vasoactive intestinal contractor (VIC) is a member of the endothelin (ET) family. We have
investigated the regional distribution of VIC/ET-2 and of ET-1 gene expression in the adult murine brain and pituitary gland. We used real-time quantitative reverse transcription-linked polymerase chain reaction. VIC/ET-2 gene expression was observed at high levels in the pituitary gland and medulla oblongata in both the mouse and rat. Moderate to low levels of expression were observed in other brain regions. On the contrary, ET-1 gene expression was quite low in the pituitary gland in comparison with the levels observed in the cerebral cortex, striatum, and midbrain. Cold injury to the mouse cerebral cortex caused a significant decrease in VIC/ET-2 gene expression in this structure, whilst expression of the ET-1 gene was increased. These results suggest that VIC/ET-2 may have certain physiological roles that differ from those of ET-1 in the brain and pituitary gland.


http://www.sciencedirect.com/science/article/B6WBK-4F03B28-NX/2/f06a9de8d7bda686449e098a9ccab4ad

A new method is presented to extract and identify specific DNA fragments from well preserved human bones, dating from three different time periods. Bone samples were thoroughly freed from surficial contaminating DNA. Access to the inner bone spongiosum was achieved by removing the covering bone layers of the vertebra or sternum, whereas the patella, tibia and caput of the femur or humerus were cleaved with an iron saw. After the spongiosum was taken out, extraction of nucleic acids from this "sand" like material was performed by heating at 94 [deg]C during 20 min in a buffer containing essentially minor concentrations of detergent, chelating and reducing agents. The extracts were used in various polymerase Chain Reaction (PCR) protocols to amplify different human specific DNA fragments (originating from chromosomes X and 12). From 15 out of 20 bone samples humanspecific gene fragments could thus be identified.


http://www.sciencedirect.com/science/article/B6WBK-4DFT24B-12/2/b9e7b8f11c266ae4db6b96036d23515f

Degenerate primers were designed from the conserved zone of hydA structural gene encoding for catalytic subunit of [Fe]-hydrogenase of different hydrogen producing bacteria. A 750 bp of PCR product was amplified by using the above-mentioned degenerate primers and genomic DNA of Enterobacter cloacae IIT-BT 08 as template. The amplified PCR product was cloned and sequenced. The sequence showed the presence of an ORF of 450 bp with significant similarity (40%) with C-terminal end of the conserved zone (H-cluster) of [Fe]- hydrogenase. hydA ORF was then amplified and cloned in-frame with GST in pGEX4T-1 and overexpressed in a non-hydrogen producing Escherichia coli BL-21 to produce a GST-fusion protein of a calculated molecular mass of about 42.1 kDa. Recombinant protein was purified and specifically recognized by anti-GST monoclonal antibody through Western blot. Southern hybridization confirmed the presence of this gene in E. cloacae IIT-BT 08 genome. In vitro hydrogenase assay with the overexpressed hydrogenase enzyme showed that it is catalytically active upon anaerobic adaptation. In vivo hydrogenase assay confirmed the presence of H2 gas in the gas mixture obtained from the batch culture of recombinant E. coli BL-21. A tentative molecular mechanism has been proposed about the transfer of electron from electron donor to H-cluster without the mediation of the F-cluster.

http://www.sciencedirect.com/science/article/B6WBK-4F03B28-1J5/2/1985f42488d1c113c8d7a0d48db70048

The gene for steroid 18-hydroxylase (P-450C18) has been recently assigned to encode corticosterone methyl oxidases Type I and Type II which were previously postulated to catalyze the final two steps in the biosynthesis of aldosterone in humans. Molecular genetic analysis of the P-450C18 gene in three patients from three different families affected with CMO II deficiency has indicated that a point mutation of (181Arg -> Trp) in exon 3 and one of (386Val -> Ala) in exon 7 occur exclusively in the gene of the patients. Analysis of PCR products by restriction enzymes (Hapll and Hphl) has indicated that the patients are homozygous and the unaffected parent is heterozygous for both mutations, in accordance with the established concept that CMO II deficiency is inherited in an autosomal recessive manner. These data clearly provide the molecular genetic basis for the characteristic biochemical phenotype of CMO II clinical variants.


http://www.sciencedirect.com/science/article/B6WBK-4FB91BN-2/2/51af3038e2ad52e6cf836b11a05f9262

A new category of methicillin-resistant Staphylococcus aureus (MRSA), called community-acquired MRSA (CA-MRSA), has emerged worldwide. In contrast to previous MRSA, most CA-MRSA carries the Panton-Valentine leukocidin (PVL) genes (lukPVSF) as a virulence genetic trait. Sequence analysis of the lukPVSF gene of a Japanese isolate demonstrated that the gene has more similarity to methicillin-susceptible S. aureus from France than MRSA from the United States. Based on the sequences, we developed a real-time PCR assay for the three key genes of CA-MRSA; that is, lukPVSF, mecA (for methicillin resistance), and spa (for S. aureus). Dual or triple assay for lukPVSF, mecA, and spa in one test tube became possible. The detection limit of the assay with probe and SYBR Green methods was between 2.7 and 2.7 X 101 CFU/ml. The assay detected PVL-positive MRSA in clinical (blood) isolates.


Three mRNA species for human muscle phosphofructokinase containing heterogeneous 5' untranslated sequences were identified through cDNA cloning. Type A mRNA was essentially the same as that reported previously ([2]). Type B mRNA was considered to be the major gene product, which contained an extra non-coding sequence within the 5' untranslated region of type A mRNA. Amplification of mRNA by polymerase chain reaction revealed that types A and B mRNAs shared a common precursor RNA, and were alternatively spliced. Type C mRNA, homologous to the cDNA sequence from a placenta library ([4]), was considered to be under the
control of an alternative promotor.


A preadipocyte clonal line has been established from porcine subcutaneous tissue. This line, designated PSPA, showed a fibroblastic phenotype and kept on growing under a preadipose condition even after reaching confluence. When confluent cultures were stimulated with insulin, dexamethasone, biotin, pantothenate, and octanoate, growth was arrested, and the cells exhibited a marked increase in lipogenesis. However, adipose conversion was not induced upon exposure of PSPA cells to a standard hormonal mixture of mouse 3T3-L1 cells, and they continued dividing as did the preadipocytes in growth medium. By serially omitting each individual adipogenic agent from the PSPA differentiation medium, it was determined that octanoate was one of the most essential but the only factor able to induce growth arrest. Octanoate supplementation to 3T3-L1 medium increased the triglyceride accumulation of PSPA cells accompanied by growth arrest. Both RT-PCR and Western blot analysis supported the idea of octanoate as a potential agent with the antiproliferative activity requisite for porcine preadipocytes to enter terminal differentiation.


We have analyzed the exon 9, 13, 14, 15, and 16 of cardiac [beta] myosin heavy chain gene in 96 Japanese patients with hypertrophic cardiomyopathy by using PCR-DNA conformation polymorphism analysis. The analysis revealed a sequence variation of the exon 16 in one patient. The sequence variation of a G to C transversion with replacement of Asn by Lys at the codon 615 was confirmed by sequencing and by dot-blot hybridization with an allele-specific oligonucleotide probe. Because the missense mutation was found at the residue conserved through birds to humans, this mutation was suggested to be a cause of hypertrophic cardiomyopathy in the patient. This is the first report of a mutant cardiac [beta] myosin heavy chain gene in the Japanese population.


Human T-cell leukemia virus type 1 (HTLV-1) is etiologically linked with HTLV-1-associated diseases. HTLV-1 proviral load is higher in persons with adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis than in asymptomatic carriers. However there are little data available on the factors controlling HTLV-1 proviral load in carriers. To study the
effect of genetic background on HTLV-1 proviral load, we employed a mouse model of HTLV-1 infection that we had established. Here we analyzed nine strains of mice and found there is a great variation of proviral load among mouse strains that is not necessarily dependent on major histocompatibility complex. The antibody response is also different among these strains. To our knowledge, this is the first demonstration of the importance of the genetic background other than major histocompatibility complex controlling the HTLV-1 proviral load.


http://www.sciencedirect.com/science/article/B6WBK-46FPR19-10/2/b37c85ed6c9bff0f6474c639cc965e6


http://www.sciencedirect.com/science/article/B6WBK-464P997-5/2/6840ce99102fd375bd66d63e50f05b


http://www.sciencedirect.com/science/article/B6WBK-46RN7B4-F/2/4604f269b7c100eb0b60503d7ec39d01

Hypervariable segments of mitochondrial DNA (mtDNA) (HV1 and HV2) were analyzed in Klinefelter's syndrome and compared to normal population data. One pair of samples consisting of a Japanese mother and affected son with Klinefelter's syndrome (involved in a criminal case), and seven unrelated DNA samples from Caucasian Klinefelter males (two involved in criminal cases and five diagnosed) were collected in Japan and the United States. The diagnosis of Klinefelter's syndrome was established previously by multiplex XY-STR typing detecting two X alleles and one Y allele in the samples. Haplotype analysis of the mtDNA sequence in Klinefelter males was found to be identical, unique, and specific, as it was not found in the normal population. Astonishingly, family data exhibited that the haplotype of the mtDNA in the son was apparently different from the mother's, suggesting that the mtDNA of Klinefelter male would not be inherited from mother to son. Our data indicate that possible interaction of the sex chromosome and the mtDNA exists, and suggests that the specific mtDNA haplotype could cause the abnormal cell to fertilize and reproduce itself.


http://www.sciencedirect.com/science/article/B6WBK-4FHJF00-6/2/98c53cda45d86613e44598e8f1d38814
Efficient regulation of transgene would greatly facilitate the analysis of gene function in biological systems for basic research and clinical applications. The tetracycline-regulatable system (TRS) has proven to be a promising tool for such purposes. Despite their widespread application, a number of challenges are still associated with the use of TRS, including clonal variability in the regulation and copy number. We have recently constructed a novel human artificial chromosome (HAC) called 21[Δ]qHAC. By housing a TRS-based DNA-PKcs expression cassette in this HAC, we were able to circumvent the problems associated with conventional TRS-based vectors. We achieved tight control of DNA-PKcs expression and rescued the radiosensitive phenotype of DNA-PKcs-deficient CHO cells. The combined use of HAC and the TRS serves as a model for controllable and fixed copy number expression vectors. Our study also demonstrates the suitability of the HAC to accommodate multi-subunit constructs such as that of the TRS.


In a previous work, we have reported that the ionic nature of the outward current recorded in MCF-7 cells was that of a K+ current. In this study, we have identified a Ca2+-activated K+ channel not yet described in MCF-7 human breast cancer cells. In cells arrested in the early G1 (depolarized cells), increasing [Ca2+]i induced both a shift in the I-V curve toward more negative potentials and an increase in current amplitude at negative and more at positive potential. Currents were inhibited by r-iberiotoxin (r-IbTX, 50 nM) and charybdotoxin (ChTX, 50 nM). These data indicate that human breast cancer cells express large-conductance Ca2+-activated K+ (BK) channels. BK current-density increased in cells synchronized at the end of G1, as compared with those in the early G1 phase. This increased current-density paralleled the enhancement in BK mRNA levels. Blocking BK channels with r-IbTX, ChTX or both induced a slight depolarization in cells arrested in the early G1, late G1, and S phases and accumulated cells in the S phase, but failed to induce cell proliferation. Thus, the expression of the BK channels was cell-cycle-dependent and seems to contribute more to the S phase than to the G1 phase. However, these K+ channels did not regulate the cell proliferation because of their minor role in the membrane potential.


Genetic impairment was revealed in idiopathic cardiomyopathy and the responsible DNA locus was estimated. Mitochondrial DNA were amplified from autopsied cardiac specimens from three patients who died from hypertrophic or dilated cardiomyopathy by using polymerase chain reaction (PCR). By using two novel methods for PCR gene amplification, the pleioplasmic existence of multiple populations of differently deleted mitochondrial DNA in all specimens from the patients was confirmed. Mitochondrial DNA with a 7,436 bp deletion which commonly existed among the specimens was sequenced and the direct repeat at each edge of deletion was identified as (CATCAACAAACC) which was located in ATPase 6 gene and in the D-loop region. From our results mitochondrial DNA mutations could also be an important contributory factor to cardiomyopathy.
Inflammatory bone diseases are characterized by the presence of pro-inflammatory cytokines that regulate bone turnover. Osteoprotegerin (OPG) is a soluble osteoblast-derived protein that influences bone resorption by inhibiting osteoclast differentiation and activation. In the present study, we demonstrate that interleukin-1[beta] and tumor necrosis factor alpha induce OPG mRNA production and OPG secretion by osteoblast-like MG-63 cells. Maximum induction of OPG secretion by either cytokine requires activation of the p38 mitogen activated protein kinase (MAPK) pathway but neither the p42/p44 (ERK) nor the c-Jun N-terminal MAPK pathways. Induction of OPG mRNA by either cytokine is also p38 MAPK dependent. Taken together, these data indicate that cytokine-induced OPG gene expression and protein secretion are differentially regulated by specific MAP kinase signal transduction pathways.

HP (2-20) is a peptide derived from the N-terminus of Helicobacter pylori ribosomal protein L1 that has been shown to have antimicrobial activity against various species of bacteria. When we tested the effects of HP (2-20), we found that this peptide displayed strong activity against pathogens from a patient with gallstones, but it did not have hemolytic activity against human erythrocytes. We also found that HP (2-20) had potent activity against cefazolin sodium-resistant bacterial cell lines, and that HP (2-20) and cefazolin sodium had synergistic effects against cell lines resistant to the latter. To investigate the mechanism of action of HP (2-20), we performed fluorescence activated flow cytometry using pathogens from the patient with gallstones. As determined by propidium iodide (PI) staining, pathogenic bacteria treated with HP (2-20) showed higher fluorescence intensity than untreated cells, similar to melittin-treated cells, and that HP (2-20) acted in an energy- and salt-dependent manner. Scanning electron microscopy showed that HP (2-20) caused significant morphological alterations in the cell surface of pathogens from the patient with gallstones. By determining their 16S rDNA sequences, we found that both the pathogens from the patient with gallstones and the cefazolin sodium-resistant cell lines showed 100% homology with sequences from Pseudomonas aeruginosa. Taken together, these results suggest that HP (2-20) has antibiotic activity and that it may be used as a lead drug for the treatment of acquired pathogens from patients with gallstones and antibiotic-resistant cell lines.
Insulin gene transcription is critical for the maintenance of pancreatic [beta]-cell differentiation and insulin production. In this study, we found that the basic helix-loop-helix transcription factor Mad, which usually acts as a repressor to c-Myc, enhances insulin gene transcription. In isolated rat islets adenoviral overexpression of Mad augmented insulin mRNA expression and insulin protein content, as well as glucokinase and GLUT2 mRNA expression. Also, Mad overexpression upregulated insulin promoter activity in [beta]-cell-derived cell lines, MIN6 and [beta]TC1, as well as in non-insulin producing liver cell line, HepG2. Mad overexpression in rat islets enhanced PDX-1 expression and its DNA binding activity. We found that Mad mediated increased PDX-1 expression by an E-box dependent transcriptional regulation of the PDX-1 gene. That the effects of Mad on insulin expression were mediated through PDX-1 was further substantiated by studies showing inhibition of insulin promoter activation by Mad in the presence of mutated PDX-1 binding site. Although Mad functions as a negative regulatory factor for multiple target genes, these studies establish the fact that Mad can also function as a positive regulatory factor for insulin gene transcription. Such regulation of insulin expression by Mad with modulation of PDX-1 expression and DNA binding activity could offer useful therapeutic and/or experimental tools to promote insulin production in appropriate cell types.


http://www.sciencedirect.com/science/article/B6WBK-45NSFW1-2G/2/381215b614bc7970bd72eb6324ff87b5


http://www.sciencedirect.com/science/article/B6WBK-4DXKBG4-192/2/f9de18cd111f88b3b145b767f5df121b

Pulsed field gel electrophoresis (PFGE) allows separation of large restriction fragments from bacterial genome. Restriction fragments obtained by digestion of Staphylococcus aureus DNA with rare cutting enzymes (Sma I, and Csp I) were separated by PFGE. To arrange the physical order of the fragments generated by digestion with one enzyme, probes were prepared by nonspecific priming and polymerase chain reaction (PCR), using individual fragments of the other enzymatic digest as a template. Probes were then used for Southern hybridization to the PFGE separated fragment distribution of the two infrequent cleaving enzymes (Sma I and Csp I). Using probes generated from four Sma I fragments and five Csp I fragments as individual templates, a partial physical order of Csp I fragments of the genome of S. aureus ISP8 has been determined in relation to a previously published Sma I map of S. aureus genome.


http://www.sciencedirect.com/science/article/B6WBK-4D2FHTM-B/2/2ac2dfdaebbb020008ee264398edffb9

The human transcriptome is constituted of a great majority of intron-containing and a minority of
intron-lacking mRNAs; given the different processing these transcripts undergo, they are expected to carry, intermingled with coding properties, very different editing information. Here we applied a computational approach to compare intronless and intron-containing coding sequences. Hexamer composition comparison allowed the definition of over- and under-represented motifs in intronless genes; surprisingly, experimental testing revealed that intron-lacking coding sequences are enriched rather than depleted in elements with splicing enhancement ability. Similarly, we show evidence that intronless transcripts display a significantly higher frequency of both shuttling and non-shuttling SR protein binding sites compared to intron-containing sequences. These observations suggest that SR proteins (and possibly other splicing factors) play a role in cellular processes distinct from splicing.


http://www.sciencedirect.com/science/article/B6WBK-49CMF9N-5/2/306ee4bf6b00a92c2dc5b8558c413bb9

Single nucleotide polymorphisms (SNPs) are linked to phenotypes associated with diseases and drug responses. Many techniques are now available to identify and quantify such SNPs in DNA or RNA pools, although the information on the latter is limited. The majority of these methodologies require prior knowledge of target sequences, normally obtained through DNA sequencing. Direct quantitation of SNPs from DNA sequencing raw data will save time and money for large amount sample analysis. A high throughput DNA sequencing assay, in combination with a SNP quantitative algorithm, was developed for the quantitation of a SNP present in HCV RNA sequences. For a side-by-side comparison, a Pyrosequencing assay was also developed. Quantitation performance was evaluated for both methods. The direct DNA sequencing quantitation method was shown to be more linear, accurate, sensitive, and reproducible than the Pyrosequencing method for the quantitation of the SNP present in HCV RNA molecules.


http://www.sciencedirect.com/science/article/B6WBK-4DFT24B-11/2/df1e7e06b7a0c9d09b5a08a02a2c45f3

The ergosterol pathway in fungal pathogens is an attractive antimicrobial target because it is unique from the major sterol (cholesterol) producing pathway in humans. Lanosterol 14[alpha]-demethylase is the target for a major class of antifungals, the azoles. In this study we have isolated the gene for this enzyme from Cryptococcus neoformans. The gene, ERG11, was recovered using degenerate PCR with primers designed with a novel algorithm called CODEHOP. Sequence analysis of Erg11p identified a highly conserved region typical of the cytochrome P450 class of mono-oxygenases. The gene was present in single copy in the genome and mapped to one end of the largest chromosome. Comparison of the protein sequence to a number of major human fungal pathogen Erg11p homologs revealed that the C. neoformans protein was highly conserved, and most closely related to the Erg11p homologs from other basidiomycetes. Functional studies demonstrated that the gene could complement a Saccharomyces cerevisiae erg11 mutant, which confirmed the identity of the C. neoformans gene.

http://www.sciencedirect.com/science/article/B6WBK-4BY3VD5-5/2/3b11ec6ad6a05a32b72fdc6bd6fb5d11

The BP8 variant of the 5L rat hepatoma cell line is completely devoid of aryl hydrocarbon receptor (AHR) and is a useful model to examine AHR function. Previous studies showed that BP8 cells, when transfected with mouse AHR, exhibit induction of a plasmid-based reporter even in the absence of exogenous ligands. We transfected BP8 cells with full-length human AHR and found that presence of the AHR alone was sufficient to induce substantial CYP1A1 and CYP1B1 mRNA without any exogenous AHR ligand. An AHR antagonist, 3,4-dimethoxyflavone, inhibited CYP1A1 and CYP1B1 expression in a dose-dependent manner. When we transfected BP8 cells with a mutated human AHR that is defective in ligand binding, expression of CYP1A1 and CYP1B1 was diminished but not abolished. Inhibition by the AHR antagonist along with the diminished response to the mutated AHR indicates that BP8 cells contain some agent that acts as an agonist ligand for the AHR.


http://www.sciencedirect.com/science/article/B6WBK-466TBJB-4/2/b3ffaad9f86e4f4871e6cb90ba6785be


http://www.sciencedirect.com/science/article/B6WBK-4F31M8Y-B/2/ado70c9a59a862c72870be4ad256d394

The frequency of p73 mutation is low in hematologic malignancies as well as solid tumors. Aberrant DNA methylation of multiple promoter associated CpG islands is a frequent phenomenon in acute lymphoblastic leukemia (ALL). In the present study, we scanned for mutations in the exons 4, 5, 6, and 7 of p73 gene. Using PCR-based methylation analysis, we have explored the prevalence of methylation of this pathway in a cohort of children with ALL (N = 120). One pediatric patient showed mutation in exon 4, two showed mutation in exon 5, and none of the patients showed mutation in exons 6 and 7. Methylation of p73 gene is absent in the patients studied. Expression level of p73 mRNA was also examined in 40 ALL samples using reverse transcriptase/polymerase chain reaction. All the patients showed p73 mRNA expression. However, p73 overexpression was observed in 58% of pediatric patients as demonstrated by immunocytochemistry and Western blot analysis. Further, mutation of p73 has been correlated with p73 mRNA and p73 protein status. The results show the presence of overexpressed protein in the samples with mutated p73 gene. Thus, it is presumed that mutation of p73 might lead to production of defective p73 protein and this might have a role in the process of leukemogenesis of ALL. Methylation of p73 does not play a role in pediatric ALL patients of our population. This report is the first demonstrating the presence of p73 gene mutations in exons 4 and 5 with overexpression of p73 protein and absence of p73 methylation in pediatric ALL patients of eastern Indian population.

http://www.sciencedirect.com/science/article/B6WBK-4C005HH-5/2/9f6c0c865cda96d495b9bfcdbe832669

The outcome of hepatitis C virus (HCV) infection varies among individuals, but the genetic factors involved remain unknown. We conducted a population-based association study in which 238 Japanese individuals positive for anti-HCV antibody were genotyped for 269 single nucleotide polymorphisms (SNPs) in 103 candidate genes that might influence the course of infection. Altogether, 50 SNPs in 32 genes were listed. Genetic polymorphisms in IL4, IL8RB, IL10RA, PRL, ADA, NFKB1, GRAP2, CABIN1, IFNAR2, IFI27, IFI41, TNFRSF1A, ALDOB, AP1B1, SULT2B1, EGF, EGFR, TGFB1, LTBP2, and CD4 were associated with persistent viremia (PP<0.05). The sorted genes allow us to draw novel hypotheses for future studies of HCV infection to ultimately identify bona fide genes and their variations.


http://www.sciencedirect.com/science/article/B6WBK-4DNJ2YB-1YC/2/662fd9dd4424828e26cafe0ae8fd54bb

A DNA polymerase purified from the thermoacidophilic archaeabacterium Sulfolobus acidocaldarius was used to perform automated DNA amplification at 70[deg]C as well as site directed mutagenesis by Polymerase Chain Reaction (P.C.R.). The yield of amplification performed at optimum MgCl2 concentration for the Taq or the S. acidocaldarius DNA polymerase, for the same DNA target, was equivalent. The ability of S. acidocaldarius DNA polymerase to perform P.C.R. under less stringent requirement of MgCl2 concentration gives this enzyme a non-negligable advantage over the Taq DNA polymerase.


http://www.sciencedirect.com/science/article/B6WBK-4DNJ8MR-3DK/2/e4c0a91bf3f454e51360699292f2126

Degenerate oligonucleotide primers complementary to the highly conserved subdomains III and VIII of subclass III tyrosine kinase receptors (TKr-III) were utilized to amplify rat aortic cDNA by polymerase chain reaction. Most of the cloned DNA products were rat platelet-derived growth factor receptor [beta] and macrophage-colony stimulating growth factor receptor cDNAs. Screening of the clones with probes coding for the receptor-specific kinase insert domain allowed the identification of a novel putative TKr-III cDNA, which hybridized with a ~6.1 kb mRNA with a distinctive tissue distribution. In situ hybridization on rat tissues and Northern analysis of cultured cells indicate that endothelial cells express a novel putative TKr-III mRNA.

http://www.sciencedirect.com/science/article/B6WBK-48TMBM6-3/2/31741fad291d8ae8c121d5ea671c6c7f

In our recent study, we found that the Ca2+ antagonist, nilvadipine caused significant preservation of photoreceptor cells in The Royal College of Surgeons (RCS) rats [Invest. Ophthalmol. Vis. Sci. 43 (2002) 919]. Here, to elucidate the mechanisms of nilvadipine-induced effects we analyzed altered gene expression of 1101 genes commonly expressed in rodent by DNA microarray analysis in the retinas of nilvadipine-treated and untreated RCS rats and SD rat. In the total number of genes, the expression of 30 genes was altered upon administration of nilvadipine to RCS rats, including several genes related to the apoptotic pathway and other mechanisms. Remarkably, neurotrophic factors, FGF-2 and Arc, known to suppress the apoptosis in the central nervous system, were up-regulated. These changes were also confirmed by real-time quantitative (Taqman) RT-PCR and Western blot analysis. Therefore, our present data suggested that administration of nilvadipine to RCS rats increases the expression of endogenous FGF-2 and Arc in retina, and potentially has a protective effect against retinal degeneration.


http://www.sciencedirect.com/science/article/B6WBK-4DXRXVK-CS/2/dcc1616310b40be49e217c28328427f5

We analyzed the mitochondrial DNA of blood cells of 5 patients from a Chinese family with myoclonic epilepsy and ragged-red fiber disease. The results showed that in all the affected individuals there was a point mutation from A to G at the 8344th nucleotide pair, which was located in the tRNALys gene. No such a mutation was found in mtDNA of either unaffected members of that family or other healthy Chinese subjects. These findings are consistent with the recent report of Shoffner. (Cell 1990, 61:931-937), and confirm that the point mutation is indeed the cause of this disease.


http://www.sciencedirect.com/science/article/B6WBK-4BWYNYWY-1/2/76cf3cd4493b3aac46d360ddbfk9e26

Despite a recent breakthrough in human islet transplantation for treating diabetes mellitus, the limited availability of insulin-producing tissue is still a major obstacle. Here, we studied whether adult pancreatic acinar cells have the potential to transdifferentiate into islet or [beta] cells. Pancreatic acini were isolated from 7- to 8-weeks-old male Sprague-Dawley rats and cultured in suspension. Within 1 week, most of the acinar cells lost amylase expression and converted to cells with a duct cell phenotype. Insulin-positive cells were also observed, mainly at the periphery of the acini-derived spheroids. Insulin gene and protein expression was increased. Presence of a few insulin-positive cells coexpressing cytokeratins suggests that a spontaneous acinar to ductal cell transdifferentiation process was further going on towards [beta] cells. This study provides the first evidence that adult pancreatic acinar cells could be differentiated into insulin-expressing cells.
Orexins/hypocretins are recently discovered neuropeptides, synthesized mainly in the lateral hypothalamus of the brain. Orexins regulate various functions including sleep and appetite. We recently reported increased amount of orexin A in the phenylketonuria (PKU) mouse brain. Whether this is caused by overexpression of the precursor for orexins, prepro-orexin was studied in the PKU mouse brain. Microarray expression analysis revealed overexpression of orexin gene in the brain of PKU mouse. Quantitative real-time RT-PCR showed increased level of prepro-orexin mRNA in the PKU mouse brain. In addition, expression of genes associated with cell signal and growth regulation was also affected in the PKU mouse brain, as observed by microarray analysis. These data suggest that up-regulation of orexin mRNA expression is the possible factor for inducing high orexin A in the brain of PKU mouse. The metabolic environment in the brain of PKU mouse affects normal expression of other genes possibly to result in pathophysiology seen in the PKU mouse, if documented also in patients with PKU.
without cloning. In Patient 1, a 7-bp directly repeated sequence of 5'-ATCCCCA-3' was found at the boundaries of deleted segment spanning 7,039 bp between the ATPase 6 and the cytochrome b genes. In Patients 2, 3, and 4, a 13-bp sequence of 5'-ACCTCCCTCACCA-3' was found in the boundaries of deleted segment spanning 4,977 bp between the ATPase 8 and the ND5 genes. In Patient 5, a 3-bp sequence of 5'-CCT-3' was found in the boundaries of deleted segment spanning 3,717 bp between the ATPase 6 and the ND5 genes. Similar directly repeated sequences may contribute to mitochondrial DNA deletions in human degenerative diseases.


http://www.sciencedirect.com/science/article/B6WBK-48BKRDT-9/2/cd28a1b449c7e2640bb4bb7185d0aab5

Oocyte-specific histone H1 is expressed during oogenesis and early embryogenesis. It has been described in mice and some nonmammalian species, but not in humans. Here, we identified the cDNA in unfertilized human oocytes using direct RT-nested PCR of a single cell. Sequencing of this cDNA indicated an open reading frame encoding a 347-amino acid protein. Expression was oocyte-specific. Homology was closest with the corresponding gene of mouse (H1oo; 42.3%), and, to lesser extent, with that of Xenopus laevis (B4; 25.0%). The gene, named osH1, included five exons as predicted by the NCBI annotation project of the human genome, although the actual splicing site at the 3' end of exon 3 was different by 48 nucleotides from the prediction. The presence of polyadenylation signals and successful amplification of cDNA by RT-PCR using an oligo(dT) primer suggested that the osH1 mRNA is polyadenylated unlike somatic H1 mRNA. Our technique and findings should facilitate investigation of human fertilization and embryogenesis.


http://www.sciencedirect.com/science/article/B6WBK-47MHXV4-K/2/ce3b6d6cf1fe58ae521f37872e68a2c2

Transcription enhancer factor (TEF/TEAD) is a family of four transcription factors that share a common TEA-DNA binding domain and are involved in similar cellular functions, such as cell differentiation and proliferation. All adult tissues express at least one of the four TEAD genes, so this family of transcription factors may be of widespread importance, yet little is known about their regulation. Here we examine the factors that regulate TEAD activity in CHO cells. RT-PCR indicated the presence of TEAD-1, TEAD-3, and both isoforms of TEAD-4, but not TEAD-2. Quantitative measurements showed that TEAD-4 is most abundant, followed by TEAD-3, then TEAD-1. We examined the relative effects of nuclear and cytosolic Ca2+ on TEAD activity, since
TEAD proteins are localized to the nucleus and since free Ca2+ within the nucleus selectively regulates transcription in some systems. Chelation of nuclear but not cytosolic Ca2+ increased TEAD activity two times above control. Inhibition of mitogen-activated protein kinase (MAPK) also increased TEAD activity, while cAMP decreased TEAD activity, and protein kinase C had no effect. Together, these results show that nuclear Ca2+, MAPK, and cAMP each negatively regulate the activity of the TEAD transcription factor.


http://www.sciencedirect.com/science/article/B6WBK-4D634VD-4/2/6942e33d2a58679ac45e71fb3abe6ea6

The de novo methylation activity is essential for embryonic development as well as embryonic stem (ES) cell differentiation, where the intensive and extensive DNA methylation was detected. In this study, we investigated the effects of a demethylating agent, 5-azacytidine (5-AzaC), on differentiated ES cells in order to study the possibility of reversing the differentiation process. We first induced differentiation of ES cells by forming embryoid bodies, and then the cells were treated with 5-AzaC. The cells showed some undifferentiated features such as stem cell-like morphology with unclear cell-to-cell boundary and proliferative responsiveness to LIF. Moreover, 5-AzaC increased the expressions of ES specific markers, SSEA-1, and alkaline phosphatase activity as well as ES specific genes, Oct4, Nanog, and Sox2. We also found that 5-AzaC demethylated the promoter region of H19 gene, a typical methylated gene during embryonic differentiation. These results indicate that 5-AzaC reverses differentiation state of ES cells through its DNA demethylating activity to differentiation related genes.


http://www.sciencedirect.com/science/article/B6WBK-4DS93NB-P/2/70e13c828ca46861120bd7aabe4c1f1

It is well established that certain subpopulations of human adult stem cells can generate hepatocyte-like cells when transplanted into adult immunosuppressed mice. In the present study, we wanted to explore whether xeno-transplantation of human cord blood CD34+ (hCBCD34+) cells during pre-immune stages of development in immunocompetent mice might also lead to human-mouse liver chimerism. Freshly isolated hCBCD34+ cells were xeno-transplanted into non-immunosuppressed mice by both intra-blastocyst and intra-fetal injections. One and four weeks after birth, immunostaining for different human-specific hepatocyte markers: human hepatocyte-specific antigen, human serum albumin, and human [alpha]-1-antitrypsin indicated the presence of human hepatocyte-like cells in the livers of transplanted animals. Detection of human albumin mRNA further corroborated the development of pre-immune human-mouse chimeras. The current report, besides providing new evidence of the potential of hCBCD34+ cells to generate human hepatocyte-like cells, suggests novel strategies for generating immunocompetent mice harboring humanized liver.

Bladder cancer is the fourth and eighth most common cancer in men and women in the United States, respectively. Survivin, a member of inhibitor of apoptosis protein (IAP) gene family, is deregulated in a wide range of malignancies, including carcinoma of the bladder urothelium. Recent advances have identified survivin as a novel intervention target to induce apoptosis in cancer cells by phytochemicals or synthetic agents. Silibinin is a naturally occurring flavanone, isolated from milk thistle extract, and has been shown to possess cancer prevention/intervention potential against various cancers. In several animal and human studies, it is found to be safe and non-toxic. Human bladder transitional-cell papilloma RT4 cells were treated with silibinin and analyzed for survivin protein and mRNA levels by Western blotting and real-time RT-PCR, respectively. Silibinin treatment of cells for 24 h at 100 μM dose resulted in ~50% decrease in survivin protein level; however, treatment at 200 μM dose for 24 and 48 h showed a complete loss in survivin protein without any change in actin used as loading control. Employing RT-PCR analysis we also observed that silibinin causes a strong to complete decrease in survivin mRNA levels. In other studies, down-regulation of survivin by silibinin was associated with a very strong and prominent caspases-9 and -3 activation as well as PARP cleavage. Quantitative apoptotic assay showed that silibinin decreased survivin levels and caspases-PARP cleavages, in accord with a strong apoptotic death and growth inhibition of RT4 cells. Together, these findings suggest that more studies are needed to investigate in vivo effect of silibinin on survivin expression and associated biological effects in bladder cancer that could provide useful information for silibinin efficacy in the prevention/intervention of human bladder cancer.


[beta]-Amyloid peptide (A[beta]), a major component of senile plaques, the formation of which is characteristic of Alzheimer's disease (AD), is believed to induce inflammation of the brain mediated by microglia, leading to neuronal cell loss. In this study, we performed an oligonucleotide microarray analysis to investigate the molecular events underlying the A[beta]-induced activation of macrophages and its specific suppression by the A[beta]-specific-macrophage-activation inhibitor, RS-1178. Of the approximately 36,000 genes and expressed sequence tags analyzed, eight genes were specifically and significantly upregulated by A[beta] treatment with interferon[gamma] (IFN[gamma]) alone (p<0.002). We found that the gene for a well-characterized lipogenic enzyme, stearoyl coenzyme A desaturase-1 (SCD-1), was specifically upregulated by A[beta] treatment and was suppressed to basal levels by RS-1178. Although the underlying mechanisms remain unknown, our results suggest the presence of a link between AD and SCD-1.

This study reports the identification and sequence of a full-length cDNA for a new member of bovine prolactin-related protein (bPRP-VII) and its quantitative and localized expression in the placenta. A full-length bPRP-VII cDNA was cloned with a 929-nucleotide open-reading-frame corresponding to a protein of 238 amino acids. The predicted amino acid sequence shares 63% homology with bPRP-I and 70% with bPRP-VI. bPRP-VII has eight cysteine residues with four disulfide bonds, which is more abundant than that of other bPRPs. RT-PCR detected bPRP-VII only in the placenta. In the placenta, mRNA was expressed in the cotyledon and intercotyledonary tissues throughout gestation. Quantitative real-time RT-PCR analysis exhibited a high expression of bPRP-VII mRNA in the fetal membrane at Day 27 of gestation. In the placentome on Day 60 of gestation, in situ hybridization analysis evidenced bPRP-VII mRNA in binucleate cells. bPRP-VII gene produced a mature protein in mammalian cell expression system. Approximately 29 kDa protein was confirmed in this by the Western blot analysis with FLAG epitope tag. Expression profiles and localization were similar to those of bPRP-I. Although the functional data remain to be examined, a new member of the bPRP-VII gene was cloned. In addition to bPRP-I, bPRP-VII may take on an important functional role in implantation.


http://www.sciencedirect.com/science/article/B6WBK-466WSWJ-P/2/63c3effad63c78c2bf152ff02ff7ad7


http://www.sciencedirect.com/science/article/B6WBK-4F14WBF-5/2/71bd029a9cb743ba8349b2fa55bdff1f

The aim of the present study was to determine if the bone marrow (BM) [beta]2m-/Thy-1+ stem cells isolated from common bile duct ligated (CBDL) rats possess hepatocyte-like characteristics in their global gene expression profiles. The Affymetrix RG U34A arrays were used to conduct transcriptomic profiling on BM [beta]2m-/Thy-1+ stem cells isolated from CBDL and control rats as well as primary hepatocytes. Forty-one probe sets were up-regulated more than 2-fold in CBDL-derived [beta]2m-/Thy-1+ BM stem cells compared to control BM stem cells. Twenty-seven probe sets were present in both CBDL-derived [beta]2m-/Thy-1+ BM stem cells and control hepatocytes but absent in control [beta]2m-/Thy-1+ BM stem cells, including Tcf1 and Dbp. Compared to the control [beta]2m-/Thy-1+ BM stem cells, CBDL-derived [beta]2m-/Thy-1+ BM stem cells shared more commonly expressed genes with hepatocytes. Overall, CBDL-derived [beta]2m-/Thy-1+ stem cells displayed a different transcriptomic fingerprint compared with [beta]2m-/Thy-1+ BM stem cells isolated from control rats; and CBDL-derived [beta]2m-/Thy-1+ stem cells started to express some hepatocyte-like genes.


http://www.sciencedirect.com/science/article/B6WBK-4CN9M8J-
Bone marrow comprises heterogeneous cell populations, of which certain progenitors have demonstrated the ability to differentiate into multiple mesenchymal cell lineages. This study demonstrates the bone marrow stromal cells (BMSCs) with intrinsic plasticity to differentiate into hepatocyte-like phenotypes under in vitro induction of hepatocyte growth factor (HGF). BMSCs isolated from rat femurs and tibias were cultured and passaged 3-4 times in the presence of HGF. Cells were harvested on days 0, 10, and 20 and subjected to examination of any hepatocyte characteristics by flow cytometry, RT-PCR, Western blot, and immunocytochemistry. Expression of albumin and [alpha]-fetoprotein at both mRNA and protein levels was detectable on day 10. By contrast, c-Met mRNA was significantly decreased in BMSC in the course of HGF induction. Here BMSC was shown to differentiate into hepatocyte-like phenotypes given the HGF induction, as an alternative source for adult stem cell transplantation in liver repair.


http://www.sciencedirect.com/science/article/B6WBK-4CJ461D-C/2/41f134a7150ce0c6809d868979c7c6ad

Recently, the G72 gene was reported to be associated with schizophrenia in the French Canadian and Russian populations. Here, we report the results obtained from the study of six single-nucleotide polymorphisms (SNPs: rs3916965, rs3916967, rs2391191, rs1935062, rs778293, and rs3918342), which span an 82-kb region covering the complementary DNA sequences of G72 and G30, in 537 schizophrenia cases and 538 controls of the Han Chinese. In this work, we have identified statistically significant differences in allele distributions of two markers rs3916965 (P=0.019) and rs2391191 (P=0.0010), and a highly significant association between haplotype AGAC of the G72/G30 locus (P=1.7 x 10-4) and schizophrenia. Our data provide further evidence that markers of the G72/G30 genes are associated with schizophrenia in a non-Caucasian population.


http://www.sciencedirect.com/science/article/B6WBK-4C0V4YB-2/2/1e02e1f99926c7bcd6a9903c9788405e

Activated hepatic stellate cells (HSCs) produce cyclooxygenase-2 (COX-2) protein to induce vascular endothelial growth factor (VEGF) production that participates in angiogenesis in injured liver. To reveal the unknown regulatory mechanism, we used hypoxic atmosphere mimicking injured-tissue microenvironment to induce VEGF expression in a rat hepatic stellate cell line (T6-HSCs). The present study showed that hypoxia up-regulated the protein levels of COX-2 and hypoxia-inducible factor-1-[alpha] (HIF-1[alpha]), but rapidly effected degradation of von Hippel-Lindau (vHL) protein. As a result, expression of VEGF in HSCs was markedly elevated; and pretreatment with COX-2 inhibitors (nimesulide or indomethacin) could significantly ameliorate the angiogenic event. Collectively, hypoxic HSCs increased accumulation of HIF-1[alpha] protein and induced VEGF expression in a time-dependent manner. Inhibition of COX-2 activities would prevent vHL protein from degradation and suppress HIF-1[alpha] up-regulation. Thus, vHL/HIF-1[alpha] has a regulatory role in COX-2-mediated VEGF production in hypoxic stellate cells in injured liver.
Liver-intestine cadherin (LI-cad) is a non-classical cadherin, which is expressed during intestinal development, but absent in normal liver tissue. Our earlier investigation has detected overexpression of LI-cad in gastric adenocarcinoma and indicated its association with lymph node metastasis. Herein, we found in RT-PCR and TaqMan Q-PCR that LI-cad was identified in HCC cell lines, HuH-7, Hep-3B, and PLC/PRF/5, but not in MIHA and HepG2 non-tumorigenic cells. Immunofluorescence cytochemistry assay revealed that the LI-cad was predominantly expressed in cytoplasm of HCC cells, contrary to that of E-cad immunostain at the plasma membrane region. By testing against 18 pairs of HCC and adjacent non-tumor tissues, 13 cases (72.2%) showed over expression of LI-cad in HCC tissues, 2 cases (11.1%) were similar, and 3 cases did not yield detectable signal. None of the 6 normal liver specimens tested was positive with LI-cad. Taken together, LI-cad could be a potential disease marker for HCC.

The genome of Streptococcus pyogenes, an important human pathogen, encodes homologs of the principal bacterial heat shock proteins DnaK and GroESL, as well as HrcA, a negative regulator of dnaK and groESL expression in other Gram-positive bacteria. Using nuclease protection assays to measure dnaK/groESL mRNA abundance and a "non-polar" insertion to disrupt hrcA, we demonstrate that heat shock triggers a 4- to 8-fold increase in dnaK and groESL-specific mRNAs within 5 min of the temperature shift and that HrcA is a negative regulator of S. pyogenes dnaK/groESL mRNA abundance in unstressed S. pyogenes. Although the loss of HrcA elevated dnaK and groESL mRNA levels under non-heat shock conditions, the relative abundance of these RNAs increased further in heat shocked S. pyogenes, suggesting an additional element contributing to their synthesis or stability.

It is well established that transforming growth factor-[beta]1 (TGF-[beta]1) can induce the transformation of fibroblasts to myofibroblasts. The molecular mechanisms of the phenotypic change remain unknown. The effect of TGF-[beta]1 on the expression of K+ channels in cultured rat vascular fibroblasts was investigated by using the patch-clamp technique and quantitative RT-PCR. In fibroblasts, the only voltage-dependent outward K+ current that can be
electrophysiologically detected is non-inactivating. In myofibroblasts, induced by the treatment of fibroblasts with TGF-[beta]1, we report the emergence of an additional transient outward K+ current. The TGF-[beta]1-induced outward current is inhibited by 4-aminopyridine. KV2.1, the transcript for a non-inactivating potassium channel gene, was detected by quantitative RT-PCT in both cultured fibroblasts and myofibroblasts. In contrast, the transcript of the transient IA gene, KV4.1, can be detected only in myofibroblasts. The results suggest that TGF-[beta]1-induced phenotypic transformation of vascular fibroblasts to myofibroblasts is accompanied by the induction of IA channels.


The super induction of spermidine/spermine N1-acetyltransferase (SSAT), has been implicated in the cytotoxic response of human solid tumors to the bis(ethyl)polyamines. The SSAT response is a phenotype specific response and is modulated at the level of increased steady-state mRNA levels and enzyme protein. The human genomic region (4,095 bases) containing the coding sequence of SSAT has been cloned and localized to the Xp22.1 region. Primer extension analysis indicates the transcription of SSAT starts 179 bases upstream from the translational start site and appears to be under the control of a "TATA-less" promoter. The availability of this human clone will facilitate the direct functional examination of the SSAT gene.


We report the construction of a cDNA clone encoding a functional GM2-activator protein. The sequence of the complete 5’ end of the coding region was determined by direct nucleotide sequencing of a fragment generated by multiple RACE PCR procedures from Hela cell cDNA. Specific oligonucleotides were synthesized from these data which allowed us to produce a PCR fragment that contained the complete coding sequence of the protein. This was then cloned into a mammalian expression vector. The ability of purified hexosaminidase A ([beta]-N-acetylhexosaminidase, EC 3.2.1.52) to hydrolyse labeled GM2 ganglioside was enhanced 10-fold more by the addition in the assay mix of lysate from transfected COS-1 cells than by the addition of identical amounts of lysate from mock transfected cells. Direct sequencing of PCR fragments from two sources also identified three polymorphisms.


The zebrafish no tail gene (ntl) is indispensable for the formation of the notochord and tail
structure. Here, we show the presence of an intronless ntl gene in zebrafish, which we designated cryptail (ctl). ctl could not be found in any zebrafish genomic resources examined and was only just cloned by a PCR-based approach that relied on its lack of introns and homology to ntl. The amplifiable region of ctl was confined to the transcribed region of ntl. ctl thus appeared to have been generated by reverse transcription of ntl mRNA, like a processed pseudogene. ctl was very polymorphic even in individual fish, but had no missense mutations. This may suggest that ctl has a physiological function in zebrafish.


http://www.sciencedirect.com/science/article/B6WBK-4F03B28-1HW/2/f604a547234a29ee49084fd44196bf7e

To understand the role of mitochondria in carcinogenesis, we compared the amount of deleted mtDNAs between human hepatic tumors and surrounding cirrhotic portion of the liver of ten patients by using polymerase chain reaction (PCR). Multiple mtDNA deletions were detected in cirrhotic portion, but no deletions were detected in the tumor portion. Direct sequencing of the fragments revealed a 7,079-bp deletion (nucleotide position 8,992-16,072) involving no direct repeated sequences and a 7,436-bp deletion (position 8,649-16,084) involving a 12-bp directly repeated sequence of 5'-CATCAACAACCG-3' exists in both the ATP6 gene and the D-loop region. These mtDNA mutations could be one of the endogenous factors that induce somatic mutations in nuclear genome and etiologically contribute to human carcinogenesis.


http://www.sciencedirect.com/science/article/B6WBK-4DWVYB4-C/2/8ff62e1aae2daa84e748af0a626cf25d

Myostatin belongs to the transforming growth factor beta superfamily and has been shown to function as an inhibitor of skeletal muscle proliferation and differentiation. To gain insight into the molecular mechanisms of myostatin function during myogenesis, differential display reverse transcription PCR was employed to identify altered gene expressions associated with myostatin inhibitory function in chicken fetal myoblasts (CFMs). In this work, we have identified seven up-regulated and 12 down-regulated genes in myostatin stimulated CFMs. Those genes are involved in myogenic differentiation, cell architecture, energy metabolism, signal transduction, and apoptosis. The down-regulation of muscle creatine kinase B, troponin C, and myosin regulatory light chain is in agreement with the myostatin negative role in myocyte differentiation. In addition, the expression alteration of skeletal muscle-specific cardiac ankyrin repeat protein and the bcl-2 related anti-apoptotic protein Nr-13 suggests possible unique roles for myostatin in regulating myogenesis by controlling cofactors participated transcriptional regulation and apoptosis.


http://www.sciencedirect.com/science/article/B6WBK-46MD2WB-

http://www.sciencedirect.com/science/article/B6WBK-4D2X20W7Z/2/0ead9c3e76acc8644d372b295f81cb23

To distinguish biological molecular processes of osmotic stress occurring in inner medulla, we utilized microarrays to monitor expression profiles. RNAs from three segments (cortex, outer medulla, and inner medulla) of mouse kidney were isolated and applied to microarrays. We found 35 genes expressed highly in inner medulla. Next, microarrays for the RNAs from mouse medullary collecting duct cell line (mIMCD) cells and osmotically adapted mIMCD cells (HT cells) were performed (designed as resistant to 1270 mOsm/H2O). Of 35 genes highly expressed in inner medulla, 6 genes such as; B-cell translocation gene protein (BTG), myc-basic motif homologue, gelsolin, cell surface glycoprotein, laminin [beta]2, and tubulo-interstitial nephritis antigen, were also expressed highly in HT cells. Using real-time PCR, we confirmed the expression of six genes. Additionally acute osmotic stress induced the BTG. By comparing the inner medulla to a mIMCD3, we identified genes which respond to acute and chronic hyperosmotic stress.


http://www.sciencedirect.com/science/article/B6WBK-4B3JS9YS/2/12c2fbeb7fdddb679c6851171e4035d

Two interleukin 13 receptors (IL-13Rs) have been identified as IL-13R[alpha]1 and IL-13R[alpha]2. IL-13R[alpha]1 is composed of a heterodimer consisting of IL-13R[alpha]1 and IL-4 receptor [alpha] (IL-4R[alpha]) as a signaling subunit. In contrast, IL-13R[alpha]2 is known as a decoy receptor for IL-13. In this study, we investigated the expression of IL-13Rs on human fibroblasts. IL-13R[alpha]2 was significantly up-regulated after stimulation with tumor necrosis factor-[alpha] (TNF-[alpha]) and/or IL-4. In contrast, IL-13R[alpha]1 was constitutively detectable and was not up-regulated. After the induction of IL-13[alpha]2 by IL-4, STAT6 phosphorylation through IL-13R[alpha]1 by IL-13 was inhibited. We also detected large intracellular pools of IL-13R[alpha]2 in fibroblasts quantitatively. Furthermore, mobilization of the IL-13R[alpha]2 protein stores from the cytoplasm to the cell surface was prevented by an inhibitor of protein transport, brefeldin-A. These results indicate that TNF-[alpha] and IL-4 synergistically up-regulate the expression of IL-13R[alpha]2 decoy receptor on human fibroblasts by inducing gene expression and mobilizing intracellular receptors, and thus may down-regulate the IL-13 signaling.


http://www.sciencedirect.com/science/article/B6WBK-4D2XF007Z/2/de173d0771f8a2c41217f60982ac5e35
Mullerian inhibiting substance (MIS), also known as anti-Mullerian hormone, is a glycoprotein belonging to transforming growth factor [beta] superfamily. In mammals, MIS is responsible for regression of Mullerian ducts, anlagen of the female reproductive ducts, in the male fetus. However, the role of MIS in gonadal sex differentiation of teleost fishes, which do not have the Mullerian ducts, has yet to be clarified. To address the role of MIS on gonadal sex differentiation in fishes, we isolated a MIS cDNA from the Japanese flounder testis and examined the expression pattern of MIS mRNA in gonads of both sexes during sex differentiation period. In this study, we present the first demonstration of sexually dimorphic expression of MIS mRNA during sex differentiation in teleost fishes, similarly to amniote vertebrates which possess the Mullerian ducts.


http://www.sciencedirect.com/science/article/B6WBK-487KHGR-2/2/f70a87fb0ea3d638ce86b0d2c2b99bf4

In some tissues 17[beta]-estradiol (E2) is known to increase endothelial NOS expression. In the present study we examined the effects of E2 on estrogen receptors (ER[alpha] and [beta]) and inducible nitric oxide synthase (iNOS) expression and analyzed the mechanisms in rat peritoneal macrophages. Reverse-transcription polymerase chain (RT-PCR) and transient transfection experiments using a reporter plasmid that contained a luciferase gene under the transcriptional control of an estrogen-responsive elements revealed that peritoneal macrophages are responsive to E2 and express both ER[alpha] and ER[beta] mRNAs. Incubation with E2 leads to an increased ER[beta] mRNA expression. When rat peritoneal macrophages were incubated with physiological concentrations of E2, E2 induced a dose-dependent increase in NO production. E2 significantly affected secretion at concentration levels of more than 10-11 M, and its maximum effect was at a concentration of 10-8 M. RT-PCR reactions showed that increases in NO secretion were due to an increase in iNOS mRNA. Coincubation with ICI 182,780, an estrogen-receptor antagonist, inhibited the influence of E2 on NO production and iNOS expression. Thus E2 stimulated iNOS expression by a classic receptor-mediated pathway. We hereby prove that E2 increases the inOS expression in macrophages and this effect appears to be the consequence of ER activation.


http://www.sciencedirect.com/science/article/B6WBK-4B0PR53-3/2/a7fc3bb2adcf279808d955efb68559b9

The ability to simultaneously isolate intact DNA-free RNA, genomic DNA, and proteins from a biological specimen can be useful in cloning genes and analyzing gene expression. Equilibrium density gradient centrifugation with CsCl is a useful tool for fractionating, quantitatively separating, and characterizing RNA, DNA, and the total quota of proteins, respectively, based on differences in their buoyant densities. In the present study we have reexamined the rarely used cesium salt, cesium trifluoroacetate, for the same purpose. A significant advantage of CsTFA lies in the fact that, unlike in CsCl, RNA can be recovered from a single, soluble fraction of the CsTFA gradient. Furthermore, unlike CsCl, CsTFA is freely soluble in ethanol so that co-precipitation of the salt in the recovered RNA upon alcohol precipitation does not take place. Hence, the RNA is recovered with minimum manipulations. The one-step separation of cellular macromolecule...
classes free of each other in small amount of starting materials provides a major advantage over other methods currently in use.


http://www.sciencedirect.com/science/article/B6WBK-4F91Y8K-D/2/9e9ef06df1d696365fe0ed4f6a635e39

Several independent linkage studies have demonstrated that the 1q22 region is likely to harbor candidate schizophrenia susceptibility genes. Recently, some genetic variants within CAPON have been reported as exhibiting significant linkage disequilibrium to schizophrenia in Canadian familial schizophrenia pedigrees. We examined nine single nucleotide polymorphisms (SNPs), which span an approximately 236-kb region of CAPON, in 664 schizophrenia cases and 941 controls in the Chinese Han population. We detected a significant difference in allele distributions of SNP rs348624 (P = 0.000017). Moreover, the overall frequency of haplotypes constructed from three SNPs including rs348624 showed significant difference between cases and controls (P = 0.000025). Our findings indicate that CAPON gene may be a candidate susceptibility gene for schizophrenia in Chinese Han population, and also provide further support for the potential importance of NMDAR-mediated glutamatergic transmission in the etiology of schizophrenia.

Biochemical Medicine and Metabolic Biology (1)


http://www.sciencedirect.com/science/article/B6WBN-4C4NSCN-3C/2/af9123b76c5bb8f87eec0eefd003e1a4

We analyzed DNA from 13 males with ornithine transcarbamylase (OTC) deficiency for gene deletions and known point mutations using the polymerase chain reaction (PCR), allelle-specific oligonucleotide (ASO) hybridization, and Southern blotting with full-length OTC cDNA and exon-specific probes. Three patients were found to have deletions: one was missing the whole OTC gene; a second patient had a deletion of both exon 7 and 8; and the third had a deletion of exon 9. Only one of the remaining 10 patients had a known point mutation consisting of a G-to-A change in nucleotide 422 of the sense strand resulting in a glutamine substitution for arginine at amino acid 109 of the mature OTC protein. This study describes the integration of various molecular methods to screen OTC-deficient patients for deletions and points mutations. Two new deletions within the OTC gene are described.

Biochemical Pharmacology (39)

http://www.sciencedirect.com/science/article/B6T4P-3YJYDBS-G/2/b151c4f5fb7ac58ca9ac9803d3ec3f28

The muscles of the corpus cavernosum of the penis relax in response to stimulation of non-adrenergic, non-cholinergic nerves or nitric oxide (NO)-donating drugs to elicit erection. It is generally assumed that NO mediates this effect via activation of soluble guanylyl cyclase and a subsequent increase in cyclic guanosine 3',5'-monophosphate concentration. However, there are no data on the expression of this enzyme in human corpus cavernosum. The purpose of the present study was the molecular characterization of NO-sensitive guanylyl cyclase in human corpus cavernosum. RNA was extracted from tissue samples obtained from seven patients undergoing penile prosthetic surgery or correction of penile deviation. Reverse transcriptase-polymerase chain reaction (RT-PCR) with specific primers for the subunits of NO-sensitive guanylyl cyclase was performed, and PCR products were subcloned and sequenced. Specific amplification products encoding the [alpha]1, [beta]1, [alpha]2, and [beta]2 subunits were detected. In addition, we isolated a transcript encoding a novel variant [beta]2 subunit. To test whether this novel transcript arises by alternative splicing or whether it is encoded by a separate gene, a 4000-bp clone of the corresponding genomic DNA sequence was isolated. Sequence analysis suggests that the novel [beta]2 variant arises by alternative splicing from the same gene as the [beta]2 subunit on chromosome 13. In conclusion, our findings suggest the presence of different subunit mRNAs of NO-sensitive guanylyl cyclase in human corpus cavernosum.


http://www.sciencedirect.com/science/article/B6T4P-4754BR7-X8/2/233a213b2f8eee4eb07a99ceb3c2bf28

Stably expressed human and rat phenol UDP-glucuronosyltransferases (UGTs) of the UGT1 complex (HlugP1, HlugP4 and 3-methylcholanthrene-inducible rat UGT1A1, the latter considered to be an orthologous enzyme to HlugP1) have been used to investigate the role of UGTs in paracetamol glucuronidation. Kinetic analysis of recombinant UGTs was compared to that of total UGT activities in liver microsomes. Paracetamol was found to be an overlapping substrate of several UGTs. It shows higher affinity for HlugP1 and rat UGT1A1 (apparent Km values of 2 and 3 mM, respectively) than for HlugP4 (Km = 50mM) and other UGTs present in liver microsomes (Km values of > 12mM). Glucuronidation of paracetamol with HlugP1 contrasts with that of 6-hydroxychrysene and of 4-methylumbelliferone, which are conjugated with higher affinity by HlugP4 than by HlugP1. Due to the wide tissue distribution of rat UGT1A1, paracetamol glucuronidation was also investigated in extra-hepatic rat and human tissues. Paracetamol UGT activity was present and inducible by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat kidney, lung and spleen. It was also detected in human kidney. A selective cDNA probe for exon 1 of HlugP1 cross-reacted with mRNA from both human liver and kidney. The results demonstrate that paracetamol is conjugated by HlugP1 and its rat orthologue UGT1A1 with higher affinity than by HlugP4 and other UGTs.

UDP-glucuronosyltransferases (UGTs), in addition to their role in overall pharmacokinetics, play important roles in local protection of cells against toxins and in the control of endogenous receptor ligands. UGT1A6, which conjugates planar phenols, appears to be expressed in many organs, but information on cell-specific expression in these organs is controversial or absent. Therefore, a non-isotopic in situ hybridization method was developed and applied to localize UGT1A6 expression in rat testis and brain. It was found that UGT1A6 is expressed in Sertoli cells and spermatogonia of rat testis and in brain neurons, in particular in hippocampal pyramidal cells and Purkinje cells of the cerebellum.


Arylamine N-acetyltransferase 1 (NAT1) conjugates several aromatic amines and their N-hydroxylated metabolites by N- or O-acetylation. NAT1 genotype and phenotype is known to be variable in human populations. In this study, we set out to measure the functional relevance of the frequent NAT1 gene variants for the activity in human red blood cells. Healthy German volunteers (N = 314) were genotyped for NAT1 alleles *3, *4, *10, *11, *14, and *15 using polymerase chain reactions and restriction fragment length pattern analysis, and NAT1 enzyme kinetic parameters were measured in a subset of 105 individuals using p-aminobenzoic acid as specific substrate. There was no functional difference between NAT1 alleles *4 and *10. In particular, there was no trend of increasing activity from NAT1*4/*4 to *4/*10 and *10/*10. Carriers of the NAT1*11 and *14 alleles had a statistically significant lower enzyme activity compared with carriers of the *3, *4, or *10 alleles. Compared with the wild-type genotype NAT1*4/*4, activity of the NAT1*11/*11, NAT1*11/*10, and NAT1*11/*4 genotypes was reduced by 20.7%, 35.7%, and 31.5%, respectively. Activity of the NAT1*10/*14 and NAT1*4/*14 genotypes was reduced by 49.8% and 55.6%, respectively. The difference in NAT1 activity between the *4/*11 and *4/*14 genotypes was also significant (P NAT1*15/*15 genotype had no detectable enzyme activity. In conclusion, functional consequences of NAT1 mutations were tested in a large population. Activity in carriers of NAT1 alleles *3, *4, and *10 did not differ, alleles NAT1*11 and *14 appeared to be low activity alleles, and allele NAT1*15 had no activity.


Screening of a foetal brain genomic DNA library allowed to isolate a 10-kb fragment of the gene encoding the human [alpha]2B-adrenergic receptor, that contained 5.5 kb of the 5'-flanking region, the open reading frame and 2.9 kb of the 3'-flanking region. The 1-kb fragment upstream from the start codon was rich in GC, lacked consensus TATA or CAAT box, but contained several Sp1-binding sites. Other potential cis-regulatory elements found in the 5'-flanking region included AP2, USF, Stat-6, NF[kappa]B and Olf-1. A single canonical polyadenylation signal (AATAAA) was found at position +3252/+3257 and the polyadenylation site was 3274 nucleotides.
downstream from ATG. Transfection experiments with chimeric luciferase constructs containing various truncated fragments of the 5'-region showed that the fragment -3160/+3 exhibited promoter activity in all tested cell lines and permitted the definition of a minimal 200-bp promoter (-603/-411) containing three putative Sp1-binding sites and two initiator elements. Transcriptional activity of this region was inhibited by the addition of mithramycin, a specific inhibitor of Sp1 binding to GC-rich sequences. The search for sequence variants within a fragment covering 1.7 kb of 5'-flanking region and the coding region allowed us to identify five novel single nucleotide polymorphisms. Interestingly, the G/C substitution at position -98 relative to the start codon was common and in complete linkage with a previously identified insertion/deletion polymorphism in the coding region which was showed to affect [alpha]2B-adrenergic receptor function. Based on transfection data and computer-assisted sequence analysis, the -98 G/C single nucleotide polymorphism was located within a portion of the 5'-UTR (-127/+3) affecting luciferase activity and it created additional putative binding site for Sp1. However, G/C substitution had no significant incidence on promoter activity in BHK-21 or HeLa cells.


http://www.sciencedirect.com/science/article/B6T4P-3V3B14D-9/2/074c7c6cc47131c9bc3f8f6c0a946c98c

The induction of cytochrome P450 3A (CYP3A) protein and mRNA by RU486 [17[beta]-hydroxy-11[beta]-{4-dimethylaminophenyl}-17[alpha]-1-propyl-stra-4,9-dien-3-one] treatment and food deprivation in female rat liver was studied using Western blotting and competitive reverse transcription-polymerase chain reaction (RT-PCR). CYP3A apoprotein levels increased in response to food deprivation and to RU486 treatment, and the combination of RU486 treatment plus food deprivation had an apparent additive effect. Food deprivation and RU486 treatment also caused increases in CYP3A1, CYP3A18, and CYP3A23 mRNA, and the combined effects of these treatments on each of these mRNA forms were synergistic. CYP3A2 mRNA was not detected in any of the treatment groups, and there was a lack of concordance between CYP3A9 mRNA levels and the specific messages corresponding to the other CYP3A isoforms. CYP3A9 mRNA levels were highest in food-deprived animals, whereas RU486 inhibited CYP3A9 mRNA expression and suppressed the induction effect of food deprivation. Food deprivation and RU486 treatment each separately caused increased microsomal diazepam C3-hydroxylase activity, and the combined effects of these treatments on this monooxygenase were additive. In contrast, the [N-methyl-14C]erythromycin demethylase activity of the fasted, RU486-treated group of rats did not differ from that of the untreated group, and kinetic analyses revealed that both groups of animals exhibited similar Km and Vmax values. These results suggest that CYP3A9 may be primarily responsible for erythromycin N-demethylation and that the isoforms induced by the combination of fasting and RU486 administration are CYP3A1, CYP3A23, and, to a lesser extent, CYP3A18.


http://www.sciencedirect.com/science/article/B6T4P-44PDMB9-M/2/6c1c99bec1e33749f5e322b21150bd8

The activity, expression and localization of the UDP-glucuronosyltransferases (UGTs) were investigated in human placenta at term. UGT activity (measured with the substrate 4-methylumbelliferone (4-MU)) was observed in all 25 placentas sampled and maximum velocity
(Vmax) ranged 13-fold from 5.1±0.9 to 66.9±17.5 nmol/min/mg protein (mean±SD). Substrate affinity (Km) ranged 5-fold from 246+/−24 to 1124+/−422 [μM]. Using reverse transcriptase-polymerase chain reaction (RT-PCR), expression of the isoforms UGT2B4, 2B7, 2B10, 2B11 and 2B15 was observed in all (12/12) placentas sampled and expression of UGT2B17 was noted in 8/12 placentas. Northern analysis of the UGT2B7 isoform in 12 placentas revealed a 10-fold difference in expression with RT-PCR variability and the 13-fold variation observed in UGT activity. The presence of UGT2B4 and 2B7 proteins (52 and 56 kDa, respectively) was demonstrated by Western blotting. The sites of placental UGT2B transcription (in situ hybridization) and protein expression (immunohistochemistry) were located in the syncytium of the placental trophoblasts bordering the placental villi. UGT1A proteins could not be observed with immunohistochemistry or Western blotting and expression could not be observed with RT-PCR. Our discovery of UGT expression and activity at the site of maternal-fetal exchange is consistent with a role for UGTs in detoxification of exogenous and endogenous ligands and the maintenance of placental function through clearance and regulation of steroid hormones.

Dabholkar, M., K. Thornton, et al. (2000). "Increased mRNA levels of xeroderma pigmentosum complementation group B (XPB) and Cockayne's syndrome complementation group B (CSB) without increased mRNA levels of multidrug-resistance gene (MDR1) or metallothionein-II (MT-II) in platinum-resistant human ovarian cancer tissues." Biochemical Pharmacology 60(11): 1611.


Tumor tissue specimens from human ovarian cancer patients were assessed for relative mRNA abundance levels of several genes thought to be involved in the development of in vitro drug resistance in this disease. Higher mRNA levels of Xeroderma pigmentosum group B (XPB), which links DNA repair with DNA transcription, and of Cockayne's syndrome group B (CSB), which is essential for gene-specific repair, were observed in tumor tissues that were clinically resistant to platinum-based chemotherapy, as compared with tissues from patients responding to therapy. In a cohort of 27 patients, mRNA levels of XPB averaged 5-fold higher in platinum-resistant tumors (P = 0.001); and for CSB, mRNA levels averaged 6-fold higher but with greater variability (P = 0.033). Concurrently, these platinum-resistant tumor tissues did not exhibit significantly higher mRNA levels for the MDR1 (multidrug-resistance) gene (P = 0.134) or of the metallothionein-II (MT-II) gene (P = 0.598). Since these platinum-resistant tumors also show higher mRNA levels of ERCC1 and XPA, platinum resistance appears to be associated with concurrent up-regulation of four genes (XPA, ERCC1, XPB, and CSB). These four genes participate in DNA damage excision activity, gene-specific repair, and linkage of DNA repair with DNA transcription. These data suggest that concurrent up-regulation of genes involved in nucleotide excision repair may be important in clinical resistance to platinum-based chemotherapy in this disease.


http://www.sciencedirect.com/science/article/B6T4P-48JK73P-1/2/9163e3a2a0a8c213ea96269be37451c9

We carried out a time-course study on the effects of a single intramuscular (i.m.) dose (0.5 x 50) of sarin (O-isopropyl methylphosphonofluoridate), also known as nerve agent GB, on the mRNA expression of acetylcholinesterase (ACHE) in the brain of male Sprague-Dawley rats. Sarin inactivates the enzyme ACHE which is responsible for the breakdown of the neurotransmitter acetylcholine (ACH), leading to its accumulation at ACh receptors and overstimulation of the
cholinergic system. Rats were treated with 50 [mu]g/kg of sarin (0.5 x 50) in 1 mL saline/kg and terminated at the following time points: 1 and 2 hr and 1, 3, and 7 days post-treatment. Control rats were treated with normal saline. Total RNA was extracted, and northern blots were hybridized with cDNA probes for AChE and 28S RNA (control). Poly-A RNA from both treated and control cortex was used for reverse transcription-polymerase chain reaction (RT-PCR)-based verification of the data from the northern blots. The results obtained indicate that a single (i.m.) dose of sarin (0.5 x 50) produced differential induction and persistence of AChE mRNA levels in different regions of the brain. Immediate induction of AChE transcripts was noted in the brainstem (126+/-6%), cortex (149+/-4%), midbrain (153+/-5%), and cerebellum (234+/-2%) at 1 hr. The AChE expression level, however, increased over time and remained elevated after a decline at 1 day in the previously shown more susceptible brainstem. The transcript levels remained elevated at a later time point (3 days) in the midbrain, after a dramatic decline at day 1 (110+/-2%). In the cortex, transcript levels came down to control values by day 1. The cerebellum also showed a decline of the elevated levels observed at 2 hr (275+/-2%) to control values by day 1. RT-PCR analysis of the AChE transcript at 30 min in the cortex showed an induction to 213+/-3% of the control level, confirming the expression pattern obtained by the northern blot data. The immediate induction followed by the complex pattern of the AChE mRNA time-course in the CNS may indicate that the activation of both cholinergic-related and unrelated functions of the gene plays an important role in the pathological manifestations of sarin-induced neurotoxicity.


http://www.sciencedirect.com/science/article/B6T4P-3W788WD-9/2/9c91d9f6ec37096eac51809f9816fb66

The effect of P-glycoprotein inhibition on the uptake of the HIV type 1 protease inhibitor saquinavir into brain capillary endothelial cells was studied using porcine primary brain capillary endothelial cell monolayers as an in vitro test system. As confirmed by polymerase chain reaction and Western blot analysis, this system functionally expressed class I P-glycoprotein (pgp1A). P-Glycoprotein isoforms pgp1B or pgp1D could not be detected. The uptake of saquinavir into endothelial cells could be described as the result of a diffusional term of uptake and an oppositely directed saturable extrusion process. Net uptake of saquinavir into cultured brain endothelial cells could be increased significantly up to 2-fold by SDZ PSC 833 in a dose-dependent manner, with an 50 of 1.13 [mu]M. In addition, the HIV protease inhibitor ritonavir inhibited p-glycoprotein-mediated extrusion of saquinavir with an 50 of 0.2 [mu]M, indicating a high affinity of ritonavir for p-glycoprotein. In conclusion, we showed that the HIV protease inhibitor ritonavir is a more potent inhibitor of P-glycoprotein than the multidrug resistance (MDR)-reversing agent SDZ PSC 833. The inclusion of this drug in combination regimens may greatly facilitate brain uptake of HIV protease inhibitors, which is especially important in patients suffering from AIDS dementia complex.


http://www.sciencedirect.com/science/article/B6T4P-3X6SBXC-9/2/755c9f49e9de93bbf0c5833d9bada326

Nitric oxide (NO) produced in endothelial cells has been implicated in the regulation of blood pressure, regional blood flow, inhibition of platelet aggregation, and endothelial and vascular
smooth muscle cell proliferation. In a variety of cardiovascular disease states, such as atherosclerosis, arterial hypertension, and restenosis, expression of endothelial NO synthase (NOS-III) and endothelial NO production appear to be altered. Thus, NOS-III is an attractive target for cardiovascular gene therapy for which adenoviral vectors are one of the most effective vector systems. Therefore, a recombinant adenoviral vector expressing NOS-III (adenovirus type 5 [Ad5] cytomegalovirus [CMV] NOSIII) was constructed and biochemically and pharmacologically characterized both in vitro and in intact cells. Ad5CMVNOSIII-derived recombinant NOS-III was successfully expressed, as shown by immunoprecipitation and immunocytochemistry, and biologically active, as shown by functional assays in human primary umbilical vein and EA.hy926 endothelial cells, as well as 293 human embryonic kidney and Chinese hamster ovary cells. The Km values for NADPH and -arginine and the Ka for tetrahydrobiopterin as well as the enzyme's dependency on other cofactors were similar to recombinant reference enzyme and literature values. NOS-III expression levels correlated linearly with the multiplicity of infection with Ad5CMVNOSIII and lasted for at least 8 days. NOS-III transfection inhibited endothelial cell proliferation. In conclusion, adenovirus-mediated gene transfer of Ad5CMVNOSIII to vascular and non-vascular cells resulted in the dose-dependent expression of intact, physiologically regulated, and functionally active NOS-III.

Groschel, B., A. Kaufmann, et al. (2002). "3'-Azido-2',3'-dideoxythymidine induced deficiency of thymidine kinases 1, 2 and deoxycytidine kinase in H9 T-lymphoid cells." Biochemical Pharmacology 64(2): 239.

http://www.sciencedirect.com/science/article/B6T4P-462BRBX-9/2/a394466934fa28d45b3a1fbdeea56d52

Continuous cultivation of T-lymphoid H9 cells in the presence of 3'-azido-2',3'-dideoxythymidine (AZT) resulted in a cell variant cross-resistant to both thymidine and deoxycytidine analogs. Cytotoxic effects of AZT, 2',3'-didehydro-3'-deoxythymidine as well as different deoxycytidine analogs such as 2',3'-dideoxycytidine, 2',2'-difluoro-2'-deoxycytidine (dFdC) and 1-[ss]-D-arabinofuranosylcytosine (Ara-C) were strongly reduced in H9 cells continuously exposed to AZT when compared to parental cells (>8.3-, >6.6-, >9.1-, 5 x 104-, 5 x 103-fold, respectively). Moreover, anti-HIV-1 effects of AZT, d4T, ddC and 2',3'-dideoxy-3'-thiacytidine (3TC) were significantly diminished (>222-, >25-, >400-, >200-fold, respectively) in AZT-resistant H9 cells. Study of cellular mechanisms responsible for cross-resistance to pyrimidine analogs in AZT-resistant H9 cells revealed decreased mRNA levels of thymidine kinase 1 (TK1) and lack of deoxycytidine kinase (dCK) mRNA expression. The loss of dCK gene expression was confirmed by western blot analysis of dCK protein as well as dCK enzyme activity assay. Moreover, enzyme activity of TK1 and TK2 was reduced in AZT-resistant cells. In order to determine whether lack of dCK affected the formation of the active triphosphate of the deoxycytidine analog dFdC, dFdCTP accumulation and retention was measured in H9 parental and AZT-resistant cells after exposure to 1 and 10 [mu]M dFdC. Parental H9 cells accumulated about 30 and 100 pmol dFdCTP/106 cells after 4 hr, whereas in AZT-resistant cells no dFdCTP accumulation was detected. These results demonstrate that continuous treatment of H9 cells in the presence of AZT selected for a thymidine analog resistant cell variant with cross-resistance to deoxycytidine analogs, due to deficiency in TK1, TK2, and dCK.


http://www.sciencedirect.com/science/article/B6T4P-47724F9-1Y/2/7890cb76dd712de6b243b7a7537985f1
The p80cdc25 protein is a protein phosphatase directly involved in p34cdc2 protein kinase activation by dephosphorylation. The cdc25B gene is one of three human cdc25 homologs which can complement the temperature-sensitive cdc25 mutation of Schizosaccharomyces pombe, and is expressed at high levels in human cell lines, particularly in some cancer cells. A fusion protein of glutathione-S-transferase (GST) and the catalytic domain of cdc25B protein was constructed and found to retain phosphatase activity in the manner of a p80cdc25 phosphatase by using a chromogenic substrate, p-nitrophenylphosphate. Two benzoquinoid antitumor compounds, dnacin A1 and dnacin B1, inhibited phosphatase activity in a non-competitive manner.


http://www.sciencedirect.com/science/article/B6T4P-3YVDRK0-1J/2/ff2e3ed71bdc942c629afe7259839804

TAN-1518 A is a cytotoxic agent with suppressive activity against Meth A fibrosarcoma in vivo. This compound inhibits calf thymus DNA topoisomerase I (Topo I) but does not stimulate cleavable complex formation in the nuclei of Chinese hamster ovary (CHO)-K1 cells, suggesting that it inhibits Topo I in a manner different from that of camptothecin (CPT). To clarify the mode of action of TAN-1518 A, we examined its effects on the eukaryotic microorganism Schizosaccharomyces pombe (S. pombe), which does not require Topo I as an essential factor for growth. TAN-1518 A inhibited purified S. pombe Topo I as potently as did CPT. TAN-1518 A, unlike CPT, did not stimulate Topo I-induced DNA cleavage; instead, it inhibited CPT-induced cleavable complex formation. We constructed a S. pombe strain, IR9, that produced excess Topo I. IR9 was hypersensitive to CPT, but its growth was not affected by TAN-1518 A. The CPT-mediated death of IR9 cells was reduced dramatically in the presence of TAN-1518 A. These findings clearly demonstrate that TAN-1518 A is a specific inhibitor of Topo I in eukaryotic cells and also suggest that this agent inhibits some earlier step(s) that occurs before the formation of cleavable complex on DNA strands in the catalytic cycle of this enzyme.


http://www.sciencedirect.com/science/article/B6T4P-43T1MD9-C/2/5d7b126b6d0d277b9221cdf345ff26de

The levels and activities of pulmonary microsomal CYP1A1 and CYP1A2 in 40-day-old male and female, and 120-day-old male offspring of pregnant rats treated with five weekly 0.1 \( \mu \)g/kg doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during gestation and lactation were compared with those in age-matched offspring of untreated dams. The CYP1A1-preferential activity, ethoxyresorufin O-deethylase (EROD), was comparably induced 5.3- and 6.4-fold in 40-day-old male and female offspring, respectively, but was not induced in 120-day-old male offspring, of TCDD-treated dams. Similarly, CYP1A1 protein was induced in 40-day-old female or male offspring of untreated dams but was undetectable in 120-day-old offspring of untreated or treated dams. CYP1A2 activity, as measured by the bioactivation of 2-amino-3,4-dimethylimidazo[4,5-f]quinoxoline (MeIQ) to mutagens in the Ames assay, was elevated 11.1- and 5.5-fold in 40-day-old female and male offspring, respectively, of TCDD-treated dams, but was unaffected by TCDD exposure in 120-day-old offspring. CYP1A2 protein was undetectable in 40-day-old male or female offspring of untreated dams or in 120-day-old male offspring of treated or untreated dams; it was detected in 40-day-old offspring of treated dams, at a level that was higher
in females than in males. The results show that gestational and lactational exposure to TCDD causes long-lasting and gender-preferential induction of CYP1A1 as well as CYP1A2 in the lungs of rat offspring.


http://www.sciencedirect.com/science/article/B6T4P-3YW28BX-C/2/e7365c183910b9ebc865dce7041bb8df

The present study investigated the role of growth hormone (GH) in hepatic CYP3A18 and CYP3A9 expression in prepubertal and adult male rats. For comparison, the effects of GH on CYP3A2 expression were also measured. Initial experiments demonstrated that CYP3A18 mRNA levels were greater during puberty and adulthood than during the prepubertal period, CYP3A9 mRNA was not expressed until puberty and its expression increased in adulthood, and CYP3A2 mRNA levels were relatively constant from prepuberty to adult life. Hypophysectomy, which results in the loss of multiple pituitary factors including GH, increased CYP3A2 and CYP3A18 mRNA expression 3- to 4-fold, but it did not affect CYP3A9 mRNA levels or CYP3A-mediated testosterone 2[beta]- or 6[beta]-hydroxylase activity in adult rats. GH administered as twice daily s.c. injections (0.12 [mu]g/g body weight) to hypophysectomized or intact adult rats did not affect CYP3A18 or CYP3A9 mRNA expression. The same treatment decreased CYP3A2 mRNA and protein and testosterone 2[beta]- and 6[beta]-hydroxylase activity levels in intact but not hypophysectomized rats. However, in intact prepubertal rats, intermittent GH administration decreased CYP3A18 and CYP3A2 mRNA levels, but a higher dosage (3.6 [mu]g/g) was required to suppress CYP3A2. Overall, the present study demonstrated that: (a) the constitutive expression of CYP3A18, CYP3A9, and CYP3A2 does not require the presence of GH, (b) CYP3A18 is more sensitive than CYP3A9 to GH modulation in adult rats; and (c) CYP3A2 is less sensitive to the suppressive influence of GH during the prepubertal period than during adult life.


http://www.sciencedirect.com/science/article/B6T4P-47795G1-R7/2/3825282594ece34ccff8b045f937b8ed6

Cyclooxygenase (COX), a key enzyme in the formation of prostanoids, is known to exist in two isoforms: an inducible enzyme (COX 2) and a constitutive form (COX 1). Both enzymes are inhibited by non-steroidal anti-inflammatory drugs (NSAID), but only marginal selectivity has thus far been reported. In this study, we report on a novel selective inhibitor of COX 2, CGP 28238 (6-(2,4-dinuorophenoxy)-5-methyl-sulfonylamino-1-indanone). Human washed platelets were used as a source of COX 1. For IL-1 stimulated rat mesangial cells we demonstrated the almost exclusive presence of COX 2 in western blot and mRNA analysis. Therefore these two model systems were chosen for selectivity testing. With an 50 value of 15 nM, CGP 28238 blocked COX 2 activity in a similar concentration range to that of other potent NSAID such as indomethacin and diclofenac (50 = 1.17-8.9 nM). However, in contrast to these reference NSAIDs, CGP 28238 was at least 1000-fold less potent in inhibiting COX 1. Using other cell systems reported to express COX 1 or COX 2, we obtained a similar selectivity for COX 2. Thus, on the basis of our findings, CGP 28238 is a novel, highly potent and selective inhibitor of COX 2 and may be a lead compound for a new generation of potent anti-inflammatory drugs with an improved side-effect profile.

Caco-2 cells are a widely used model in drug development to study intestinal drug transport and metabolism. Recently, serotonin (5-hydroxytryptamine, 5-HT) has been characterized as a highly selective substrate of human UDP-glucuronosyltransferase UGT1A6 [Krishnaswamy S, Duan SX, von Moltke LL, Greenblatt DJ, Court MH. Validation of serotonin (5-hydroxytryptamine) as an in vitro substrate probe for human UDP-glucuronosyltransferase (UGT) 1A6. Drug Metab Disp 2003; 31:133-9], an isoenzyme which conjugates planar phenols and is inducible by Ah receptor agonists and by oxidative/electrophile stress. To gain more insight into intestinal 5-HT disposition, uptake and metabolism of this neurotransmitter was studied in Caco-2 cell monolayers. It was found that 5-HT was taken up from the basolateral and to a lesser extent from the apical surface. It was mainly excreted basolaterally as 5-HT glucuronide. 5-HT UGT activity and UGT1A6 mRNA were induced by Ah receptor agonists and by oxidative stress generated by tert-butylhydroquinone and by isomeric thymoquinone, a potential antitumor agent and constituent of Nigella sativa seeds, commonly used as a condiment in the Middle East. While UGT1A6 induction was clearly detectable in NAD(P)H:quinone oxidoreductase 1 (NQO1)-deficient Caco-2 cells, it was not induced in NQO1-efficient HT-29 colon adenocarcinoma cells. The results suggest that - in addition to its detoxification function - intestinal UGT1A6 contributes to intestinal homeostasis of 5-HT from dietary sources and from release by enterochromaffin cells.

Kohle, C., B. Mohrle, et al. (2003). "Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians." Biochemical Pharmacology 65(9): 1521.

http://www.sciencedirect.com/science/article/B6T4P-488GB0P-1/2/d2ef1d1c022f0a8b4bfff2e9f98202b16

Polymorphisms of drug metabolizing enzymes are frequently associated with diseases and side effects of drugs. Recently, a TATA box mutation of UGT1A1 (UGT1A1*28), a common genotype leading to Gilbert's syndrome, and several missense mutations of other UDP-glucuronosyltransferase 1 (UGT1) family members have been described. Furthermore, co-occurrence of UGT1A1*28 and UGT1A6*2 has been observed. In order to elucidate the basis for co-occurrence of UGT1 mutations, fluorescence resonance energy transfer techniques were developed for rapid determination of polymorphisms of three UGT isoforms (UGT1A1*28, 1A6*2, and 1A7*2/*3). Hundred healthy Caucasians and 50 Egyptians were genotyped. All genotypes followed the Hardy-Weinberg equilibrium. Only three major haplotypes were found, including a haplotype consisting of allelic variants of all three isoforms (29% in Caucasians and 22% in Egyptians), all leading to reduced UGT activity. Frequent haplotypes containing several UGT1 allelic variants should be taken into account in studies on the association between diseases, abnormal drug reactions, and UGT1 family polymorphisms.


http://www.sciencedirect.com/science/article/B6T4P-3TCW06S-
The coincidence of mutated alleles of CYP2C18 and CYP2C19 was studied in 154 Japanese subjects. The mutant alleles of CYP2C18 studied were CYP2C18m1 (T204 -> A substitution in exon 2) and CYP2C18mFR (A-460 -> T substitution in the 5'-flanking region), and those of CYP2C19 were CYP2C19m1 (G689 -> A substitution in exon 5) and CYP2C19m2 (G636 -> A substitution in exon 4). They were identified by polymerase chain reaction and restriction fragment length polymorphism. The results indicate that genotypes of CYP2C18m1 and CYP2C18mFR are completely coincident with those of CYP2C19m2 and CYP2C19m1, respectively. The finding suggests that the mutations of CYP2C18 and CYP2C19 examined in the present study are very closely linked with each other (i.e. CYP2C18m1 vs CYP2C19m2 and CYP2C18mFR vs CYP2C19m1), at least in a Japanese population.


http://www.sciencedirect.com/science/article/B6T4P-3VW752G-G/2/22b3868497f26d25f3efac5b75532e6c

The cytochromes P450 are a large family of haemoproteins which have a major role in the oxidative metabolism of a wide range of xenobiotics and some endogenous compounds. In this study the presence of individual members of the CYP1, CYP2 and CYP3 P450 families has been investigated by reverse transcriptase polymerase chain reaction in different regions of normal human brain consisting of frontal and temporal cortices, mid brain, cerebellum, pons and medulla. All the P450s were identified in specific regions of brain with CYP1A1 and CYP2C being the most frequently expressed forms of P450. Sequencing identified the CYP2C PCR product as CYP2C8. This study indicates that individual P450 mRNAs are present in human brain and are found in specific brain regions. The distribution of individual P450s in different regions of human brain is likely to be highly important in determining the response of the brain to toxic foreign compounds.


http://www.sciencedirect.com/science/article/B6T4P-48FCJWV-2/2/bc0ec075063967b03ab8922aa6058ccb

Cytochrome P450 (CYP) drug metabolising enzymes CYP1A1 and CYP1B1 are regulated through the ligand-activated aryl hydrocarbon (Ah) receptor. Differential expression of CYP1A1 and CYP1B1 mRNA and protein has previously been reported in human tissues with the presence of the message often extrapolated to indicate the presence of protein. The aim of this study was to clarify these potentially misleading findings, by analysing components of the Ah receptor pathway (CYP1B1, CYP1A1, Ah receptor and ARNT) using a combination of quantitative real-time RT-PCR and immunoblotting. Three human cell lines (MOG-G-CCM, MCF7 and HEPG2) known to differentially express CYP1A1 and CYP1B1 mRNA and protein were exposed to the Ah receptor agonist 3-MC, and basal and inducible levels of CYP1A1, CYP1B1, Ah receptor and ARNT were determined. The key finding of this study was the demonstration of equivalent levels of CYP1B1 mRNA in both the treated and untreated MOG-G-CCM cell lines, with expression of the corresponding CYP1B1 protein only after exposure to an Ah receptor agonist. This finding suggests that a post-transcriptional mechanism is involved in the regulation of CYP1B1. In addition, the expression pattern of CYP1B1 mRNA and protein in the MOG-G-CCM cells highlights this cell line as a potential model for studying CYP1B1 expression in human
tissue.


http://www.sciencedirect.com/science/article/B6T4P-42D81KP-C/2/2a382a799e4dc95fa73c21b0300163b1

Ribonuclease H (RNase H), an enzyme that cleaves an RNA sequence base-paired with a complementary DNA sequence, is proposed to be the mediator of antisense phosphorothioate oligonucleotide (S-oligo) lethality in a cell. To understand the role of RNase H in the killing of the parasitic protozoan Leishmania by antisense S-oligos, we expressed an episomal copy of the Trypanosoma brucei RNase H1 gene inside L. amazonensis promastigotes and amastigotes that constitutively express firefly luciferase. Our hypothesis was that S-oligo-directed degradation of target mRNA is facilitated in a cell that has higher RNase H activity. Increased inhibition of luciferase mRNA expression by anti-luciferase S-oligo and by anti-miniexon S-oligo in these stably transfected promastigotes overexpressing RNase H1 was correlated to the higher activity of RNase H in these cells. The efficiency of killing of the RNase H overexpressing amastigotes inside L. amazonensis-infected macrophages by anti-miniexon S-oligo was higher than in the control cells. Thus, RNase H appears to play an important role in the antisense S-oligo-mediated killing of Leishmania. Chemical modification of S-oligos that stimulate RNase H and/or co-treatment of cells with an activator of RNase H may be useful for developing an antisense approach against leishmaniasis. The transgenic Leishmania cells overexpressing RNase H should be a good model system for the antisense-mediated gene expression ablation studies in these parasites.


http://www.sciencedirect.com/science/article/B6T4P-3RJPBNK-5/2/502410cf1e0b7196243353e6330f84c7

We have examined the expression of three prostaglandin (PG) receptors, EP2, EP4, and FP, in a nonpigmented ciliary epithelial cell line (ODMC1-2) and in human ciliary muscle (HCM) cells. Total RNA preparations from either ODMCl-2 or HCM cells were subjected to reverse transcription-polymerase chain reaction (RT-PCR) with sense and antisense primers for each of the three PG receptors. The RT-PCR generated DNA products of predicted sizes corresponding to the EP2, EP4, and FP receptors in both ODMCl-2 and HCM cells. PCR products corresponding to each receptor were hybridized with specific 32P-labeled probes and, for further confirmation, digested with appropriate restriction enzymes. Pharmacological studies with the EP2 receptor-selective agonist butaprost resulted in a significant increase in the cyclic AMP level in ODMCl-2 cells. The stimulation of cyclic AMP in ODMCl-2 cells by PGE2 and 11-deoxy PGE1, the respective EP1/EP2/EP3/EP4 and EP2/EP3/EP4 receptor agonists, was concentration-dependently inhibited by the EP4 receptor-selective antagonist AH23848. These results conclusively demonstrate the presence of both mRNA and protein for EP2, EP4, and FP receptors in ODMCl-2 and HCM cells.

against human and rodent tumor cells: Evidence for an alternate transport system?" Biochemical Pharmacology 51(3): 301.

http://www.sciencedirect.com/science/article/B6T4P-3VXH2BS-G/2/2e5642f6371b9d9b269b3809f20202b9

The efficacy of taxol against a wide range of sensitive and refractory solid tumors has prompted extensive investigation into the factors that influence its cytotoxicity. Our preliminary observations indicated that taxol had a superior antitumor effect against human cells (Daudi, K562, 2008, 2008/C13*, 2780 and C70) compared with its effect against rodent cells (WS, WR, NIH3T3, and CHO). Although verapamil, an inhibitor of P-glycoprotein function, markedly increased the efficacy of taxol against the rodent cells (WS, WR, and CHO), the expression of P-glycoprotein was found only at low levels in the WR cells. In addition, levels of the multidrug resistance-associated protein (MRP), as assessed by reverse transcriptase-polymerase chain reaction analysis, were found to be higher in the human than in the rodent cells, although MRP mRNA was not detected by northern blotting. Transport studies indicated that the reduced sensitivity of the rodent cells to taxol was due to decreased intracellular taxol levels and reduced intracellular binding. However, no correlation was found between the intracellular binding of taxol and the intracellular levels of [alpha]- and [beta]-tubulin, or the intracellular concentration of polymerized tubulin. These studies were extended further by assessing the binding of taxol to semi-purified microtubule proteins from WS, CHO and 2008/C13* cells in vitro. The microtubule protein preparations from WS, CHO and 2008/C13* cells, which have a 50-fold difference in their sensitivity to taxol, were found to bind equal amounts of radiolabeled taxol, and this binding was inhibited (80%) in the presence of unlabeled taxol. These results lead us to propose the presence in the rodent cells of an alternative taxol transport system that is distinct from the P-glycoprotein and MRP systems.


http://www.sciencedirect.com/science/article/B6T4P-3RJ912M-3/2/a304bc8fedeaa2992f4108e2945382bb9

Taxol-resistant clones from a human ovarian carcinoma cell line (2008) were selected by an initial exposure to 0.05 [mu]M (2008/13) or 0.5 [mu]M (2008/17) taxol. Thereafter, a series of clones with increasing taxol resistance were derived from the 2008/17 and 2008/13 cells by stepwise sequential exposure to increasing concentrations of taxol. The 2008/17 clones displayed a classical P-glycoprotein-mediated drug-resistance phenotype. In contrast, the 2008/13 clones followed the classical P-glycoprotein-mediated resistance phenotype until a 245-fold taxol-resistant clone (2008/13/2) was obtained, which was followed by a further increase in the degree of resistance but significant down-regulation of P-glycoprotein expression in the 252-fold taxol-resistant 2008/13/4 cells. This clone (2008/13/4) also accumulated significantly higher intracellular levels of taxol than those expressing the P-glycoprotein. No correlation between the expression of the multidrug resistance-associated protein and taxol resistance was observed. Verapamil increased the sensitivity of all drug-resistant clones to taxol, and this was probably related to the ability of verapamil to increase the intracellular concentration of taxol (except in the case of 2008/13/4 cells). The 2008/17 clones were highly cross-resistant to Adriamycin(R), etoposide, and vincristine. They also displayed a low level of cross-resistance to camptothecin but were not cross-resistant to cisplatin. The taxol-resistant 2008/13 clones displayed a similar pattern of crossresistance for all drugs (except Adriamycin). The 2008/13 clones were only 2- to 4-fold cross-resistant to Adriamycin. The levels of [alpha]-tubulin and [beta]-tubulin were similar in the parental 2008 and taxol-resistant 2008/13/4 cells. Furthermore, the in vitro binding of [3H]taxol to semipurified microtubule preparations derived from the parental 2008 and the taxol-
resistant 2008/13/2 and 2008/13/4 cells was similar. These results show that in human ovarian carcinoma cells resistance to taxol can be acquired via as yet undescribed mechanisms.


http://www.sciencedirect.com/science/article/B6T4P-45FYNH5-2/2/8250adc13e081033bf8db169a042757b

Paclitaxel, an antimitotic, anticancer agent, induces cell cycle arrest in the mitotic phase by binding to the [beta]-tubulin subunit and forming highly stable microtubule polymers that resist depolymerization. The overexpression of the P-glycoprotein (P-gp) and/or alteration in the cellular microtubules is associated with the development of paclitaxel resistance. However, we have established a paclitaxel-resistant human ovarian carcinoma subline (2008/13/4) wherein the degree of resistance could not be correlated with overexpression of P-gp, alterations in the [alpha]- and [beta]-tubulin isotypes, or changes in the drug-binding affinity of the microtubules. mRNA differential display analysis revealed the overexpression of sorcin, a calcium-binding protein in the 2008/13/4 cells. However, no detectable changes in the intracellular calcium levels were detected in the parental and the paclitaxel-resistant variant. Furthermore, co-treatment with A23187, a calcium ionophore, did not alter the cytotoxicity of paclitaxel against the parental and the paclitaxel-resistant cells. Transfection of the parental 2008 cells with full-length sorcin cDNA induced a low level (3-5-fold) of paclitaxel resistance. In addition, transfection of human breast cancer cells with the full-length sorcin cDNA also led to the induction of a low level of paclitaxel resistance in the transfectants. Although the overexpression of sorcin did not produce high levels of paclitaxel resistance, the results obtained present compelling evidence of the involvement of sorcin in developing low-level paclitaxel resistance in a variety of tumor cells. The precise biochemical mechanism(s) by which sorcin overexpression induces low-level paclitaxel resistance is currently under investigation.


http://www.sciencedirect.com/science/article/B6T4P-45YO4G7-1/2/8d56a21e4828d4aa53b6d1a41748d5a9

Exposure of cultured glomeruli to benzo[a]pyrene (BaP), a carcinogenic hydrocarbon, modulates mesangial and visceral epithelial cell proliferation in vivo and in vitro. The present studies were conducted to characterize mitogenic signaling profiles of cultured glomeruli following repeated cycles of BaP challenge. Enhanced rates of DNA synthesis were observed by the third passage in randomly cycling cultures after single or repeated carcinogen exposure. This response was characterized by upregulation of mitogenic sensitivity during early cell cycle transit, and increased cell numbers under restrictive growth conditions. The mitogenic response to platelet-derived growth factor (0.5 to 25 ng/mL), acidic fibroblast growth factor (2.5 to 10 ng/mL), basic fibroblast growth factor (0.05 to 5 ng/mL), epidermal growth factor (0.5 to 5 ng/mL), or conditioned medium was not enhanced by hydrocarbon challenge. BaP-treated cultures exhibited anchorage-independent growth and increased expression of hepatocyte growth factor mRNA and E-cadherin protein. Binding of activator protein-1 to DNA was enhanced in BaP-treated cells, but this change did not involve truncation or mutation of the c-jun delta region. Collectively, the data demonstrate that repeated cycles of BaP injury alter mitogenic signaling profiles in cultured glomerular cells. These alterations may contribute to deregulation of proliferative control following carcinogen exposure in vivo.

http://www.sciencedirect.com/science/article/B6T4P-3V394WK-6/2/4c5a7f0002ba5dd1209da0a9a4dc9c39

The discordance between P-glycoprotein (P-gp) expression and functionality [as measured by the efflux of doxorubicin (DOX)] was analyzed in a DOX-sensitive human breast cancer cell line (HTB-123) with high reactivity against four P-gp specific monoclonal antibodies (C219, MRK-16, UIC2, and 4E3). Reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting analyses confirmed the overexpression of MDR1 mRNA and P-gp in this cell line. However, incubation of cells with efflux blockers, verapamil (VPL) or dipyridamole (DPD), did not enhance cellular (DOX) accumulation or cytotoxicity. Upon incubation with 12-O-tetradecanoylphorbol-13-acetate (TPA), HTB-123 cells retained less DOX than control cells and were sensitive to the efflux blockers verapamil or dipyridamole. These observations suggest that 12-O-tetradecanoylphorbol-13-acetate-induced P-gp phosphorylation may be associated with induction of P-gp-mediated drug efflux in the HTB-123 cell line.


http://www.sciencedirect.com/science/article/B6T4P-3YVDRS5-56/2/4b872a7f93820d37917101ea87ea28eb

The expression of drug resistance-associated mdr-1, GST [p], and topoisomerase II genes was analyzed in cell cycle phase enriched populations of doxorubicin-resistant murine leukemic P388/R-84 cells. Flow cytometric analysis of bromodeoxyuridine (BrdU) incorporation and staining with anti-BrdU antibodies was used to confirm the purity of cell cycle phase enriched populations obtained by centrifugal elutriation. Doxorubicin (DOX) and daunorubicin (DNR) accumulation was significantly lower in S-phase cells, and coincubation with verapamil (VPL) or chlorpromazine (CPZ) enhanced DOX and DNR accumulation more in S-phase than in G1- and G2/M-phase cells. While the cellular content of mdr-1 and topoisomerase II mRNAs changed, GST [p] mRNA content remained constant during the cell cycle. S-phase cells had about 3-fold higher mdr-1 mRNA content than G1- and G2/M-phase cells. In G1 cells, P-glycoprotein expression, as determined by C219 monoclonal antibody, was 12% less than that of S and G2/M cells. Topoisomerase II mRNA content increased with the progression of cell cycle and peaked in G2/M cells. These observations suggest that cell cycle stage related changes in expression of drug resistance markers may have a major bearing on chemosensitivity of drug-resistant cells.


http://www.sciencedirect.com/science/article/B6T4P-475TFC1-DJ/2/fde633c9a408a50e237c3ff26644051

The murine Cyp2a-4 and Cyp2a-5 genes encode P450 isoforms catalysing testosterone 15[alpha]-hydroxylase and coumarin 7-hydroxylase (COH) activities, respectively. Two days after
the administration of a hepatotoxic dose of cerium chloride (2 mg/kg i.v.), COH activity was increased 3.2-fold in the liver of DBA/2 mice. Three and 4 days after the cerium treatment, coinciding with the occurrence of overt liver damage, there was a dramatic decrease in COH activity. The activities of testosterone 15[alpha]-hydroxylase and the Cypla-1-mediated 7-ethoxyresorufin O-deethylase (EROD) were decreased in response to cerium. Much less pronounced changes in the enzyme activities occurred in the C57BL/6 mouse liver. Northern blot analysis showed a 21-fold increase in the hepatic Cyp2a-4/5 mRNA in the DBA/2 mice at day 2, whereas no increase occurred in the C57BL/6 mice. Also in the kidneys the increase in COH activity and in Cyp2a-4/5 mRNA was marked only in the DBA/2 mice. A polymerase chain reaction-mediated analysis method utilizing a unique Pstl restriction site in the Cyp2a-5 cDNA was used to differentiate between the highly homologous Cyp2a-4 and Cyp2a-5 mRNAs. Cerium was found to increase the amount of hepatic and renal Cyp2a-4 and Cyp2a-5 mRNA only in the DBA/2 mice. These data indicate that the Cyp2a-4/5 complex is regulated in a different way in DBA/2 and C57BL/6 mice and that some association exists between the development of liver damage and COH induction.


http://www.sciencedirect.com/science/article/B6T4P-408TGM7-8/2/e86e0e943bf134e0845cea0e75c3c67b

Troglitazone, a novel thiazolidinedione drug used to treat non-insulin-dependent diabetes mellitus, is a selective ligand for the peroxisome proliferator-activated receptor-[gamma] (PPAR[gamma]). Recent results indicate that PPAR[gamma] activation by thiazolidinediones regulates adipose tissue- and monocyte/peritoneal macrophage-derived cytokine expression in vitro. We evaluated whether troglitazone may also negatively regulate cytokine expression in the liver, which harbors the majority of the body's resident macrophages but which only weakly expresses PPAR[gamma]. Lean C57BL6 mice and genetically obese KKAy mice were chronically treated with troglitazone (100 mg/kg/day for 2 weeks). At the end of treatment, hepatic expression of tumor necrosis factor (TNF)-[alpha] and interleukin (IL)-6 mRNA was quantitatively determined by kinetic polymerase chain reaction both under basal conditions and after stimulation with lipopolysaccharide (LPS). Both untreated lean and obese mice exhibited low levels of baseline TNF-[alpha] and IL-6 mRNA expression and responded with a dramatic increase in hepatic cytokine transcripts and TNF-[alpha] protein expression following a challenge with LPS. Similar to the effects on white adipose tissue, troglitazone not only down-regulated the baseline levels of hepatic TNF-[alpha] and IL-6, but also greatly attenuated the inducing effects of LPS. The extent of this inhibitory effect of troglitazone was higher in obese KKAy mice than in lean mice and was also reflected by markedly down-regulated hepatic TNF-[alpha] protein expression. These data demonstrate that chronic administration of troglitazone is associated with a greatly attenuated responsiveness towards inducers of hepatic TNF-[alpha] and IL-6 production. The possible biological consequences of these effects, however, have not yet been assessed.


http://www.sciencedirect.com/science/article/B6T4P-475TDND-H0/2/1b83c772c3ef8c92d302bd20f2aa469

EO9, a new bioreductive indoloquinone alkylating agent, requires activation by a two-electron
reduction, which can be catalysed by the NAD(P)H: quinone oxidoreductase DT-diaphorase (DTD) (EC 1.6.99.2). Seven human and four murine tumor cell lines from different histological origins were evaluated for their DTD enzyme activity (evaluated using dichlorophenolindophenol and EO9 as substrates), DTD gene expression and chemosensitivity to EO9. In general the cell lines could be divided into two groups: leukemic cells which were relatively resistant to EO9 (50 [ges] 0.5 [mu]M) and had no measurable DTD activity, and solid tumor cells, which were more sensitive to the drug (50 90 nmol/min/mg). The expression of the DTD gene was measured by semiquantitative PCR in the human cell lines and an excellent correlation between gene expression and enzyme activity was observed (r2 = 0.94). A higher DTD gene expression also correlated with higher chemosensitivity to EO9. Protection of chemosensitivity to EO9 by dicoumarol, a strong and specific inhibitor of DTD activity, was dependent on duration of exposure and concentration of dicoumarol. Inhibition was best observed by short exposure to dicoumarol and EO9 together, demonstrating that bioactivation of EO9 by DTD is essential. In conclusion, DTD activity and expression appear to predict sensitivity to EO9 in a variety of cell lines. Evaluation of activity or expression in patients' tumor samples might predict the response to EO9.


Genistein has been reported to be a natural chemopreventive in several types of human cancer. In our prior study, soy isoflavones were shown to induce cell cycle arrest and apoptosis of bladder cancer cells in the range of human urine excretion. This study was designed to identify the novel molecular basis underlying anti-angiogenic activities of soy isoflavones. An immortalized E6 and five human bladder cancer cell lines were studied by immunoassay, flow cytometry, functional activity, reverse transcription-polymerase chain reaction, immunoblotting, and transwell co-culture in vitro. The efficacy of soy isoflavones on angiogenesis inhibition in vivo was examined by nude mouse xenograft and chick chorioallantoic membrane bioassay. Factors analyzed included angiogenic factors, matrix-degrading enzymes, and angiogenesis inhibitors. Genistein was the most potent inhibitor of angiogenesis in vitro and in vivo among the isoflavone compounds tested. It may also account for most of the reduced microvessel density of xenografts observed and the suppressed endothelial migration by soy isoflavones. Genistein exhibited a dose-dependent inhibition of expression/excretion of vascular endothelial growth factor165, platelet-derived growth factor, tissue factor, urokinase plasminogen activator, and matrix metalloprotease-2 and 9, respectively. On the other hand, there was an up-regulation of angiogenesis inhibitors--plasminogen activator inhibitor-1, endostatin, angiostatin, and thrombospondin-1. In addition, a differential inhibitory effect between immortalized uroepithelial cells and most cancer cell lines was also observed. Altogether, we discovered that tissue factor, endostatin, and angiostatin are novel molecular targets of genistein. The current investigation provides further evidence in support of soy-based foods as natural dietary inhibitors of tumor angiogenesis.

Functional studies have shown that 6-chloro-9-[(3-methyl-2-butenyl)oxy]-3-methyl-1H-2,3,4,5-tetrahydro-3-benzazepine (SKF 104078) has very low affinity for prejunctional [alpha]2-adrenoceptors ([alpha]2-AR) in the guinea pig atrium. In this study, we have cloned guinea pig homologues of the human [alpha]2-C10, [alpha]2-C2 and [alpha]2-C4 AR subtypes and have studied them in isolation by heterologous expression in cultured mammalian cells. Oligonucleotide primers, designed from conserved areas of the human [alpha]2-ARs were used in a polymerase chain reaction (PCR) with template cDNA synthesized from guinea pig atrial mRNA. Three PCR products were obtained that shared identity with the three human [alpha]2-AR subtypes. A guinea pig (gp) genomic library was screened with a cDNA clone encoding a portion of the gp-[alpha]2A, and genes containing the complete coding sequences of the guinea pig [alpha]2A, [alpha]2B, and [alpha]2C AR subtypes were obtained. These guinea pig genes were subcloned into a eukaryotic expression plasmid and were expressed transiently in COS-7 cells. The binding of the [alpha]2-selective antagonist [3H]MK-912 to membranes prepared from these cells was specific and of high affinity with Kd values of 810 pM for gp-[alpha]2A, 2700 pM for gp-[alpha]2B and 110 pM for gp-[alpha]2C. Competition for the binding of [3H]MK-912 by SKF 104078 indicated that it was of moderately high affinity (~ 100 nM) but that it was not selective for any of the guinea pig [alpha]2-AR subtypes. Co-expression of guinea pig [alpha]2-AR subtypes with a cyclicAMP-responsive chloramphenicol acetyltransferase (CAT) reporter gene resulted in agonist-dependent modulation of CAT activity. For the gp-[alpha]2A, a biphasic response was obtained with low concentrations of noradrenaline (NE) decreasing forskolin-stimulated CAT activity and high concentrations causing a reversal. For the gp-[alpha]2B, NE produced mostly potentiation of forskolin-stimulated activity, and for the gp-[alpha]2C, NE caused mainly inhibition. Overall, the pharmacology of the cloned guinea pig [alpha]2-AR subtypes was in agreement with data obtained for the native guinea pig receptors and was functionally similar to that of the cloned human [alpha]2-AR subtypes.


http://www.sciencedirect.com/science/article/B6T4P-44RNPVM-N/2/0b57bbe531e9cece564be658dc5bc36f

We investigated the mitochondrial gene expression related to cardiac function and ventricular fibrillation (VF) in ischemic/reperfused nondiabetic and diabetic myocardium. To identify potentially more specific gene responses we performed subtractive screening, Northern blotting, and reverse transcription-polymerase chain reaction (RT-PCR) of mitochondrial genes expressed after 30 min ischemia followed by 120 min reperfusion in isolated rat hearts that showed VF or did not show VF. Cytochrome oxidase B subunit III (COXBIII) and ATP synthase subunit 6, studied and selected out of 40 mitochondrial genes by subtractive screening, showed an expression after 30 min ischemia (no VF was recorded) in both nondiabetic and diabetic subjects. Upon reperfusion, the down-regulation of these genes was only observed in fibrillated hearts. Such a reduction in signal intensity was not seen in nonfibrillated myocardium. In additional studies, nondiabetic and diabetic hearts, without the ischemia/reperfusion protocol, were subjected to electrical fibrillation, and a significant reduction in COXBIII and ATPS6 mRNA signal intensity was observed indicating that VF contributes to the down-regulation of these genes. Cardiac function (heart rate, coronary flow, aortic flow, left ventricular developed pressure) showed no correlation between the up- and down-regulation of these mitochondrial genes in both nondiabetic and diabetic ischemic/reperfused myocardium. Our data suggest that COXBIII and ATPS6 may play a critical role in arrhythmogenesis, and the stimulation of COXBIII and ATPS6 mRNA expression may prevent the development of VF in both nondiabetic and diabetic ischemic/reperfused myocardium.
Agonist-mediated regulation of \([\beta2]\)-adrenoceptors in mononuclear leukocytes has been examined at the protein but not at the mRNA level. In the present study, incubation of mononuclear leukocytes with the \([\beta2]\)-agonist (-)-isoproterenol (10-6 M) for up to 42 hr led to a maximum decrease in both \([\beta2]\)-adrenoceptor mRNA concentration and total receptor number of ca. 56 and 70%, respectively. The decrease in the mRNA level, however, was slower than for the protein level. After 4 hr of incubation with the \([\beta2]\)-agonist, the protein level decreased to a minimum of 65% of the initial amount, while an incubation of 8 hr was necessary to reach a similar decrease in the level of mRNA (69% of the initial level). Measurements of mRNA stability revealed a reduction in the half-life of \([\beta2]\)-adrenoceptor mRNA from 2.7 to 1.1 hr following 4 hr of incubation with (-)-isoproterenol. Our data clearly demonstrate that treatment of human mononuclear leukocytes with (-)-isoproterenol induces a \([\beta2]\)-adrenoceptor down-regulation together with a slower time course of mRNA down-regulation which is partly due to a reduction of mRNA stability.

Sister of P-glycoprotein (spgp) is a gene that is closely related to the P-glycoprotein family (Pgps). This class of proteins belongs to the superfamily of ATP-binding cassette transporters and is known for its involvement in pharmacological drug interactions. Therefore, this study investigated the distribution of spgp expression in different tissues known for their high levels of Pgps expression such as brain, liver, kidney, small- and large-gut mucosa. Analysis was done by using the reverse transcription-polymerase chain reaction. In addition to a high expression in the liver, we were able to demonstrate a significant spgp expression in brain grey cortex, small- and large-gut mucosa. Although Pgps are expressed in the kidney and brain capillary endothelial cells, no expression of spgp was detected in these tissues, which might indicate that spgp has no function in the blood-brain barrier and is not involved in the renal excretion of drugs.

3'-Phosphoadenosine 5'-phosphosulfate (PAPS) is the high-energy "sulfate donor" for reactions catalyzed by sulfotransferase (SULT) enzymes. The strict requirement of SULTs for PAPS suggests that PAPS synthesis might influence the rate of sulfate conjugation. In humans, PAPS is synthesized from ATP and SO42- by two isoforms of PAPS synthetase (PAPSS): PAPSS1 and PAPSS2. As a step toward pharmacogenetic studies, we have resequenced the entire coding sequence of the human PAPSS1 gene, including exon-intron splice junctions, using DNA samples from 60 Caucasian-American and 58 African-American subjects. Twenty-one genetic
polymorphisms were observed--1 insertion-deletion event and 20 single nucleotide polymorphisms (SNPs)--including two non-synonymous coding SNPs (cSNPs) that altered the following amino acids: Arg333Cys and Glu531Gln. Twelve pairs of these polymorphisms were tightly linked, and a total of twelve unequivocal haplotypes could be identified--two that were common to both ethnic groups and ten that were ethnic-specific. The Arg333Cys polymorphism, with an allele frequency of 2.5%, was observed only in DNA samples from Caucasian subjects. The Glu531Gln polymorphism was rare, with only a single copy of that allele in a DNA sample from an African-American subject. Transient expression in mammalian cells showed that neither of the non-synonymous cSNPs resulted in a change in the basal level of enzyme activity measured under optimal assay conditions. However, the Glu531Gln polymorphism altered the substrate kinetic properties of the enzyme. The Gln531 variant allozyme had a 5-fold higher Km value for SO42- than did the wild-type allozyme and displayed monophasic kinetics for Na2SO4. The wild-type allozyme (Glu531) showed biphasic kinetics for that substrate. These observations represent a step toward testing the hypothesis that genetic variation in PAPS synthesis catalyzed by PAPSS1 might alter in vivo sulfate conjugation.

Biochimica et Biophysica Acta (BBA) - Bioenergetics(2)


http://www.sciencedirect.com/science/article/B6T1S-3XY2R40-V/2/8efad893aa9330cd634ab7c0795ef80f

Recent studies have shown that the NADPH oxidase participates in the generation of superoxide anion in non-phagocytic cells. Here we report the isolation and nucleotide sequence of a cDNA for the cytochrome b-558 [alpha]-subunit of the NADPH oxidase in rat vascular smooth muscle cells (VSMCs). The coding region of the cDNA was 93% homologous to mouse and 81% to human in nucleotide sequence and 96% homologous to mouse and 89% to human in the deduced amino acid sequence. Our results provide a tool with which to explore the mechanism of superoxide anion generation in rat VSMCs and other non-phagocytic cells.


http://www.sciencedirect.com/science/article/B6T1S-3X88CMS-6/2/abf193d42329a999f430f975e2326f5

Uncoupling protein 1 (UCP1) is of demonstrated importance in mammalian thermogenesis, and early hypotheses regarding the functions of the newly discovered UCP homologues, UCP2, UCP3 and others, have focused largely on their potential roles in thermogenesis. Here we report the amino acid sequences of two new UCPs from ectothermic vertebrates. UCPs from two fish species, the zebrafish (Danio rerio) and carp (Cyprinus carpio), were identified in expressed sequence tag databases at the European Molecular Biology Laboratory. cDNAs from a C. carpio 'peritoneal exudate cell' cDNA library and from a D. rerio 'day 0 fin regeneration' cDNA library were obtained and fully sequenced. Each cDNA encodes a 310 amino acid protein with an average 82% sequence identity to mammalian UCP2s. The fish UCP2s are about 70% identical
to mammalian UCP3s, and 60% identical to mammalian UCP1s. Carp and zebrafish are ectotherms - they do not raise their body temperatures above ambient by producing excess heat. The presence of UCP2 in these fish thus suggests the protein may have function(s) not related to thermogenesis.

Biochimica et Biophysica Acta (BBA) - Biomembranes (8)


http://www.sciencedirect.com/science/article/B6T1T-47MHVW8-1/2/ad5df9df889b3031177ea54c342aae8

The aim of this work is to develop a prokaryotic system capable of expressing membrane-bound receptors in quantities suitable for biochemical and biophysical studies. Our strategy exploits the endogenous high-level expression of the membrane protein bacteriorhodopsin (BR) in the Archaeon Halobacterium salinarum. We attempted to express the human muscarinic acetylcholine (M1) and adrenergic (a2b) receptors by fusing the coding region of the m1 and a2b genes to nucleotide sequences known to direct bacterio-opsin (bop) gene transcription. The fusions included downstream modifications to produce non-native carboxyl-terminal amino acids useful for protein identification and purification. bop mRNA and BR accumulation were found to be tightly coupled and the carboxyl-terminal coding region modifications perturbed both. m1 and a2b mRNA levels were low, and accumulation was sensitive to both the extent of the bop gene fusion and the specific carboxyl-terminal coding sequence modifications included. Functional a2b adrenergic receptor expression was observed to be dependent on the downstream coding region. This work demonstrates that a critical determinant of expression resides in the downstream coding region of the wild-type bop gene and manipulation of the downstream coding region of heterologous genes may affect their potential for expression in H. salinarum.


http://www.sciencedirect.com/science/article/B6T1T-482YJ0R-2/2/7d813b567b42c3afef1ee5c053af4b5b1

We investigated the influence of intracellular pH (pHi) on [14C]-glycocholate (GC) uptake by human hepatoblastoma HepG2 cells that express sodium-independent (mainly OATP-A and OATP-8), but not sodium-dependent, GC transporters. Replacement of extracellular sodium by choline (Chol) stimulated GC uptake but did not affect GC efflux from loaded cells. Amiloride or NaCl replacement by tetraethylammonium chloride (TeACl) or sucrose also increased GC uptake. All stimulating circumstances decreased pHi. By contrast, adding to the medium ammonium or imidazole, which increased pHi, had no effect on GC uptake. In Chinese hamster ovary (CHO) cells expressing rat Oatp1, acidification of pHi had the opposite effect on GC uptake, that is, this was reduced. Changes in extracellular pH (pHo) between 7.40 and 7.00 had no effect on GC uptake at pHi 7.30 or 7.45 when pHo=pHi. Inhibition was not proportional to the pHo-pHi
difference. Intracellular acidification decreased Vmax, but had no effect on Km. In sum, sodium-independent GC transport can be affected by intracellular acidification, possibly due both to modifications in the driving forces and to the particular response to protonation of carrier proteins involved in this process.


http://www.sciencedirect.com/science/article/B6T1T-3W7XB4D-V/2/95a17135ef17cb848c138f62eeb2c316

We have cloned a cDNA for vacuolar proton-translocating pyrophosphatase of Chara corallina that is one of the closest green algae to the land plants. The deduced protein consists of 793 amino acid residues. Its sequence is 71% identical to the H+-pyrophosphatases of land plants, and is less than 46% identical to those of marine alga and phototrophic bacterium.


http://www.sciencedirect.com/science/article/B6T1T-4CX6SD7-1/2/7a298c5d9d3ed297f5866ac50c3dbff6

In this work, we studied the mRNA distribution of CNG-A3, an amiloride-sensitive sodium channel that belongs to the cyclic nucleotide-gated (CNG) family of channels, along the rat nephron. The possible involvement of aldosterone in this process was also studied. We also evaluated its expression in rats subjected to diets with different concentrations of sodium or to alterations in aldosterone plasma levels. Total RNA isolated from whole kidney and/or dissected nephron segments of Wistar rats subjected to low- and high-sodium diets, furosemide treatment, adrenalectomy, and adrenalectomy with replacement by aldosterone were analyzed by the use of Western blot, ribonuclease protection assay (RPA) and/or reverse transcription followed by semi-quantitative polymerase chain reaction (RT-PCR). CNG-A3 sodium channel mRNA and protein expression, in whole kidneys of rats subjected to high-Na+ diet, were lower than those in animals given a low-salt diet. Renal CNG-A3 mRNA expression was also decreased in adrenalectomized rats, and was normalized by aldosterone replacement. Moreover, a CNG-A3 mRNA expression study in different nephron segments revealed that aldosterone modulation is present in the cortical thick ascending loop (cTAL) and cortical collecting duct (CCD). This result suggests that CNG-A3 is responsive to the same hormone signaling as the amiloride sensitive sodium channel ENaC and suggests the CNG-A3 may have a physiological role in sodium reabsorption.


http://www.sciencedirect.com/science/article/B6T1T-3SBVWPT-B/2/0731fe1eaae37b90ab5ca97f4ecbf673

The Na+/dicarboxylate cotransporter, NaDC-1, and the Na+/sulfate cotransporter, NaSi-1, share
43% sequence identity, but they exhibit no overlap in substrate specificity. A functional chimera, SiDC-4, was prepared from NaDC-1 and NaSi-1 by homologous recombination and expressed in Xenopus oocytes. SiDC-4 contains putative transmembrane domains 1-4 of NaSi-1 (amino acids 1-139) and putative transmembrane domains 5-11 of NaDC-1 (amino acids 141-593). SiDC-4 retains the substrate specificity of NaDC-1, which suggests that the substrate recognition domain is found in the carboxy-terminal portion of the protein, past amino acid 141. However, residues that affect substrate affinity and inhibition by furosemide and flufenamate are found in the amino terminal third of the protein. The cation binding properties of SiDC-4, including a stimulation of transport by lithium, differed from both parental transporters, suggesting that cation binding is determined by interactions between the amino- and carboxy-terminal portions of the protein. We conclude that the substrate recognition site of NaDC-1 and NaSi-1 is found in the carboxy-terminal portion of the protein, past amino acid 141, but residues in the amino terminus can affect substrate affinity, inhibitor sensitivity, and cation selectivity.


Background and Aims This study aimed at functional characterization of the tight junction protein occludin using the occludin-deficient mouse model. Methods Epithelial transport and barrier functions were characterized in Ussing chambers. Impedance analysis revealed the ionic permeability of the epithelium (Re, epithelial resistance). Conductance scanning differentiated transcellular (Gc) and tight junctional conductance (Gtj). The pH-stat technique quantified gastric acid secretion. Results In occludin+/+ mice, Re was 23±5 Ω cm2 in jejunum, 66±5 Ω cm2 in distal colon and 33±5 Ω cm2 in gastric corpus and was not altered in heterozygotic occludin+/- or homozygotic occludin-/- mice. Additionally, [3H]mannitol fluxes were unaltered. In the control colon, Gc and Gtj were 7.6±1.0 and 0.3±0.1 mS/cm2 and not different in occludin deficiency. Epithelial resistance after mechanical perturbation or EGTA exposition (low calcium switch) was not more affected in occludin-/- mice than in control. Barrier function was measured in the urinary bladder, a tight epithelium, and in the stomach. Control Rt was 5.8±0.8 k[Ω] cm2 in urinary bladder and 33±6 k[Ω] cm2 in stomach and not altered in occludin-/- mice. In gastric corpus mucosa, the glandular structure exhibited a complete loss of parietal cells and mucus cell hyperplasia, as a result of which acid secretion was virtually abolished in occludin-/- mice. Conclusion Epithelial barrier characterization in occludin-deficiency points against an essential barrier function of occludin within the tight junction strands or to a substitutional redundancy of single tight junction molecules like occludin. A dramatic change in gastric morphology and secretory function indicates that occludin is involved in gastric epithelial differentiation.


Taurine was shown recently to increase the frequency at which 2-cell mouse conceptuses develop into blastocysts in vitro. For this reason and because taurine helps cells adapt to external stresses, we studied transport of this and related amino acids by preimplantation mouse conceptuses. The most conspicuous component of taurine transport in conceptuses at the 1-cell...
through blastocyst stages of development was both Na+- and Cl--dependent. This Na+- and Cl--dependent transport system interacted relatively strongly with [beta]- but not [alpha]-amino acids. By these criteria, transport system [beta] is responsible for Na+-dependent taurine transport in preimplantation mouse conceptuses. Moreover, detection of mRNA encoding the taurine transport protein (TAUT) in early conceptuses supports the theory that TAUT is a major component of system [beta]. Transport of taurine by system [beta] in 1-cell conceptuses was slower in hypotonic than in hypertonic media, whereas the reverse was true for system [beta] in blastocysts. In contrast, hypotonically stimulated Na+-independent taurine transport was, of course, more rapid in hypotonic than in hypertonic media in both 1-cell conceptuses and blastocysts. Transport via this hypotonically stimulated process also showed no sign of saturation by up to 10 mM taurine. Hypotonically stimulated taurine transport appeared transiently in 1-cell conceptuses under hypotonic conditions until they had recovered their initial volumes. Hence, we suggest that a decrease in taurine uptake via system [beta] and an increase in taurine exodus via the Na+-independent, nonsaturable transport process could contribute to the regulatory volume decrease in 1-cell conceptuses in hypotonic medium. Since taurine uptake by system [beta] in blastocysts is, however, higher in hypotonic than in hypertonic media, taurine uptake by system [beta] in blastocysts might intensify a tendency to increase cell volume in hypotonic medium. Such an increase in taurine uptake could further favor anabolic changes associated with cell swelling. In addition to contributing to regulation of cellular volume and perhaps metabolism, the hypotonically stimulated Na+-independent transport processes in early embryos have novel characteristics. Hypotonically stimulated Na+-independent taurine transport was inhibited by niflumate, N-ethylmaleimide and NaN3 but not by furosemide, iodoacetate, KCN, ouabain or [alpha]- or [beta]-amino acids. Furthermore, 4,4'-diisothiocyanostilbene-2,2'-disulfonate inhibited this transport in 1-cell conceptuses but not in blastocysts. Hence, different hypotonically stimulated Na+-independent taurine transport processes appear to be present in 1-cell conceptuses vs. blastocysts. The functions of these and other instances of developmental regulation of expression of transport processes in preimplantation conceptuses remain largely to be elucidated. Moreover, neither of the hypotonically stimulated Na+-independent taurine transport processes in conceptuses appears to have been detected in other types of cells. Instead, these processes may be unique to preimplantation conceptuses.


http://www.sciencedirect.com/science/article/B6T1T-3V3RHKC-1F/2/205e3de09ca4b5d874f957ace7023940

Recent evidence that insulin-like growth factor-1 (IGF-1) influences certain properties of H4IIE hepatoma cells independent of insulin led us to examine whether H4IIE cells express IGF-1 receptors. Competitive binding experiments demonstrated IGF-1, but not insulin or IGF-II, could compete with [125I]IGF-1. Chemical crosslinking detected a protein with an apparent mass of 175 kDa and its identity as the IGF-1 receptor [alpha]-subunit was confirmed by Western blotting. The apparent molecular mass of this protein decreased to 135 kDa following deglycosylation. Immunofluorescence microscopy verified that both insulin and IGF-1 receptors were present, although measurement of IGF-1 receptor quantity revealed they were less abundant than insulin receptors. Binding of IGF-1 was low in growing cells and higher in a quiescent cell population. Scatchard analysis confirmed that receptor density was increased in non-growing H4IIE cells while there was no apparent difference in receptor affinity. Western blot analysis and RT-PCR revealed that both protein and mRNA levels were elevated as cell growth ceased. Interestingly, addition of insulin to quiescent H4IIE cells, which stimulates cell proliferation, further increased IGF-1 receptor protein levels with a peak at 12-24 h. Distinct modes of regulating IGF-1 receptor expression are indicated.

http://www.sciencedirect.com/science/article/B6T1V-3VS7M79-6/2/afce3483aeb46098819917f3c808d9ed

We report a unique isoform of PLC[beta]4 in rat, PLC[beta]4c, that has an additional 37-nucleotide exon inserted between nucleotides 3459-3460 of the previously published PLC[beta]4a coding sequence. This insertion results in replacement of 22 amino acid residues at the carboxyl terminal tail of PLC[beta]4a with 41 unique residues. A human EST for PLC[beta]4 also contains this exon and this exon was mapped to within a 5.5 kb intron of the human PLC[beta]4 gene. PLC[beta]4c is the third PLC[beta]4 isoform to be identified which has a unique carboxyl-terminal tail. PLC[beta]4b differs from PLC[beta]4a by truncation 162 amino acid residues from the carboxyl terminus which are replaced with 10 distinct amino acid residues. Reverse transcription-polymerase chain reaction experiments show that both PLC[beta]4a and PLC[beta]4c mRNA are expressed throughout the rat brain and that PLC[beta]4c mRNA is highly expressed in the eye and cerebellum. RNase protection assays demonstrate that both PLC[beta]4a and PLC[beta]4c transcripts are abundant in the cerebellum. The different carboxyl terminal tails of PLC[beta]4 isoforms may allow for differential targeting and subcellular localization, contributing to regulation of PLC[beta]4-mediated signal transduction.


http://www.sciencedirect.com/science/article/B6T1V-414N39C-3/2/ff33786d47c58dafa5a00d394f6e21f81

We previously reported the structure of the human hexokinase type I (HKI) gene and provided direct evidence of an alternative red blood cell-specific exon 1 located in the 5' flanking region of the gene. Three unique HKI mRNA species have also been described in human spermatogenic cells. These mRNAs contain a testis-specific sequence not present in somatic cell HKI, but lack the sequence for the porin-binding domain necessary for HKI to bind to porin on the outer mitochondrial membrane. The present study reports a new mRNA isoform, hHKI-td, isolated from human sperm. hHKI-td mRNA contains both a testis-specific sequence at the 5' end common to the three other mRNA isoforms and an additional unique sequence. Screening of a cosmid library and analysis of the cosmids containing the HKI gene revealed that testis-specific sequences are encoded by six different exons. Five of these exons are located upstream from the somatic exon 1 (5.6-30 kb) and one within intron 1. This study shows that a single human HKI gene spanning at least 100 kb encodes multiple transcripts that are generated by alternative splicing of different 5' exons. Testis-specific transcripts are probably produced by a separate promoter that induces the expression of the HKI gene in spermatogenic cells.
Overexpression of the Fli-1 gene has been shown to be involved in retrovirus-induced mouse tumors. Cloning of the 5' flanking sequence of the mouse and human Fli-1 exon 1 was performed. At least two major transcription initiation sites were localized respectively at 143 and 114 nucleotides upstream of the previously defined mouse Fli-1 cDNA 5' end. The sequences flanking the CAP sites show good conservation between human and mouse (94%). The promoter region contains a potential TATA box lying 30 bp from one of the major identified CAP sites. Several conserved elements, such as GATA, EBS, GC rich, AP-2, AP-3 elements and a repetition of GA were observed next to the two major CAP sites. Furthermore, this latter was shown to form a H-DNA structure in vitro by S1 nuclease sensitivity experiments. The highly conserved 5' non-translated region of exon 1 is predicted to form a very stable hairpin structure which could regulate the Fli-1 expression at the post-transcriptional level. In Cas-Br-E-induced tumors all the proviruses are found clustered within 35 nucleotides directly upstream the Fli-1 ATG start codon, thus deleting the hairpin structure from the transcript. Promoter activity was tested using the CAT reporter gene transfected in mouse and human erythroid cell lines. No promoter activity could be detected with various mouse Fli-1 promoter-CAT constructs containing 600 bp of the 5' flanking region, the complete exon 1, the 5' end of intron 1 and/or retroviral LTR sequence. Constructions of the human homologue containing nearly 1.5 kbp of Fli-1 5' flanking region was also inactive in transfected cells. These results suggest that multiple levels of regulation might control the Fli-1 expression.

Alveoli form, in part, by subdivision (septation) of saccules constituting the gas exchange region of the immature lung. Rat lungs septate from postnatal day 4 through 14 but alveoli in male rats continue to form, by other means, until about age 44 days. In rats, we sought to identify genes involved in septation. Using differential display reverse transcription polymerase chain reaction, we cloned a cDNA, rA5D3, that had 1270 bp with an open reading frame encoding a putative polypeptide of 165 amino acids containing potential leucine zipper motifs and phosphorylation sites. Database searches indicated rA5D3 was a novel gene. rA5D3 RNA relative concentration increased 1.7-fold between postnatal days 4 and 8, decreased 4-fold between days 8 and 14, declined significantly thereafter but was still detected post alveolus formation. If the expression of rA5D3 protein resembles its RNA, its peak (postnatal day 8) is well after the onset of septation, suggesting a role other than the initiation of septation.
The N7 of guanine is thought to be the primary target for adduct and crosslink formation between cisplatin and DNA. However, reactive sites in DNA other than the N7 of guanine may also participate in the formation of adducts with cisplatin. The possibility that these interactions arise and form DNA polymerase blocking lesions was investigated by primer extension reactions with Taq DNA polymerase. To differentiate between damage produced at relatively weak sites from those formed at the N7 of guanine, a modified DNA template was synthesised with the N7 of guanine replaced with a carbon atom. This was achieved in a PCR designed to incorporate 7-deazaguanine instead of normal guanine. The sequence specificity of cisplatin damage in the modified and unmodified DNA substrates was compared (after linear amplification) by DNA sequencing gel analysis. For concentrations of cisplatin (1 to 5 [µM]) that induce blocking lesions in normal DNA, no significant damage was observed in the modified DNA. This confirmed that the N7 of guanine is the major site of adduct formation in normal DNA. At higher concentrations of cisplatin (50 [µM] and 100 [µM]), lesions were found at AA dinucleotides and other novel sites in the modified DNA. These results indicate that the N7 of guanine is not required in the formation of some cisplatin adducts.

Chan, J. Y. and M. Kwong (2000). "Impaired expression of glutathione synthetic enzyme genes in mice with targeted deletion of the Nrf2 basic-leucine zipper protein." Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1517(1): 19.

http://www.sciencedirect.com/science/article/B6T1V-41WBB32-2/2/c06719a63d5759a71844dccc66eefeb30

Transcriptional activation of genes that play a role in detoxification of xenobiotics and defense against oxidative stress is mediated in part by the antioxidant response element (ARE). For example, it has been shown that the promoters for both the heavy and light chain [gamma]-glutamylcysteine synthetase (GCSH and GCSL) genes require the ARE. CNC-bZIP factors, together with small Maf proteins, have been shown to bind as heterodimers to the NF-E2/AP-1 element, which is similar to the consensus sequence for the ARE. Nrf1 and Nrf2, two widely expressed CNC-bZIP factors, have been implicated in the regulation of genes involved in oxidative stress response. In this study, we examined the effect of nrf2 mutation on the expression of genes involved in glutathione synthesis. We observed that transcripts for gcsH and gcsL genes were decreased in nrf2-/- fibroblasts and livers. Correspondingly, glutathione levels were decreased in Nrf2 deficient livers and fibroblasts. By transient transfection studies in nrf2-/- fibroblasts, we show that transcriptional activation of reporter constructs bearing the human GCSL promoter, as well as the functional ARE of GCSH promoter, required the activator protein Nrf2. By electrophoretic mobility shift assay, recombinant Nrf2 binds the ARE of the GCSL and GCSH promoters. Overexpression of Nrf2 cDNA restored glutathione (GSH) levels in nrf2-/- fibroblasts, which correlated with increased steady state levels of gcsH and gcsL transcripts. These results establish a link between Nrf2 transcription factor and GSH biosynthesis.

Chavan, S. S., W. Tian, et al. (2002). "Characterization of the human homolog of the IL-4 induced gene-1 (Fig1)." Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression 1576(1-2): 70.

http://www.sciencedirect.com/science/article/B6T1V-45MDW5K-4/2/16fb71d7006666fd2c4016ac369e4612

Mouse interleukin-four induced gene-1 (mFig1) maps to a region of susceptibility for systemic lupus erythematosus (SLE) that includes the Sle3 locus. To begin examining this relationship in humans, we have isolated and characterized the human homolog of mFig1. Human Fig1 (hFig1) has the same eight exon genomic structure as mFig1. The predicted 63-kDa protein, like mFig1, contains a signal peptide, a large internal sequence that is most similar (43% identical over 484
amino acids) to -amino acid oxidase (LAAO), and a carboxy terminal domain with no similarity to known genes. When compared to the LAAO crystal structure, hFig1 conserves key residues thought to be involved in catalysis and binding of the flavin adenine dinucleotide cofactor. Surprisingly, the carboxy terminal domains of hFig1 and mFig1 have little similarity (Fig1, hFig1 RNA is induced by interleukin-4 (IL-4) in B lymphocytes, and is primarily found in immune tissues. Finally, hFig1 maps to the predicted mFig1 syntenic region on human chromosome 19q13.3-19q13.4, a hot spot for susceptibility to several autoimmune diseases, including SLE.


http://www.sciencedirect.com/science/article/B6T1V-442Y84S-2/2/674e71f15d4e738c5439a905ae9828bc

We report the cloning and sequence of a novel gene, BALGR, which is coding for a candidate G protein-coupled receptor (GPCR) that is distantly related to the histamine, adrenergic, serotonin and dopamine receptors. The coding region of the human BALGR gene predicts a seven transmembrane domain receptor of 451 amino acids. BALGR has 42% amino acid identity to a Medaka fish 'orphan' GPCR. BALGR gene has been conserved throughout the mammalian evolution as indicated by Southern blot analysis. BALGR gene has been assigned to chromosome 1 by typing a panel of somatic cell hybrids and its exon/intron organization has been predicted. As determined by semiquantitative RT-PCR, expression of BALGR is relatively the highest in the human brain. A high level of BALGR transcript is also detected in testes. Within the brain, Northern blot analysis revealed relatively high expression in frontal and temporal lobes, occipital pole, amygdala and hippocampus. The preferential expression of BALGR in the areas of the human brain associated with cognition, learning and memory, and its conservation in evolution, indicate a potentially important biological function for this biogenic amine-like receptor and its putative neurotransmitter ligand.


http://www.sciencedirect.com/science/article/B6T1V-42M1CPP-7/2/e1bea1e95ef63f3c7f55fb3395850587

Sequencing of rat and human vascular endothelial growth factor (VEGF) cDNA clones has previously identified a 3' untranslated region of approximately 1.9 kb, although the apparent site of polyadenylation differed in the two species, despite a high degree of sequence conservation in the region. Neither site is preceded by a canonical AAUAAA polyadenylation signal, a situation frequently found in genes that are subject to alternative polyadenylation. We have sequenced 2.25 kb of the 3' region of the mouse VEGF gene and mapped the usage of potential polyadenylation sites in fibroblasts cultured under both normoxic and hypoxic conditions. We find that two sites for polyadenylation are present in the mouse VEGF gene but the majority of transcripts contain the longer form of the 3'UTR and that their usage is not effected by environmental oxygen tension.

Studies investigating the mechanisms that govern the expression of the human angiotensin II (Ang II) type 1 receptor (hAT1R) gene have progressed slowly due to the lack of human cell lines that express the AT1R. Recently, however, an immortalized human trophoblast cell line (HTR-8/SVNeo) was demonstrated to respond to Ang II. Therefore, we utilized this cell line to characterize the AT1R expressed on the cell surface and to investigate the mechanisms by which the hAT1R gene is regulated in these cells. HTR-8/SVNeo cells were shown to express functional high affinity AT1Rs having a Bmax value of 114±11 fmol/mg protein and a Kd value of 0.14±0.1 nM. Additionally, Ang II-induced IP3 production was mediated via the AT1R. Deletional analysis of the hAT1R promoter localized a major basal regulatory sequence within the -105 to -79 bp region, relative to the transcription start site, in HTR-8/SVNeo cells. Electrophoretic mobility shift assay (EMSA) and Chromatin Immunoprecipitation (ChIP) assay demonstrated that the transcription factors, Sp1 and Sp3, interact with this region of the hAT1R promoter in vitro and in vivo. Taken together, our data demonstrate that HTR-8/SVNeo cells express functional AT1Rs and that basal level expression of this gene is regulated, in part, by Sp1 and Sp3 in this cell line.


The structural gene HMP1 encoding a cruciform DNA binding protein from Ustilago maydis has been cloned. Gene isolation was enabled by a polymerase chain reaction procedure using primers designed from amino acid sequence obtained from the purified protein. DNA sequence determination has revealed that the gene encodes a protein containing 98 amino acids with a calculated molecular weight of 10151. Comparison of the cDNA and genomic sequences indicated the presence of a single intron in the 5' coding region of the gene. The gene was over-expressed as a translational fusion with a hexahistidine leader sequence enabling affinity purification of the protein on an immobilized metal matrix. Protein isolated after over-expression exhibited cruciform binding activity, conforming earlier purified native protein results. Sequence analysis indicated that no HMG box was present and very little homology to other known cruciform binding proteins was found. It is plausible that HMP1 represents a novel class of proteins that recognize such secondary structures.


CD151 (PETA-3/SFA-1) is a member of the Transmembrane 4 Superfamily (TM4SF) of cell-surface proteins and, like other TM4SF members CD9 and CD63, is expressed by platelets,
megakaryocytes and endothelial cells. The precise function of CD151 is unknown however complexes containing CD151 and [beta]1 integrins have been isolated from a number of cell systems and studies using anti-CD151 monoclonal antibodies have suggested a role in transmembrane signalling and cell adhesion. To further investigate the function of CD151 we have determined the genomic organisation of mouse CD151 (Cd151). Cd151 spans 4 kb and contains six coding region exons. Using 5' RACE and reverse transcriptase-polymerase chain reaction (RT-PCR) we have identified three 5' UTR splice variants which arise through alternate splicing of three exons. Splice variants were detected in a number of mouse tissues by RT-PCR. Analysis of the Cd151 genomic structure reveals a high degree of structural conservation with other TM4SF molecules supporting the theory that family members have arisen from gene duplication of a common ancestral gene. Cd151 maps to chromosome 7, in close linkage to the p gene (OCA2 in humans), and helps define a boundary in the human/mouse homology relationships.


We have isolated and characterized two cDNA clones encoding senescence marker protein-30 (SMP30), the amounts of which are known to decrease androgen-independently with aging in the livers of rats. Of these cDNA clones, one consisted of 1588 bp nucleotides and the other of 1195 bp nucleotides generated by alternative polyadenylation. These two cDNA clones shared the same open reading frame, but the larger species had 393 bp nucleotides of 3' untranslated region in addition to the first polyadenylation site of smaller species. Northern hybridization analysis showed that two species of mRNA (1.7 kb and 1.4 kb) located in the liver and kidney were consistent with these short and long forms of cDNA. The open reading frame, 897 bp could encode 299 amino acids. The estimated molecular weight and pl of the deduced polypeptide were 33 387 and 5.1, respectively. Furthermore, immunohistochemical analysis confirmed that SMP30 was preferentially localized in the hepatocytes and renal proximal tubular epithelium. Genomic Southern hybridization analysis demonstrated that SMP30 was widely conserved among higher animals. A computer-assisted homology analysis of nucleic acid and protein databases revealed no remarkable homology with other known proteins. Therefore, SMP30 seems to be a novel protein. In addition, the existence of putative A-U rich mRNA degradation signals and protein degradation signals (PEST sequence) in the structure of SMP30 may suggest important regulatory function of this unique protein manifested by changes in its concentrations.

gland, as well as in reticulocytes and bone marrow. This was an unexpected finding since the protein has been isolated only from erythrocytes. In contrast, membrane-bound cytochrome b5 mRNA was detected in all tissues tested, suggesting that the corresponding protein is ubiquitous in tissue distribution.


http://www.sciencedirect.com/science/article/B6T1V-4C0RRHF-1/2/33a00a7c216406398447c5a00aad5765

Focal adhesion kinase (FAK) gene encodes focal adhesion kinase that localizes at contact points of cells with extracellular matrix. It was shown that FAK expression is increased in a variety of malignancies, both at early and advanced stages of tumorigenesis. To understand mechanisms of FAK gene expression and regulation, we cloned and characterized the 5' promoter region of the FAK gene. The 1.2-kb fragment with FAK promoter was placed upstream of the luciferase reporter gene in a pGL3-Basic vector and transfected into different cell lines. Endogenous high-FAK-expressing cell lines showed high levels of luciferase activity in contrast to low-FAK-expressing cells, indicating on transcriptional level of FAK regulation. Serial deletion constructs revealed that a ~600 base pair region (-564 to +47) is required for the maximal FAK promoter activity. The 5'-flanking region of FAK is GC-rich and contains several potential transcription factor binding sites, including two NF-kappa B and p53 binding sites. Inhibition of NF-kappa B with NF-kappa B super-repressor decreased FAK luciferase activity. Induction with TNF-[alpha] increased luciferase activity confirming a role of NF-kappa B transcription factor in the FAK transcriptional activation. The binding of NF-kappa B and p53 transcription factors to the FAK promoter region was demonstrated by electrophoretic mobility shift assay (EMSA). Cotransfection of NF-kappa B and p53 plasmids with FAK promoter luciferase constructs demonstrate induction and inhibition, respectively, of FAK luciferase activity. The results provide a molecular basis for analysis of FAK transcriptional regulation.


Vascular endothelial growth factor (VEGF-A) is a key angiogenic growth factor which regulates vertebrate embryonic vascularization, adult physiology such as wound healing and reproduction as well as many human diseases. To understand the evolution and regulation of this gene in vertebrates, we have isolated and characterized the zebrafish vegf-A gene and compared it with VEGF-A genes of human, mouse as well as an in silico isolated VEGF-A homologue from pufferfish. Our results indicate that the zebrafish vegf-A gene is organized similarly to mammalian and Fugu VEGF-A genes, with eight exons interrupted by seven introns. However, zebrafish vegf-A introns are generally larger than mammalian introns while Fugu VEGF-A introns are much smaller. Furthermore, zebrafish exon 6 (z6) has a unique sequence while Fugu's exon 6 is highly homologous to the mammalian counterparts. Alternative splicing generates multiple vegf-A mRNA isoforms in zebrafish with Vegf121 as the dominant isoform in adult and Vegf165 as the dominant isoform in early embryos. The exon z6 containing isoform Vegf12345z678 is only detected in heart, muscle, and early embryos while another isoform Vegf-A1234577a8 is only detected in heart. Furthermore, no conserved 5' flanking sequences between zebrafish and Fugu
were observed while numerous conserved regions exist between human and mouse in this area. These results suggest both conserved and diverged functions of VEGF-A from fish to mammals since the separation of these two groups from their common ancestor about 450 million years ago and a diverged regulation of this gene since the separation of zebrafish from Fugu. These data will be valuable for future studies of VEGF-A gene regulation and function in different vertebrates.


http://www.sciencedirect.com/science/article/B6T1V-3PSGXPY-8/2/3d5f133fc126dc42c140bab9fe97642

CDC42 is a member of the ras superfamily of small GTP-binding proteins that are related through the highly conserved GTP-binding domain and are involved in signal transduction pathways. Two full-length CDC42 cDNAs have been isolated: a 2148-bp chick cochlea cDNA and a 2063-bp mouse liver cDNA. Each encodes a CDC42 protein of 191 amino acids. The avian CDC42 protein differs from the mouse at only one amino acid residue, a Thr for a Ser at position 185. Both CDC42 proteins are more similar to the ubiquitous human isoform originally isolated from placenta than to the isoform isolated from fetal brain. Using a probe from the 3'UTR of the mouse liver CDC42 cDNA, we demonstrated that the mouse gene is expressed in all tissues examined. Southern blot analysis of a mouse inter-specific backcross with this gene-specific probe identified at least three CDC42-like (Cdc42l) genes in the mouse genome. Cdc42l1 was mapped to distal mouse Chromosome 4, near Cappb1. Cdc42l2 mapped more proximal on Chromosome 4, whereas Cdc42l3 mapped to the X Chromosome. (c) 1997 Elsevier Science B.V. All rights reserved.


http://www.sciencedirect.com/science/article/B6T1V-41S4TYX-2/2/c335bb8db0ce8946dd8d727ca0db3228

In homologous recombination in bacteria, the RuvAB Holliday junction-specific helicase catalyzes Holliday junction branch migration, and the RuvC Holliday junction resolvase catalyzes formation of spliced or patched structures. RuvAB and RuvC from the hyperthermophile Thermotoga maritima were expressed in Escherichia coli and purified to homogeneity. An inverted repeat sequence with unique termini was produced by PCR, restriction endonuclease cleavage, and head-to-tail ligation. A second inverted repeat sequence was derived by amplification of a second template containing a three-nucleotide insertion. Reassociation products from a mixture of these two sequences were homoduplex linear molecules and heteroduplex heat-stable Holliday junctions, which acted as substrates for both T. maritima RuvAB and RuvC. The T. maritima RuvAB helicase catalyzed energy-dependent Holliday junction branch migration at 70[deg]C, leading to heteroduplex linear duplex molecules with two three-nucleotide loops. Either ATP or ATP[gamma]S hydrolysis served as the energy source. T. maritima RuvC resolved Holliday junctions at 70[deg]C. Remarkably, the cleavage site was identical to the preferred cleavage site for E. coli RuvC [(A/T)TT[darr](G/C)]. The conservation of function and the ease of purification of wild-type and mutant thermophilic proteins argues for the use of T. maritima proteins for additional biochemical and structural studies.
The pentafunctional arom protein of Saccharomyces cerevisiae is encoded by the ARO1 gene. Substantial elevation of the levels of the arom protein (25-fold) was achieved in yeast using a vector that exploited the ubiquitin-fusion cleavage system of yeast. However, attempts to express the N-terminal 3-dehydroquinate synthase domain (E1) or the internal 3-dehydroquinase domain (E2) using the same system did not succeed. The yeast arom protein was successfully purified from the over-expressing transformant, and was found to possess all five enzymatic activities in a ratio similar to that observed in crude cell extracts. The purified material consisted mainly of a polypeptide that co-migrated in SDS-PAGE with intact arom proteins from other species.


We have investigated the epigenetic mark in the human H19 gene. The H19 promoter is methylation-free in human sperm, but it is methylated in the paternally derived allele of most adult tissues. Consequently, the H19 gene is exclusively transcribed from the maternal allele. It was demonstrated that the differentially methylated region (DMR) located 2 kb upstream from mouse H19 is essential for the imprinting of H19. A 39 bp sequence in DMR has a high degree of similarity between humans, mice and rats. The highly conserved 15 bp core region of the consensus sequence contains four methylatable sites, and thus has been proposed as a potential imprinting mark region. In this study, fine epigenetic sequencing analysis was performed on the sperm DNA in comparison with other adult organs. Interestingly, the conserved sequence of the potential mark region was methylated in almost all the sperm genomes analyzed. Furthermore, the single dinucleotide CpG, whose methylation affects the accessibility of the element to CTCF, was methylated in the conserved core in the human sperm. These results suggest that the human core sequences may act as an imprinting center in the reciprocal monoallelic expression of H19.


NKX2.1 is a member of the NK2 family of homeodomain-containing transcriptional factors which binds to and activates the promoters of thyroid and pulmonary epithelial genes. We have cloned and sequenced twelve human lung Nkx2.1 cDNAs. To elucidate the origin of Nkx2.1 transcripts, we also cloned and sequenced a 12 kb human Nkx2.1 genomic clone. Alignment of cDNA sequences with the genomic clone showed that contrary to previous reports, the human Nkx2.1 gene is organized into three exons and two introns. The newly discovered exon I contains an ATG codon that falls in frame with the previously identified Nkx2.1 initiator ATG codon on one of the cDNAs, designated 5E. Northern blot analysis shows that an mRNA of approximately 2.5 kb in size, homologous to 5E, is expressed in both lung and thyroid. The deduced amino acid sequence of the longest open reading frame on 5E is identical to NKX2.1 with the exception of a
30 amino acid N-terminal extension. Coupled in vitro transcription/translation of the 5E cDNA confirms that the open reading frame is translated into a contiguous polypeptide of 44 kDa. Analysis of Nkx2.1 genomic DNA fragments suggest that at least two independent regions, one within the first intron and the other 5' of the first exon may mediate the basal promoter activity of the Nkx2.1 gene in lung epithelial cells.


http://www.sciencedirect.com/science/article/B6T1V-47S69G1-HS/2/07f10cf757ad3e56e7601c7fca5d8a67

We observed earlier that there are 5 nucleotide polymorphisms in the protein coding sequence of the acid [beta]-galactosidase gene between the C57BL/6J and DBA/2J strains of mice. Two of them result in amino acid substitutions. Consequences of the difference in the primary amino acid sequence were studied by introducing the two DBA polymorphisms into the C57BL cDNA, individually and in combination, by oligonucleotide-directed mutagenesis and expressing the resultant cDNAs in the COS-1 cell expression system. Introduction of one polymorphism, Asn517 -> Asp into the C57BL cDNA, did not alter the acid [beta]-galactosidase activity in the transfected COS-1 cells, while introduction of Gly539 -> Arg completely abolished the catalytic activity. When both polymorphisms were introduced together, as in the DBA mice, however, the acid [beta]-galactosidase activity was restored to that of the C57BL level. Thus, Asn517 -> Asp appears to counteract the activity-abolishing effect of Gly539 -> Arg, although it does not by itself raise the catalytic activity. All four types of cDNA generated similarly large amounts of stable mRNA in COS-1 cells. These results do not explain the significantly low acid [beta]-galactosidase activity in tissues of DBA mice, described earlier and also confirmed in this study.


http://www.sciencedirect.com/science/article/B6T1V-3XX6VB3-292/4017c231c271bd26c164325338069d52

Using degenerate oligonucleotide primers corresponding to conserved regions of the G-protein coupled receptor superfamily we carried out a low-stringency polymerase chain reaction and obtained two novel partial-length clones from a rat brain cDNA library. We used one of these clones for conventional library screening and isolated a longer cDNA clone designated as RBU-15, from another rat brain library. Although RBU-15 was truncated at its 5' end Northern blot analysis revealed that the gene was expressed in the brain and spleen. Next we isolated a full-length cDNA clone, designated as HB-954, from a human fetal brain library, using RBU-15 as a probe. The deduced amino acid sequence of HB-954 contained four putative glycosylation sites in the N-terminal part, seven transmembrane domains, and a large cytosolic domain in the C-terminal part. The protein products of RBU-15 and HB-954 likely belong to a distinctive subfamily, because no receptors in the superfamily were found to be closely related to them.

Based on the published nucleotide sequence for rat hepatocyte nuclear factor 4 (HNF-4; Sladek, F.M., Zhong, W., Lai, E. and Darnell, J.E., Jr. (1990) Genes Dev. 4, 2353-2365), we have cloned a cDNA by means of polymerase chain reaction amplification of reverse-transcribed RNA (RT-PCR). Our clone contained an extra segment of 30 bp, which was not found in the previously reported clone, in the coding region near the C-terminus. Further RT-PCR analysis demonstrated that both isoforms of HNF-4 mRNA, i.e., with or without the 30 nucleotide segment, occur in rat liver and kidney, presumably by differential splicing.


To identify the essential sequence of the promoter of the human thymidylate synthase (hTS) gene, deletion mutants were constructed and assayed for promoter activity. The essential sequence was located within 65 bp upstream from the major cap site and a sequence that reduces the promoter activity was found in a region upstream from the essential promoter sequence. We previously identified two DNA-binding nuclear factors, NF-TS2 and NF-TS3, that bind to a region around the site of initiation of translation of the hTS gene. In this study, we confirmed the binding site of these factors by gel mobility shift analysis and found that NF-TS2 is the major factor that binds to the hTS gene in HeLa cells, whereas NF-TS3 is the major factor in the TIG-1 line of human fibroblast cells. To clarify the function of these factors, we examined the effects of the binding of these factors on the promoter activity. Our findings suggest that the binding of NF-TS2 enhances the promoter activity of the hTS gene in HeLa cells, whereas the binding of NF-TS3 represses the activity of the same promoter in TIG-1 cells.


Using rat Max cDNA as probe, which had been isolated for the first time from the seminal vesicle of intact rats by reverse transcription-polymerase chain reaction, we demonstrated the castration-induced expression of the 2 kb transcript both in the ventral prostate and the seminal vesicle of rats.

Unstimulated mononuclear cells express IGF-1, PDGF-A and PDGF-B mRNA, but not a number of other genes coding for growth factors or cytokines, as we demonstrated previously. The main focus of the present investigation was to compare gene expression of mononuclear cells unstimulated in suspension with gene expression of monocytes stimulated by adherence. mRNA levels of IGF-1A and -B, PDGF-A, -B, PD-ECGF, basic FGF, acidic FGF, TGF-[alpha], TGF-[beta]1, and IGF-2 were sought for and quantified with our sensitive RT-PCR method (3n-PCR). The respective mRNAs of basic FGF, acidic FGF, TGF-[alpha] and IGF-2 were not detected, independent of the culture conditions. In suspension culture, mRNA levels of IGF-1A and -B, PDGF-A, -B, and CD18 remained unchanged. Monocyte adherence regulated IGF-1A, PDGF-A, and -B mRNA levels. In parallel, mRNA levels of the monocyte adhesion molecule CD18 increased rapidly (4.5-fold). In contrast, independent of the presence of an adherence stimulus, the mRNAs for the cytoskeletal structure protein [beta]-actin and PD-ECGF remained constant, whereas mRNA for growth factors TGF-[beta]1 and IGF-1B, respectively, was increased. Thus, monocyte adherence selectively regulates IGF-1, PDGF-A, PDGF-B and CD18 mRNAs (adherence-responsive genes) in a coordinated manner. This led us to identify two novel consensus elements within their respective functional promoters. Both motifs, an 11 bp purine-rich sequence and a 13 bp pyrimidine-rich segment, respectively, are absent from the genes that were not specifically activated by adherence. The identified elements are potential binding sites for transcription factors that may define a common basis for the regulation of the adherence-responsive genes IGF-1A, PDGF-A, PDGF-B and CD18.


The DNA polymerases from Thermus aquaticus and Thermus flavus were recently found to bind to short double-stranded DNA fragments without sequence specificity [Kainz et al. (2000) Biotechniques 28, 278-82]. In the present study, it is shown that the accumulation of amplification products during later PCR cycles also exerts an inhibitory effect on several enzymes tested. To simulate later cycle conditions, a 1.7 kb sequence from phage [lambda] DNA was amplified in the presence of various amounts of a 1 kb double-stranded DNA fragment. A 30-fold molar excess of fragments to polymerase molecules was found to be required for a complete inhibition of Taq, Tfl and Pwo DNA polymerase. This stoichiometric relation remained constant when PCR amplifications were performed using polymerase concentrations of 0.5, 1 or 1.5 U/50 [mu]l reaction volume. The amount of 1 kb DNA fragments required for a complete inhibition was similar to the product yield of the controls (no fragment added), that were run to plateau phase levels. Additionally, PCR mixtures, that were subjected to different numbers of cycles, were compared in their ability to extend 3'-recessed ends by using a hairpin extension assay. The presence of endogenous amplicon DNA accumulated in later PCR cycles was found to inhibit completely the activity of DNA polymerase. PCR mixtures still in quasi-linear phase partially extended the hairpins. In both cases, a further addition of polymerase significantly improved their function. These results indicate that the main factor contributing to the plateau phase in PCR consists of binding of DNA polymerase to its amplification products.


http://www.sciencedirect.com/science/article/B6T1V-486TC9F-2M/2/701696332d6dd9291510a59b47697b6d
We have developed a rapid and efficient nucleotide sequencing technique, named the colony direct sequencing method, which combines both the conventional cloning method for picking up a single gene and the polymerase chain reaction (PCR) method for amplifying the gene directly from a colony. In the present study, the colony direct PCR product was used both for identification of the DNA insert and for nucleotide sequencing by an automated DNA analysis system. A nucleotide sequence of 300 to 400 bp could be determined within 13 h after picking the bacterial colonies on LB medium plates. We applied this method to sequencing of junctional regions of multiple deleted mtDNAs in two siblings with inherited recurrent myoglobinuria. Mitochondrial DNA fragments with deletions were amplified by PCR and then cloned into plasmids. Among 48 white colonies propagated on LB medium plates, nine different clones were identified by PCR directly from colonies. Determination of six different junctional sequences disclosed involvement of directly repeated sequences of 2 to 12 bp in length on each side of the deletions. We believe that the colony direct sequencing method will be a powerful tool in molecular genetics for identification of a single gene among polymorphic DNAs.


http://www.sciencedirect.com/science/article/B6T1V-3SK80DC-1C/2/3b7f6bcf531bc4ee2d475914995423d3

Monoclonal antibodies (mAbs) were generated that recognize UvrA and UvrB proteins. These proteins are components of the Uvr(A)BC endonuclease, which initiates nucleotide excision repair in Escherichia coli. mAbs, which can be used for probing of structural intermediates of Uvr(A)BC endonuclease functioning, were selected for their ability to: (i) recognize different epitopes; (ii) have a high-affinity for native antigenic protein; (iii) preserve functionality of the Uvr protein in immunocomplex. The adherence of anti-Uvr mAbs with these criteria was verified by additivity and competition tests, and by their influence on the ATPase activities of UvrA and UvrB*, the functionally active proteolytic fragment of UvrB. Two out of twelve anti-UvrA and seven out of thirteen anti-UvrB/anti-UvrB* hybridoma lines were shown to satisfy these criteria. Recognition of UvrA and UvrB deletion mutant proteins by mAbs was used to map their epitopes. Epitopes of A2D1 and A2B1 mAbs were mapped to regions of amino acids 230-281 and 560-680 of UvrA, respectively. Epitopes of anti-UvrB/anti-UvrB* mAbs were assigned to the following amino acid regions of UvrB: B2A1, 8-61; B2C5 and B*2E3, 171-278; B2E2, 631-673; B3C1, 1-7 and/or 62-170; B*2B9, 473-630; B*3E11, 379-472. The ability of selected mAbs to neutralize the incision function of UvrA/BC was analyzed. The results are discussed in terms of the applicability of these mAbs to probe the structures of intermediates in the functioning of Uvr(A)BC.


http://www.sciencedirect.com/science/article/B6T1V-3TGVY68-C2/4/488984e07a6d8964de7f2c2be5942a111

The 2767 bp BamHI-HindIII fragment specifying the trpDC genes of B. japonicum I-110 was sequenced. The trpD and trpC genes each have three highly conserved 'Crawford' consensus sequences and are part of an operon with three open reading frames (ORFs). The third ORF has a predicted product with 58% amino-acid sequence identity with the gene product of E. coli moaC, a gene encoding an enzyme involved in biosynthesis of the molybdenum cofactor required for the activity of nitrate reductase and other Mo cofactor-requiring enzymes.

http://www.sciencedirect.com/science/article/B6T1V-44X09XN-1/2/f3c533e4f24f30208523437f6ddaf386

While the p53 tumor suppressor plays a crucial role in regulating cell cycle checkpoints and apoptosis by acting as either a transcriptional activator or repressor in a variety of mammalian cells, its evolutionarily conserved functions remain to be elucidated in non-mammalian species. In the present study, we determined the functional role of p53 in avian cells by analyzing the expression pattern of the chicken homologue (CDM2) of mouse double minute 2, one of the transcriptional target genes of p53. CDM2 displayed considerable conservation in the p53 binding region as well as the nuclear localization and nuclear export signals and was found to be abundantly expressed in the reproductive organs (testis and ovarian follicles) and in the immune organs (bone marrow, bursa and thymus). CDM2 expression exhibited an early serum-response pattern consistent with its mammalian counterparts and was dramatically downregulated in most of the p53-downregulated immortal chicken embryo fibroblast (CEF) cells analyzed. Expression of CDM2 was shown to be transcriptionally upregulated in the primary CEF cells where p53 was activated by either mitomycin C treatment or by the exogenous transfection of the chicken p53 cDNA. Together, the current studies demonstrate that the expression of MDM2 homologues may be biologically conserved in mammalian and avian cells.


http://www.sciencedirect.com/science/article/B6T1V-4FDJPC5-1/2/73231d594994bfc9add88c7d404518

SPLUNC1, originally named PLUNC for palate, lung and nasal epithelium clone, is a small protein which is secreted from the epithelial cells of the nasal cavity and the upper respiratory tract in humans, mice, rats and cows. SPLUNC1 is structurally homologous to the two key mediators of host defense against Gram-negative bacteria, lipopolysaccharide binding protein (LBP) and bactericidal permeability increasing protein (BPI). SPLUNC1 is therefore believed to play a role in the innate immune system. This work reports the cloning and analysis of the porcine (Sus scrofa) homologue of SPLUNC1. The SPLUNC1 cDNA was amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using oligonucleotide primers derived from in silico sequences. The porcine cDNA codes for a protein of 249 amino acids which shows a high similarity to bovine (74%) and to human (69%) SPLUNC1. The predicted S. scrofa SPLUNC1, SsSPLUNC1, polypeptide contains a putative signal peptide of 19 residues. A similar signal sequence is also found in all other members of the PLUNC family. Expression analysis by RT-PCR demonstrated a very high expression level of the porcine SPLUNC1 homologue in trachea and lung tissue only. This airway-specific expression might be of particular interest in the study of airborne diseases in pig.

We report the isolation of a novel human cDNA encoding a putative transmembrane protein, TMC. The predicted protein sequence is highly conserved evolutionarily. The cDNA clone was mapped to human chromosome 11q24-25 by fluorescence in situ hybridization. mRNA expression was observed in all tissues tested with the highest levels in testes and ovary.


The organization and nucleotide sequence of the capsular gene cluster involved in the biosynthesis of the type 33F capsular polysaccharide of Streptococcus pneumoniae have been determined. The complete type 33F operon (cap33f) is composed of 14 potential open reading frames where the last ten genes are group-specific. Putative functions have been assigned to several gene products by sequence comparison with the proteins included in the databases. A functional promoter located immediately upstream from the first gene of the cap33f gene cluster has been demonstrated. A 20 kb DNA fragment containing the cap33f genes and the operon promoter was sufficient to transform a S. pneumoniae type 3 unencapsulated mutant to the type 33F capsule.


A Chinese hamster ovary cell line hemizygous for a defective adenine phosphoribosyltransferase (aprt) gene was transfected with a plasmid, pAG100, capable of correcting the endogenous aprt mutation by targeted homologous recombination. In some experiments, pAG100 was transfected in combination with one of two 'competitor' plasmids. Competitor pCOMP-A was identical to pAG100 except that the aprt sequence on pCOMP-A had the same mutation as the endogenous aprt gene. Competitor pCOMP-B was identical to pAG100 except for a 763 bp deletion in the aprt sequence encompassing the site of mutation in the endogenous gene. Neither pCOMP-A nor pCOMP-B was capable of correcting the defect in the endogenous aprt gene via gene targeting. We asked whether cotransfection of a 4-fold excess of either competitor DNA molecule with pAG100 would reduce the efficiency of targeted correction of the endogenous aprt gene. We report that while plasmid pCOMP-B did not influence the efficiency of gene targeting by pAG100, plasmid pCOMP-A reduced the number of gene targeting events about 5-fold. These observations indicate that the initial homologous interaction between transfected DNA and a genomic target sequence occurs rapidly and that targeting efficiency is limited by a step subsequent to homologous pairing.

We have identified a human tRNACys isoacceptor matching the UCG codon. The tRNA was discovered via its ability to act in reverse transcription of a murine leukemia virus vector containing a complementary tRNA primer binding site (Lund et al., Nucleic Acids Res., 28 (2000) 791-799). The tRNACys(CGA) was detected in cell lines of human, monkey and mouse origin. The UCG codon is the most rarely used codon in human genes. The cloned human tRNACys(CGA) gene encodes an 85 nucleotide, intron-less tRNA, contains a consensus split intragenic promoter and is located at region p21.3-22.2 on chromosome 6. The integrity and functionality of the cloned tRNACys(CGA) gene was verified by in vitro transcription analysis in HeLa nuclear extracts.


We have identified certain unusually spliced cDNA species following PCR amplification of peripheral blood lymphocyte (PBL) mRNA from the hMSH2 gene. A naturally occurring transcript containing a nonsense codon due to the skipping of 5 exons was amplified from PBLs of several healthy individuals. A feature of this and another unusual splicing product was the presence of sequence motifs which bore significant similarity to mRNA instability determinants in the region immediately downstream of the stop codon. In particular, the rare tetranucleotide GAUG, previously identified in yeast as being of critical importance to the rapid degradation of nonsense-containing mRNAs was situated 23 base pairs downstream of the stop codon. Furthermore the region downstream of the stop codon was A:U rich and contained 2 copies of the AUUUA motif. As other forms of alternative splicing would not result in the same juxtaposition of stop codons and instability motifs, we suggest that the stop codons may have been deliberately introduced by the splicing process for their proximity to these destabilising motifs, and that splicing may play a role in channelling mRNAs into degradative pathways. These results are consistent with the hypothesis that nuclear factors may scan pre-mRNAs prior to splicing.


The thyroglobulin gene, the substrate for thyroid hormone biosynthesis, is not expressed in the FRT cell line, which, even though it manifests the polarised epithelial phenotype, does not express any of the thyroid functional properties. Two transcription factors, TTF-1 and Pax-8, have been implicated in thyroid specific expression of the thyroglobulin gene. FRT cells contain Pax-8 but they lack TTF-1. In this paper, we show that transfection of TTF-1 expression vectors in FRT cells results in activation of thyroglobulin gene expression. If the expression vector encoded for
TTF-1-ER, a fusion gene coding for the entire TTF-1 protein fused to the hormone-binding domain of the steroid receptor, under the control of the RSV promoter, thyroglobulin gene expression was controlled by estrogen. These data provide a direct demonstration that TTF-1 activates the chromosomal thyroglobulin promoter. Since transfection of TTF-1 expression vectors in non-thyroid cell types did not result in thyroglobulin gene expression, it is suggested that Pax-8, in addition, perhaps, to a specific cellular environment, might be required for thyroid specific expression of the thyroglobulin gene.


http://www.sciencedirect.com/science/article/B6T1V-3XY2KP0-1G/2/f878735c49ea127f9b4b1f74c0f453c2

The gene for Thermus aquaticus (Taq) DNA polymerase enzyme (Taq Pol I) was mutagenized and sixty-two candidate clones were screened for enzyme activity. Two of the clones expressed enzymes (*Taq-3 and *Taq-5) that showed very reduced 5'-3' exonuclease activity and normal DNA polymerase activity. These two enzymes showed heat resistance and storage stability similar to Taq Pol I and had similar effectiveness in PCR. Processivity of the polymerases was compared by measuring the extension of an end-labeled primer annealed to a single stranded DNA, as well as by a PCR method. The processivity of *Taq-3 and *Taq-5 was similar to Taq Pol I (50-80 nucleotides) and more processive than a Taq Pol I deficient in the 5'-3' exonuclease due to absence of the first 290 amino acids (Stoffel fragment). The results indicate two amino acid which are required for normal 5'-3' exonuclease activity in Taq Pol I (Arg-25 and Arg-74).


http://www.sciencedirect.com/science/article/B6T1V-47S6BD7-VY/2/e0ab4579d69199d36263afdec6003e91

The quantitative use of the polymerase chain reaction (PCR) is often compromised by the variability of the amplification. The most useful system for quantitation by PCR involves the use of controls which are almost identical to the target and which can be amplified using the same primers as the sequences of interest. In this paper, we use a model system consisting of differently sized targets amplifiable with varying primers to demonstrate the effects of the plateau phase of PCR on quantitation by PCR. This model confirms two commonly observed results: (i) when varying amounts of a single target are amplified, a constant maximum level of product is obtained and (ii) coamplification of different concentrations of different targets results in retention of the initial proportions. The inherent contradiction in these results is examined by replacement of the key elements of the reaction including enzyme, dNTPs or primers, none of which have an effect on the plateau. Pyrophosphate is found to exert no inhibitory effect on the reaction, nor does the exonuclease action of the enzyme cause the plateau. Levels of amplification attained during amplification are both theoretically and empirically defined as being insufficient to lead to the plateau due to competition between self-annealing of product DNAs and primer binding. We conclude that, pending further biochemical enquiry into the enzyme(s) used in the PCR, none of reasons conventionally proposed for the plateau phase of the PCR are sufficient to explain the phenomenon. This being so, we define the plateau as being a feature of the reaction as a whole and, since the onset of this phase is simultaneous for all amplicons, quantitation using the internal control system need not require exponential amplification. This therefore greatly simplifies the quantitative application of PCR.
Kynurenic acid (KA) is an endogenous glutamate receptor antagonist at the level of the different ionotropic glutamate receptors. One of the enzymes responsible for the production of KA, kynurenine aminotransferase I (KATI), also catalyses the reversible transamination of glutamine to oxoglutaramic acid (GTK, EC 2.6.1.15). The enzyme exists in a cytosolic and in a mitochondrial form because of the presence of two different KATI mRNAs coding for a protein respectively with and without leader sequence targeting the protein into mitochondria. We have cloned from a phage library of rat kidney cDNA four new KATI cDNAs containing different 5’ untranslated regions (UTRs). One of the transcripts (+14KATI cDNA) contains an alternative site of initiation of translation. The tissue distribution of the different transcripts was studied by RT-PCR. The study demonstrated that several KATI mRNAs are constitutively expressed in a ubiquitous manner, while +14KATI mRNA is present only in kidney. The translational efficiency of the different transcripts was studied in vitro and enzymatic activities were measured in transiently transfected Cos-1 cells. Each KATI mRNA exhibits a different in vitro translational efficiency, which corresponds to different levels of KAT enzymatic activity in transfected cells. Both findings correlate with the predicted accessibility of the ribosomal binding sites of the different mRNAs. The structure of the rat KATI/GTK gene was also studied. The expression of several KATI mRNAs with different 5’UTRs represents an interesting example of transcriptional/translational control on the expression of pyridoxal phosphate (PLP)-dependent aminotransferases.


The sequence specificity of DNA damage of n-bromoalkylphenanthridinium bromides, with linker chain lengths (n) of 4, 6, 8 and 10 methylene groups, was investigated in the plasmid pUC8 and in intact human cells. A linear amplification assay was used to elucidate the DNA sequence specificity of the alkylating agents. In this assay Taq DNA polymerase extends from an oligonucleotide primer up to the damage site and the products run on a DNA sequencing gel to reveal the precise sites of DNA damage. For both the plasmid and cellular experiments, the compound that caused the most damage to DNA was the n = 6 compound, followed by (in decreasing order) the n = 4, N = 8, and N = 10 compounds. There were significant differences in the sequence specificity of DNA damage between n-bromoalkylphenanthridinium bromides of different linker chain length: (1) the main sites of damage were at guanines for the n = 4, 6 and 8 compounds but at guanines and adenines for the n = 10 compound; (2) a consensus sequence of 5’-c(a/t)Ggg-3’ was obtained for the n = 4, 6 and 8 compounds but 5’-c(a/c)(G/A)(g/a)-3’ for the n = 10 compound; (3) runs of consecutive Gs were the major site of damage for the n = 4, 6 and 8 compounds, but consecutive Gs or consecutive As for the n = 10 compound; (4) for damage at single isolated guanines, the most damaged sequences were at 5’-Ga-3’ for the n = 4 compound but at 5’-Gt-3’ for the n = 6, 8 and 10 compounds. The tandemly repeated alpha RI DNA sequence was the DNA target in intact human K562 cells. In intact human cells, the compounds produced damage with similar DNA sequence selectivity to that found in plasmid DNA. The n = 4 and 6 compounds possess marginal anti-tumour activity and these compounds produced the most
damage in intact human cells. The n = 8 and 10 compounds do not demonstrate significant anti-tumour activity and these compounds resulted in the least damage in cells.


http://www.sciencedirect.com/science/article/B6T1V-3XX6V8T-10/2/a3992419254ee0201b99d5306765b1cc

The sequence specificity of the pluramycin antibiotics hedamycin and DC92-B, was established in intact human cells using a linear amplification system. In this system an oligonucleotide primer is extended by Taq DNA polymerase up to a damage site. The products are run on a DNA sequencing gel and the damage can be determined to the exact base pair. The human repetitive [alpha] RI DNA was used as the target DNA sequence for these experiments. It was found that G residues were the main site of adduct formation, for both hedamycin and DC92-B. The sequences were the most intense sites of DNA damage. A comparison of the DNA damage intensity in intact cells and purified DNA revealed that the sequence position of adduct formation was very similar in the two environments. However, a densitometric comparison of the damage intensity in the two environments revealed significant differences. Two regions were found (120 and 130 bp in length) where the damage intensity was relatively lower in intact cells compared to purified DNA. But at the boundaries of these sequences, there were regions (approx. 50-60 bp long) that were relatively more damaged in intact cells compared to purified DNA. One explanation of this phenomenon is the presence of a protecting nucleosome core on each of the 120/130 bp regions and flanking nucleosome linker regions of 50-60 bp. This postulated sequence phasing of the nucleosomes corresponds almost exactly with the major nucleosome phasing found in African green monkey cells. Also the centromere protein B binding site is found in the border region between the nucleosome core and linker DNA regions. Hedamycin and DC92-B produced nearly identical results in this human cell system.


http://www.sciencedirect.com/science/article/B6T1V-3W3178C-9/2/ad286e3b30a425b098f6995dcf962ff9f

We report a new method for the specific detection of the mutant mitochondrial DNA (mtDNA) that contains the 4977-bp deletion. We designed an oligonucleotide probe that was designated the 'ATP8/ND5 Chimera' probe: its 5'- and 3'-portions correspond to the ATP8 gene and the ND5 gene, respectively, and its middle portion includes the 13-bp direct repeat sequence that flanks the 4977-bp deletion. By Southern blot analysis, this chimeric probe specifically detected the deleted mtDNA, even in the presence of both normal mtDNA and other mtDNA deletions. The specificity of the probe was further confirmed by in situ hybridization of muscle fibers from patients with Kearns-Sayer syndrome who carry the deleted DNA in the heteroplasmic state. The deleted mtDNA was markedly accumulated in cytochrome-c oxidase (COX)-deficient ragged-red fibers. In tissues where multiple deleted mtDNAs were detected, such as muscle tissues from a patient with myotonic dystrophy and from an aged individual, the in situ hybridization detected a small number of muscle fibers that contained the deleted mtDNA. These results indicate that in situ hybridization using this chimera probe is a useful and specific method for detecting a small amount of deleted mtDNA.
We cloned the Slc14a2 gene and determined the genomic organization of the rat urea transporter UT-A. Slc14a2, the gene encoding the rat UT-A transporter, extends for more than 300 kb. The four known rat mRNA isoforms: UT-A1, UT-A2, UT-A3, and UT-A4 are transcribed from 24 exons. The Slc14a2 genomic map also accounts for 3'-untranslated sequences expressed alternatively in UT-A1, UT-A2, and UT-A3. We previously identified a TATA-less, tonicity-responsive promoter controlling the transcription of UT-A1, UT-A3, and UT-A4 from a single initiation site in the 5'-flanking region of the gene. Here, we describe a second, internal promoter in intron 12, which controls the transcription of UT-A2 starting from exon 13. This region contains a TATA motif upstream from the UT-A2 transcription start site, and shows consensus sequences for the cAMP response element (CRE) and for the tonicity enhancer (TonE) motif. Stimulation by cAMP induces UT-A2 mRNA expression in mIMCD3 cells, and luciferase activity in mIMCD3 cells transfected with those pGL3 constructs including the CRE sequences. Although long-term exposure to hypertonicity induces UT-A2 expression in mIMCD3 cells, hypertonicity does not induce significantly the activity of the promoter in intron 12. In summary, we describe the genomic structure of the rat UT-A urea transporter, encoded by the Slc14a2 gene. Our findings suggest that two promoters regulate transcription of the four UT-A isoforms, and that stimulation of transcription by vasopressin, mediated by cAMP and CRE sequences, and controlled by an intronic promoter, may contribute to the increase in UT-A2 expression during water deprivation.

Heavy metal-dependent transcriptional activation of metallothionein (MT) genes is mediated by multiple enhancer sequences, metal responsive element (MRE), located in the upstream region of the genes. Previously, we have reported purification of a zinc-dependent MRE-binding protein, zinc regulatory factor (ZRF), from HeLa cells, and have pointed to the close relationship between ZRF and mouse MRE-binding transcription factor-1 (hMTF-1) according to the analysis of partial amino acid sequences. By means of cDNA cloning and the product analyses, we show that ZRF is a variant of human MTF-1 (hMTF-1), which carries a single amino acid exchange in the zinc finger domain. Accordingly, ZRF is renamed hMTF-1b. Expression of hMTF-1b in HeLa cells is constitutive at both mRNA and protein levels, and is unaffected by treatment with cadmium (Cd). On the other hand, when cells were fractionated into nuclear extract and cytosol, a large part of the hMTF-1b was recovered in the cytosol fraction. A significant increase in the amount of nuclear hMTF-1b occurs when cells are treated with various heavy metals, including Cd, Zn, Cu and Ag, which is associated with concomitant decrease in the amount recovered in the cytosol fraction. Since immunocytochemical analysis revealed that intracellular distribution of hMTF-1b is restricted to the nucleus irrespective of the heavy metal treatment, such an increment in the nuclear extracts apparently results from promotion of nuclear retention of hMTF-1b by the heavy metal treatment. Analysis by native gel electrophoresis shows that the mobility of hMTF-1b significantly changes in association with Cd treatment, raising the possibility that a conformational change of hMTF-1b occurs in response to treatment with heavy metals in vivo.

http://www.sciencedirect.com/science/article/B6T1V-47S6C4S-13J/2/ea2b802da04709f23e361725822255a5

Different mRNAs for fibronectin arise from the variable processing of a single primary transcript. We used ribonuclease protection assay to investigate the changes occurring in fibronectin expression and the alternative splicing of mRNA precursor during aging in vitro of human diploid endothelial cells. Senescent endothelial cells release more protein and contain 4.5-fold more fibronectin mRNA than young cells. The pattern of alternative splicing of fibronectin mRNA, with the EDA and the CS1 segments largely included (35% and 77%, respectively) and the EDB segment undetectable, correlates well with previous studies at the protein level both in vitro and in vivo. No changes in the splicing pattern of fibronectin mRNA precursor were detected during endothelial cellular senescence. The increased expression of fibronectin in senescent cells may be a result of the activity of interleukin-1 [alpha], which is overexpressed in senescent endothelial cells. It could be also important in vivo during aging and in atherosclerotic lesions.


http://www.sciencedirect.com/science/article/B6T1V-3W498CG-N/2/2f826b90ca1090cd3365d46ee63e0408

The acute phase plasma protein ceruloplasmin (Cp) appears to play some role in host defense. The possibility that production of Cp in extrahepatic sites may also be essential for the activation, effector functions and cytoprotection of immune cells in localized environments has received minimal attention. Here, we have surveyed various types of human and rat immune cells for the presence of Cp mRNA using RT-PCR with primers that span exons 17-19 as an initial step in addressing this possibility. Validated Cp RT-PCR bands were obtained from RNA samples isolated from resting and activated human lymphocytes, CD4 and CD8 T-cells and B-cells. Semiquantitative RT-PCR indicated that Cp mRNA in immune cells is present at about 0.2% the level of Cp mRNA in HepG-2 human liver cell line. Various human cell lines derived from the immune system, rat splenic MNC and purified rat T-lymphocytes also constitutively express Cp gene.


http://www.sciencedirect.com/science/article/B6T1V-3VS7M79-D/2/02ba1a9fe771661aa568ad25054f20a7

In the baboon, estrogen regulated 11[beta]-hydroxysteroid dehydrogenase (11[beta]-HSD) catalyzed metabolism of cortisol and cortisone by the placenta is an important component in the sequence of events regulating the fetal pituitary-adrenocortical axis. The present study was designed to isolate and sequence the promoter region of the baboon 11[beta]-HSD-2 gene and to
produce constructs of this gene and the 1.7 kb fragment of 5'-flanking region of baboon 11[beta]-HSD-1 isolated previously in order to determine whether the promoters of these two genes were activated in human placental JEG-3 cells and whether expression could be modulated by estradiol. The 11[beta]-HSD-2 genomic DNA was isolated from a baboon kidney genomic library using a human 11[beta]-HSD-2 cDNA as a probe. The sequence of a 1.2 kb fragment of the 5'-flanking region showed extensive homology with that published by others for human 11[beta]-HSD-2, particularly in exon 1 (>95%) and in the proximal promoter (>90%). Primer extension confirmed that the baboon 11[beta]-HSD-2 gene has multiple transcriptional start sites which are preceded by a GC box. To determine promoter activity of 11[beta]-HSD-2 and -1, the 5'-flanking regions of these genes were subcloned into luciferase reporter pGL3 vectors, transiently transfected into human placental JEG-3 cells, and then incubated for 16-18 h in the presence or absence of 10-8 M 17[beta]-estradiol or 17[alpha]-estradiol. To augment the low level of estrogen receptor (ER) in JEG cells, promoter activity studies were also performed in JEG cells co-transfected with an expression vector containing the human ER cDNA. The promoters of both 11[beta]-HSD-1 and -2 were activated following transient transfection into JEG-3 cells although basal activity of 11[beta]-HSD-2 (87+/−21 RLU/[μg protein]) always exceeded (PP<0.05) basal promoter activities of 11[beta]-HSD-1 and -2 by 8.1+/−1.5 and 8.3+/−2.0 fold, respectively. Collectively, these findings indicate that the promoter region of the baboon 11[beta]-HSD-2 gene is comparable to that in the human and that the 5'-flanking region of both the baboon 11[beta]-HSD-1 and -2 genes were active when transiently transfected into JEG-3 cells and that activation could be enhanced by estradiol in the presence of an estrogen receptor.


http://www.sciencedirect.com/science/article/B6T1V-3SK80DC-K/2/73e6289a73e2eb46bae1658d1452b282

cDNA clones encoding human neurotrypsin have been isolated from a human fetal brain cDNA library using a PCR-amplified probe. The assembled cDNA sequence contains a 2625 bp open reading frame encoding a multidomain serine protease with an overall sequence identity of 82.5% to murine neurotrypsin. Surprisingly, the human neurotrypsin exhibits an additional scavenger receptor cysteine-rich repeat.


http://www.sciencedirect.com/science/article/B6T1V-45N84SN-1/2/839165d99f01abb181af60dc8cbfabe3

Copper (Cu) is an essential element required in many biological processes including cellular growth and development. The molecular mechanisms involved in copper homeostasis include proteins that play a role in Cu uptake. Genes encoding high affinity copper transporters (Ctr) have been identified in yeast, plant and mammalian cells. Analysis of copper and zinc content in growing ovarian follicles and ovulated eggs of the reptilian Podarcis sicula demonstrated that the levels of both metals rise during oocyte growth, reaching the maximum in ovulated eggs. By exploiting the remarkable evolutionary conservation of the primary structure of Ctr proteins, cDNA encoding a Ctr was isolated from the liver of the lizard P. sicula by reverse transcriptase PCR and RACE strategy by using primers designed based on consensus motifs present in mammalian Ctr.
The predicted protein sequence contains three transmembrane domains and a putative hydrophilic extracellular amino-terminal domain. Besides complementing the respiratory deficiency of yeast cells defective in high affinity Cu transport, expression of lizard Ctr11 in Hek293 cells stimulates Cu uptake. Gene expression assessed by Northern blot hybridization of RNA from different tissues of P. sicula shows the highest levels of transcript in both intestine and liver. The profile of Ctr1 mRNA in growing ovarian follicles and eggs demonstrates that the transcript accumulates during the oocyte growth and reaches the highest levels in ovulated eggs. These results suggest that lizard Ctr1 protein may function in Cu acquisition in growing oocytes and eggs.


http://www.sciencedirect.com/science/article/B6T1V-488939S-53/2/acb41d644c662ed85cb56d1dae31ed3a

We have cloned cDNA and genomic DNA fragments from Dictyostelium discoideum that represent the entire coding region of glycogen phosphorylase 1 (gp1, [alpha]-glucosyltransferase, EC 2.4.1.1). Nucleic acid sequencing of the gp1 clones revealed a single 139 bp intron separating the two exons that encode the 853 amino acids of gp1. The gp1 sequences are similar to other genes and proteins described for Dictyostelium in terms of G + C composition of coding and noncoding regions, splice junctions, intron length, codon preference and termination signals. Genomic Southern blot hybridizations suggest that gp1 exists as a single or low copy number gene in Dictyostelium. Northern analyses demonstrate that gp1 is a developmentally regulated transcript. In alignments of the gp1 peptide sequence to glycogen phosphorylase sequences from other organisms, a high degree of amino acid conservation at many active and regulatory sites was found; however, critical residues in the AMP and purine binding sites were not conserved.


http://www.sciencedirect.com/science/article/B6T1V-44P6YH5-1/2/4df17e091efb3c039c4ea232629d5928

A new isoform of the full-length murine thrombopoietin (Tpo) receptor was isolated from a murine spleen cDNA library. This isoform, c-mpl-II, differs from full-length c-mpl (c-mpl-I) by virtue of deletion of 180 nucleotides that encode 60 amino acids located in the extracellular domain of Mpl. Normal murine megakaryocytes were found to express both c-mpl-I and c-mpl-II transcripts. BaF3 cells transfected with c-mpl-I expressed a 95 kDa protein that was displayed on the cell surface and bound 125I-Tpo. BaF3 cells transfected with c-mpl-II expressed a 70 kDa protein. However, these cells were not able to bind 125I-Tpo and surface display of Mpl-II could not be detected. In summary, c-mpl-II is an isoform of murine Mpl expressed by megakaryocytes that lacks a 60 amino acid region required for surface expression of the protein.

The gene for a highly thermostable neutral proteinase (Npr) was isolated from Bacillus sp. strain EA1 by the polymerase chain reaction using consensus primers based on the sequences of npr genes from related species. The gene was sequenced and shown to be closely related to a neutral proteinase gene from Bacillus caldolyticus strain YP-T; the mature form of the enzyme differing by only a single amino acid. Enzyme samples were prepared from both the native organisms and also from recombinant Escherichia coli expressing the two npr genes. The proteinase from strain EA1 was shown to be significantly more thermostable than that from B. caldolyticus and that this difference is the result of a single amino acid substitution which is situated proximal to a region of the enzyme known to be crucial to conferring thermal stability. The phylogenetic relationship of EA1 to other Bacilli is also described.


Patients with renal and colon cancer frequently develop IgG autoantibodies toward the NY-CO-38/PDZ-73 antigen, a protein of 652 amino acids (73 kDa) which contains three copies of the PDZ protein-protein interaction domain. The gene encoding PDZ-73 mapped to chromosome 11p15.4-p15.1. Additional tissue-specific isoforms were identified: PDZ-45, which lacks the third PDZ domain and the putative PEST protein degradation motif, is expressed in kidney, colon, small intestine, brain and testis; PDZ-54 and PDZ-59, which also lack the third PDZ domains, have unique carboxyl terminal amino acids and are expressed in brain, kidney, bladder, colon cancer and renal cancer; and a putative PDZ-37 isoform, containing only the third PDZ domain, that is expressed in the central nervous system. Immunohistochemical staining with anti-PDZ 73 monoclonal antibodies showed strong cytoplasmic reactivity in epithelial cells of the small intestine, colon and kidney tubules, with a prominent apical staining pattern in cells of the small intestine. The reactivity pattern of the antibodies with various tissues correlated with the mRNA expression pattern of the PDZ-45 isoform. The existence of multiple PDZ-73 isoforms with variations in tissue distribution, PDZ domains, protein degradation sequences and carboxyl terminal structure indicate that these isoforms have distinct tissue-specific functions.


Cysteine proteinases, cathepsins B, H, K, L and S, have been implicated in several proteolytic processes during development, growth, remodeling and aging, as well as in a variety of pathological processes. For systematic analysis of cathepsin gene expression we have produced cDNA clones for mouse and human cysteine cathepsins. Northern analysis of a panel of total RNAs isolated from 16-19 different human and mouse tissues revealed the presence of mRNAs for cathepsin B, H, K, L and S in most tissues, but each with a distinct profile. Of the different cathepsin mRNAs, those for cathepsin K were clearly the highest in bone and cartilage. However, relatively high mRNA levels for the other cathepsins were also present in these tissues. To better
understand the roles of different cathepsins during endochondral ossification in mouse long bones, cathepsin mRNAs were localized by in situ hybridization. Cathepsin K mRNAs were predominantly seen in multinucleated chondroclastic and osteoclastic cells at the osteochondral junction and on the surface of bone spicules. The other cathepsin mRNAs were also seen in osteoclasts, and in hypertrophic and proliferating chondrocytes. These observations were confirmed by immunohistochemistry and suggest that all cysteine cathepsins are involved in matrix degradation during endochondral ossification.


http://www.sciencedirect.com/science/article/B6T1V-47S6C4S-13S/2/753dfd15ad4d05d52dadae20539139d8

Trehalase ([alpha]-glucoside-1-glucohydrolase, EC 3.2.1.28) was purified from silkworm pupal midgut to homogeneity by DEAE-Sepharose CL-6B and hydroxyapatite chromatography, and native gel electrophoresis. The enzyme had a molecular mass of 70 kDa. The N-terminal amino-acid sequence of the intact trehalase and its three fragments by V8 proteinase digestion was determined. Based on the amino-acid sequence, degenerate oligonucleotides were synthesized and used as primers in a polymerase chain reaction (PCR). Using a 0.8 kb PCR product as a hybridization probe, trehalase clones were isolated from the pupal midgut cDNA library. Sequence analysis revealed that the isolated trehalase cDNA contains 3103 nucleotides and comprises 579 amino acids, including a cleavable signal sequence and five potential N-glycosylation sites. Northern blot analysis clearly showed a 3.0 kb transcript in midgut, and Malpighian tubule, but not in fat body, silk gland, ovary, trachea, brain and suboesophageal ganglion.


http://www.sciencedirect.com/science/article/B6T1V-41S4TYX-8/2/8f0901e739173e9577e5235048a22ff5

We have cloned the gene for chicken 2',5'-oligoadenylate synthetase (ChOAS) by the method of polymerase chain reaction with use of ChOAS cDNA sequence. The ChOAS gene is composed of five introns and six exons containing all of the sequence of the ChOAS cDNA from the start to the stop codon. The first five exons of ChOAS gene which encode the OAS catalytic domain have a similar structure to HuOAS1 gene including the exon-intron boundaries. However, the length of introns of ChOAS gene is only 1/7 of those of HuOAS1 gene. The sixth exon of the ChOAS gene encodes the ubiquitin-like (UbL) domain of two consecutive sequence (UbL1 and UbL2) homologous to ubiquitin. ChOAS encoded in a single copy gene has at least two alleles, OAS*A and OAS*B. The differences between these two alleles are in the sixth exon of the gene; a 96-nucleotide sequence in the UbL1 portion of OAS*A is deleted from OAS*B. No OAS*B gene was detected in nine lines of chickens tested other than Leghorns. Almost the same levels of ChOAS-A and -B proteins induced physiologically in erythrocytes were detected in infant chickens (2-week-old), but in grown-up chickens (6-month-old) the level of erythrocyte OAS-B was markedly reduced in most of B/B chickens. Thus, the UbL domain of ChOAS is responsible for the maintenance of the OAS level in the tissue.
A gene encoding the precursor for a novel member of the human acyl-CoA dehydrogenase (ACD) gene family has been isolated which maps to human chromosome 11q25. The cDNA contains an open reading frame of 1248 nucleotides encoding a predicted 415-amino-acid peptide, and shares considerable sequence similarity with other members of the ACD family.


As part of an ongoing drug development programme, this paper describes the sequence specificity and time course of DNA adduct formation for a series of novel DNA-targeted analogues of cis-diaminedichloroplatinum(II) (cisplatin) (9-aminoacridine-4-carboxamide Pt complexes) in intact HeLa cells. The sequence specificity of DNA damage caused by cisplatin and analogues in human (HeLa) cells was studied using Taq DNA polymerase and a linear amplification/polymerase stop assay. Primer extension is inhibited by a Pt-DNA adduct, and hence the sites of these lesions can be analysed on DNA sequencing gels. The repetitive alphoid DNA sequence was used as the target DNA in human cells. The 9-aminoacridine-4-carboxamide Pt complexes exhibited a markedly different sequence specificity relative to cisplatin and other analogues. The sequence specificity of the 9-aminoacridine-4-carboxamide Pt complexes is shifted away from a preference for runs of guanines. The 9-aminoacridine-4-carboxamide Pt complexes have an enhanced preference for GA dinucleotides. This is the first occasion that an altered DNA sequence specificity has been demonstrated for a cisplatin analogue in human cells. A time course of DNA damage revealed that the DNA-targeted Pt complexes, consisting of four 9-aminoacridine-4-carboxamide Pt complexes and one acridine-4-carboxamide Pt complex, damaged DNA more rapidly compared to cisplatin and non-targeted analogues. A comparison of the time taken to reach half the maximum relative intensity indicated that the DNA-targeted Pt complexes reacted approximately 4-fold faster than cisplatin and the non-targeted analogues.


We report the generation and characterisation of the first transgenic mice exclusively expressing normal human [beta]-globin (hu[beta]-globin) from a 183 kb genomic fragment. Four independent lines were generated, each containing 2-6 copies of the hu[beta]-globin locus at a single integration site. Steady state levels of hu[beta]-globin protein were dependent on transgene copy number, but independent of the site of integration. Hemizygosity for the transgene on a
heterozygous knockout background (hu[beta]+/0, mu[beta]th-3/+) complemented fully the hematological abnormalities associated with the heterozygous knockout mutation in all four lines. Importantly, the rescue of the embryonic lethal phenotype that is characteristic of homozygosity for the knockout mutation was also demonstrated in two transgenic lines that were homozygous for two copies of the hu[beta]-globin locus, and in one transgenic line, which was hemizygous for six copies of the hu[beta]-globin locus. Our results illustrate the importance of transgene copy number determination and of the hemizygosity/homozygosity status in phenotypic complementation studies of transgenic mice containing large heterologous transgenes. Transgenic mouse colonies with 100% hu[beta]-globin production from the intact hu[beta]-globin locus have been established and will be invaluable in comparative and gene therapy studies with mouse models containing specific [beta]-thalassemia mutations in the hu[beta]-globin locus.


http://www.sciencedirect.com/science/article/B6T1V-45D8MV4-1/2/52d940dc5ffece72fa4979d3e26bc9ee3

Abnormal expression of the [alpha]E-catenin protein, a component of the E-cadherin/catenin cell adhesion complex, is frequently observed in human cancer cells. An inverse correlation between [alpha]E-catenin expression and tumor malignancy can be of prognostic value. Mutations of the [alpha]E-catenin gene, CTNNA1, were described in several human cancer cell lines and were found to result in aberrant cell adhesion. We have developed a polymerase chain reaction/single-strand conformation polymorphism-based method for mutation analysis of this gene in human tumor DNA. This approach enabled us to identify several polymorphisms in a set of desmoid tumors, demonstrating that this method is suitable for [alpha]E-catenin mutational analysis. On the basis of our genomic characterization data, we found that the previously reported alternative splicing of the [alpha]E-catenin gene actually generates a frame-shift, resulting in a truncated [alpha]E-catenin protein. This finding is unlike the other [alpha]-catenin family members [alpha]N-catenin and vinculin, which show in-frame alternative inserts. Furthermore, real-time quantitative reverse transcriptase-PCR analysis did not reveal relevant expression levels of this alternatively spliced [alpha]E-catenin variant neither in any human tissue or cell line tested, nor at any mouse developmental stage tested. Thus, contrary to previous notions, alternative splicing with in-frame insertion nearby the C-terminal end of the protein is not a general feature for all members of the [alpha]-catenin/vinculin family.


http://www.sciencedirect.com/science/article/B6T1V-3TGVY68-M/2/4cca8604d371e279541462de4e41424

We have isolated a Pinus sylvestris cDNA encoding a globular protein of 474 amino acids with a predicted molecular weight of 52 995 Da. The deduced amino acid sequence showed 41.9% identity and 13.6% similarity to mammalian cytosolic 3-hydroxy-3-methylglutaryl-CoA-synthase (HMGS). Treatment of Scots pine seedlings with ozone resulted in a transient increase of a 1.95 kb transcript, whereas a 1.2 kb mRNA decreased transiently, indicating a possible influence of ozone on isoprenoid biosynthesis.

http://www.sciencedirect.com/science/article/B6T1V-486TC9F-2Y/2/274905e8631602e3aea8fbeb7c50796ee

A clone of about 1 kb has been isolated from a human brain cDNA library. The clone possesses a 151 amino acid open reading frame that exhibits 72% amino acid identity with the E2 ubiquitin-conjugating enzyme encoded by the RAD6 gene of *Saccharomyces cerevisiae*. A 90% amino acid identity was observed in a central sequence surrounding a cysteine, which most likely contributes the sulfhydryl group involved in the formation of the ubiquitin-E2 thiolester linkage. Northern hybridization analyses have identified a poly(A)-containing mRNA of about 1 kb encoding the E2-like sequence in human CEM lymphoblastoid and HeLa cells, Novikoff rat hepatoma cells and S49 mouse leukemia cells. Southern hybridization analyses indicate the presence of a single gene encoding this sequence in both human cell lines, but of two or more related genes in the rodent cell lines.


http://www.sciencedirect.com/science/article/B6T1V-47MK2CX-1/2/5b1515ec24e8f2138e03c75864371953

N6-furfuryladenine (kinetin, K) was shown to have cytokinin activity and antiageing effects. It also appears to protect DNA against oxidative damage mediated by the Fenton reaction. Kinetin was identified as a natural component of DNA in plant extract, calf thymus DNA, fresh DNA preparations from human cell culture, as well as in human urine. A proposed mechanism of kinetin synthesis includes furfural, the oxidative damage product of a 2-deoxyribose moiety of DNA, which reacts with an adenine residue to form N6-furfuryladenine at DNA level. The identification of kinetin in plant cell extracts, as well as in human urine, suggests its excision from DNA by repair mechanisms. Since such a bulky modification as kinetin induces conformational changes of DNA, this could lead to mutations. Therefore, it was interesting to analyze an effect of kinetin on coding properties of DNA. Chemically synthesized oligodeoxynucleotide (20-mer) containing kinetin AAAACTGCCGTCCTGAKGAT was used as a primer. It was elongated in a polymerase chain reaction (PCR) on a template plasmid pEW1 harboring a 210-bp fragment of DNA derived from the 5' end of HIV mRNA. The PCR product of that length containing kinetin in position 17 from the 5' end was isolated and sequenced. Interestingly, DNA polymerase correctly incorporates thymine opposite of kinetin (an adenine derivative) on the complementary strand, but the misincorporations occur in a vicinity of the modified base.


http://www.sciencedirect.com/science/article/B6T1V-3W6F5WJ-3/2/0d4a7fd8d925ee6d907a2f251cb22161
Certain Bacillus licheniformis strains isolated from oil wells have been shown to produce a very effective biosurfactant, lichenysin A, which is structurally similar to another less active lipopeptide, surfactin. Surfactin, like many small peptides in prokaryotes and lower eukaryotes, is synthesized non-ribosomally by multi-enzyme peptide synthetase complex. Analysis of several peptide synthetases of bacterial and fungal origin has revealed a high degree of sequence conservation. Two 35-mer oligonucleotides derived from highly conserved motifs ('core I' and 'core II') of surfactin synthetase were used to identify the cloned putative operon of lichenysin A synthetase lchA from B. licheniformis BNP29, a strain not amenable to genetic manipulation in a BAC system (F-plasmid-based bacterial artificial chromosome) based on Escherichia coli and its single-copy plasmid F-factor. A 32.4 kb fragment containing lichenysin A biosynthesis locus was sequenced and analysed. The structural architecture of putative lichenysin A synthetase protein containing seven amino acid (aa) activation-thiolation, two epimerization and one thioesterase domains is discussed in terms of its similarity to surfactin and other peptide synthetases. The 100 aa peptide chain situated between the highly conserved signature sequences FDXX and NXYGPE(IV)X within amino acid binding domains of peptide synthetases is proposed to be a minimal block dictating the substrate specificity of the enzymes. A new operon-type structure has been localized directly upstream from the lichenysin A synthetase genes which, on the basis of sequence determination, potentially encode a four-member ABC-type transport system involved in product secretion.


DmsR protein is a member of the OmpR response regulator subfamily that activates the transcription of the dmsCBA operon in Rhodobacter sphaeroides f. sp. denitrificans. By site-directed mutagenesis some functional amino acid residues were investigated in DmsR, which consists of the N-terminal regulatory and the C-terminal DNA-binding domains and the linker connecting the two domains. The substitution of P130S in the linker caused decreases of both DNA-binding and transcriptional activator activities. Introducing additional substitutions of R129P or D131P to the DmsR-P130S derivative recovered both activities, demonstrating necessity of proline residue at one of the positions 129-131 in the linker. Substitutions of D12A, D55A, and K104M, at residues conserved in the phosphorylation region, caused no production of DMSO reductase, but retained DNA-binding ability, suggesting that unphosphorylated DmsR also has high affinity to its target nucleotide sequence of DNA. Substitutions in the C-terminal domain suggested the presence of a winged helix-turn-helix structure observed in the DNA-binding domain of the Escherichia coli OmpR.


The coding region of cDNA and genomic DNA, with its promoter region, of zebrafish metallothionein (zMT) gene homologous to the piscine MT-II was obtained. The A/T-rich promoter region contains four metal regulatory elements (MREs), three activator protein 1 (AP1) and one
specific protein 1 (Sp1) binding sites. The four MREs are organized into two clusters, a distal cluster with one MRE lying around 740 bp upstream of the transcription start point and a proximal cluster with three MREs located close to the TATA box. The metal induction ability of the promoter was assessed by transient luciferase gene expression assays in HepG2 cells. The zMT promoter was inducible by Zn²⁺, Cd²⁺, Cu²⁺ and Hg²⁺ ions in decreasing inducibility, while inert to Ni²⁺, Pb²⁺ and Co²⁺ ions, and H₂O₂ treatment in vitro. Deletion of the putative cis-acting elements in the promoter region revealed that the distal MRE (MRED) was important in mediating metal inducibility. Despite the binding of HepG2 cell nuclear protein factors to all MREs as confirmed by electrophoretic mobility shift assay (EMSA), the proximal MREs did not provide significant contribution to metal induction of zMT gene in HepG2 cells. The metal inducibility of zMT promoter required the cooperative effect of at least three MRE sites.


http://www.sciencedirect.com/science/article/B6T1V-42M1CPP-C/2/009d1ae417a42d90e7c77731964c20d4

Transposon mutagenesis was employed to isolate the gene(s) related with the biosynthesis of dipeptide antibiotic in Bacillus subtilis PY79 (a prototrophic derivative of the standard 168 strain). The blocked mutants were phenotypically selected from the transposon library by bioassay and the complete loss of biosynthetic ability was verified through ESI-mass spectrometry analysis. Four different bacilysin nonproducer mutants (Bac:-Tn10(ori-spc)) were isolated from the transposon library. The genes involved in bacilysin biosynthesis were identified as thyA (thymidilate synthetase), ybgG (unknown; similar to homocysteine methyl transferase) and oppA (oligopeptide permease), respectively. The other blocked gene was yvgW (unknown; similar to heavy metal-transporting ATPase); however, backcross studies did not verify its involvement in bacilysin biosynthesis. This gene, on the other hand, appeared to be necessary for efficient sporulation and transformation. Opp involvement was significant as it suggested that bacilysin biosynthesis is under or a component of the quorum sensing pathway which has been shown to be responsible for the establishment of sporulation, competence development and onset of surfactin biosynthesis. For verification, it was necessary to check the involvement of peptide pheromones (PhrA or PhrC) internalized by the Opp system and response regulator ComA as the essential components of this global control. phrA, phrC and comA deleted mutants of PY79 were thus constructed and the latter two genes were shown to be essential for bacilysin biosynthesis.


http://www.sciencedirect.com/science/article/B6T1V-3XX6V8T-14/2/8820c9346a8ce03607c5d88e040a9815

dlk encodes a transmembrane protein member of the EGF-like family of homeotic proteins. dlk is expressed in the same type of neuroendocrine tissues and tumors as pG2, a gene cloned because of its differential expression in human pheochromocytomas versus neuroblastomas. Human dlk and pG2 cDNAs are around 98% similar in sequence, but the predicted proteins encoded by those genes are apparently unrelated. This fact suggested the existence of polymorphic variants of the same gene. We have sequenced again several pG2 and dlk clones in parallel. We identified a pG2 cDNA species corresponding to an alternatively spliced dlk mRNA, as well as several other variant forms of dlk mRNA. One of the pG2 clones resulted to be
identical to human dlk and encode the same EGF-like protein. Pref-1, a cDNA isolated from 3T3-L1 fibroblasts, encodes a putative protein possessing an extracellular EGF-like domain similar to dlk, but a different intracellular region. Analysis of sequence data from different clones obtained in our laboratory confirmed some of the differences between dlk and Pref-1. However, the putative difference in the intracellular regions of dlk and Pref-1 was due to sequence artifacts. These data suggest that dlk, pG2 and Pref-1 are variant products of the same gene.


http://www.sciencedirect.com/science/article/B6T1V-3V8TY93-7/2/1273099a1fe0997ba4b5048b52cd135c

Alternative oxidase (AOX) is dramatically induced when the fungus Magnaporthe grisea is incubated with the fungicide SSF-126, which interacts with the cytochrome bc1 complex in the electron transport system of mitochondria. A full-length cDNA for the alternative oxidase gene (AOX) was obtained, and the deduced amino acid sequence revealed marked similarity to other AOXs, but lacks two cysteine residues at corresponding sites which are conserved in plant AOXs and play essential roles in the post-translational regulation. Northern blot experiments showed that treatment of M. grisea cells with SSF-126 induces accumulation of AOX mRNA in a dose-dependent manner, and the level was correlated with the activity of alternative respiration. H2O2 also induced the accumulation of the transcript with a short half-life (AOX gene was transcribed constitutively in unstimulated cells. Cycloheximide did not change the basal level of transcription, but induced the accumulation of the transcript, indicating that active degradation of the transcript occurs by factor(s) sensitive to cycloheximide. On the other hand, SSF-126 enhanced the transcriptional activity of AOX gene threefold compared to that of control cells, and H2O2 was also potent for enhancement of the transcription. From these results, it is concluded that the respiratory inhibitor-dependent activation of the transcription is a primary determinant for the induction of alternative respiration in M. grisea. Because we have previously shown that SSF-126 treatment of M. grisea mitochondria induced the generation of superoxide, active oxygen species are thought to be signal mediators to activate AOX gene transcription in M. grisea.

Biochimica et Biophysica Acta (BBA) - General Subjects (16)


http://www.sciencedirect.com/science/article/B6T1W-42VV80F-5/2/7f2f4fafebfc140f26c806069b97c18

The aim of this study was to characterize the cellular phenotypes of articular cartilage and meniscus in rabbits with experimentally induced osteoarthritis (OA), by histological and molecular biological techniques. OA was induced by severing the anterior cruciate ligament of the knee and rabbits were killed 2, 4 or 9 weeks following surgery. Our histological observations show a progressive destruction of extracellular matrix in both tissues. To determine whether these
morphological changes could be related to alterations in the regulation of gene expression for a subset of relevant molecules, levels of mRNA for proteinases and one inhibitor (MMP-1, -3 and -13, aggrecanase-1 and -2 and TIMP-1), matrix molecules and one chaperone (type II and X collagens, aggrecan, osteonectin, [beta]ig-h3 and BiP) were assessed by reverse transcription-polymerase chain reaction. Our results indicate that for most markers expression profiles were similar in both tissues. In particular, matrix protein gene expression remained stable or varied little during progression of OA, suggesting a poor repair capacity of the tissues. MMP gene expression increased rapidly whereas aggrecanase gene expression remained stable. These findings suggest that differential regulation of mRNA levels of MMP-1, -3 and -13 on the one hand and aggrecanase-1 and -2 on the other, occurs during OA.


http://www.sciencedirect.com/science/article/B6T1W-3YN927B-H/2/3bf3435a4aed8f0e42ba8e6c160b9f13

Colonization of the human stomach by Helicobacter pylori is associated with the development of gastritis, duodenal ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. H. pylori-antigen-binding single-chain variable fragments (ScFv) were derived from murine hybridomas producing monoclonal antibodies and expressed as a g3p-fusion protein on a filamentous M13 phage. The recombinant ScFv-phage reacted specifically with a 30-kDa monomeric protein of a H. pylori surface antigen preparation and by means of immunofluorescence microscopy the phage was shown to bind to both the spiral and coccoid forms of the bacterium. In vitro, the recombinant phage exhibited a bacteriocidal effect and inhibited specifically the growth of all the six strains of H. pylori tested. When H. pylori was pretreated with the phage 10 min before oral inoculation of mice, the colonization of the mouse stomachs by the bacterium was significantly reduced (P<0.01). The results suggest that genetic engineering may be used to generate therapy-effective phages.


http://www.sciencedirect.com/science/article/B6T1W-49031HB-2/2/119cc7ca6fe06a0f96356f2abc10ddb0

Rodent cells, widely used for the industrial production of recombinant human glycoproteins, possess CMP-N-acetylneuraminic acid hydroxylase (CMP-Neu5Ac hydroxylase; EC 1.14.13.45) which is the key enzyme in the formation of the sialic acid, N-glycolyineuraminic acid (Neu5Gc). This enzyme is not expressed in an active form in man and evidence suggests that the presence of Neu5Gc in recombinant therapeutic glycoproteins may elicit an immune response. The aim of this work was, therefore, to reduce CMP-Neu5Ac hydroxylase activity in a Chinese Hamster Ovary (CHO) cell line, and thus the Neu5Gc content of the resulting glycoconjugates, using a rational antisense RNA approach. For this purpose, the cDNA of the hamster hydroxylase was partially cloned and sequenced. Based on the sequence of the mouse and hamster cDNAs, optimal antisense RNA fragments were selected from preliminary in vitro translation tests. Compared to the parental cell line, the new strain (CHO-AsUH2), which was transfected with a 199-bp antisense fragment derived from the mouse CMP-Neu5Ac hydroxylase cDNA, showed an 80% reduction in hydroxylase activity. An analysis of the sialic acids present in the cells' own glycoconjugates revealed a decrease in the percentage of Neu5Gc residues from 4% in the
parental cells to less than 1% in the CHO-AsUH2 cell line.


http://www.sciencedirect.com/science/article/B6T1W-49W63M1-1/2/f88e7f3c16fc7281dc01f50f402d9450

We investigated the efficiency and the mechanism of action of a tetraphenyl porphyrin derivative in its photoreaction with T7 phage as surrogate of non-enveloped DNA viruses. TPFP was able to sensitize the photoinactivation of T7 phage in spite of the lack of its binding to the nucleoprotein complex. The efficiency of TPFP photosensitization was limited by the aggregation and by the photobleaching of porphyrin molecules. Addition of sodium azide or 1,3-dimethyl-2-thiourea (DMTU) to the reaction mixture moderated T7 inactivation, however, neither of them inhibited T7 inactivation completely. This result suggests that both Type I and Type II reaction play a role in the virus inactivation. Optical melting studies revealed structural changes in the protein part but not in the DNA of the photochemically treated nucleoprotein complex. Polymerase chain reaction (PCR) also failed to demonstrate any DNA damage. Circular dichroism (CD) spectra of photosensitized nucleoprotein complex indicated changes in the secondary structure of both the DNA and proteins. We suggest that damages in the protein capsid and/or loosening of protein-DNA interaction can be responsible for the photodynamic inactivation of T7 phage. The alterations in DNA secondary structure might be the result of photochemical damage in phage capsid proteins.


http://www.sciencedirect.com/science/article/B6T1W-47C4CJV-4/2/ec9cfdd03b0bf63e0bcf2fcb32388907

RE1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) mediates transcriptional repression in many neuron-specific genes by interaction with the repressor element 1/neuron-restrictive silencing element (RE1/NRSE). This element has been identified at least in 20 neuron specific genes. REST/NRSF is highly expressed in non-neuronal tissues, where it is thought to repress gene transcription. We performed a BLAST search to look for the presence of RE1/NRSE elements in the rat cytochrome P450 genes. We identified the presence of RE1/NRSE element in the cytochrome P450 genes CYP1A1, 2A2, 2E1 and 3A2. Electrophoretic mobility shift assay and supershift assays were carried out to prove functionality of these sites and detect the interaction of REST/NRSF with this sequence. Cotransfection studies in PC12 cells with a plasmid containing the RE1 element of the CYP genes, cloned upstream of the minimal type II sodium channel promoter, in the presence of REST/NRSF, showed a marked expression inhibition of the CAT reporter gene. These data suggest that the RE1 elements that exist in these four CYP genes might be a target for the REST/NRSF transcription factor and such an interaction might play a role in the negative regulation of these genes.

The present study constitutes the first finding of the calcium-binding protein S100B and of its mRNA in human milk, as revealed by a quantitative immunoluminometric assay, by Western blot analysis and by reverse transcription-polymerase chain reaction (RT-PCR) assay followed by restriction enzyme digestion. The concentration of S100B in milk is markedly higher than that observed in other biological fluids such as cord blood, peripheral blood, urine, cerebrospinal fluid and amniotic fluid. This finding could be related to a possible trophic role, which has been hypothesized for the protein.


Lsh is a member of the SNF2 family of chromatin remodelers, that regulate diverse biological processes such as replication, repair and transcription. Although expression of Lsh is highly tissue specific in adult animals, Lsh mRNA is detectable in multiple tissues during embryogenesis. In order to determine the physiologic role of Lsh during murine development and to assess its unique function in adult mice, we performed targeted deletion of the Lsh gene using homologous recombination in murine embryonic stem cells. Lsh-/− embryos occurred with the expected Mendelian frequency after implantation and during embryogenesis. However, Lsh-/− mice died within a few hours after birth. Furthermore, newborn mice were 22% lower in weight in comparison with their littermates and showed renal lesions. Thus Lsh is a non-redundant member of the SNF2 family and is essential for normal murine development and survival.


Cellular retinol-binding protein II (CRBP II) is an abundant cytosolic protein of intestinal absorptive cells. In this study, we examined whether dietary fat modulates the expression of CRBP II in the small intestine. In the rats fed a diet rich in long-chain triacylglycerols (LCT), both CRBP II mRNA and CRBP II protein levels in the jejunum were more than two-fold greater than in the rats fed a low fat diet and a diet rich in medium-chain triacylglycerols (MCT). The mRNA abundance of a retinoid X receptor (RXR[alpha]), which is thought to interact with the cis-element located in the CRBP II promoter, was elevated in the jejunum of rats fed high-LCT and high-MCT diets as compared with that of animals fed a low-fat diet, but the levels of RXP[alpha] mRNA of the LCT diet groups was similar to that of MCT diet group. These results suggest that the expression level of the CRBP II gene is not directly related to the RXR[alpha] expression, and that it might be modulated by long-chain fatty acids or their metabolites.

For an understanding of tumor-related alterations of the complex carbohydrate pattern of carcinomas, it is indispensable to monitor the expression profile of the various glycosyltransferases. The objective of this contribution was to perform an evaluation of the usefulness and the limits of the microarray approach for the identification of enzymes responsible for carbohydrate synthesis with differential expression in carcinomas. Expression profiles of colonic carcinomas were studied by oligonucleotide arrays using a novel strategy: colonic tissue of healthy individuals was compared with early staged colonic carcinomas; 'pure' cell populations were obtained by laser microdissection; RNA samples for hybridization with the oligonucleotide arrays were prepared by in vitro transcription without additional amplification. Expression of 39 glycosyltransferases and of 10 sulfotransferases in colonic tissues was analyzed by Affymetrix GeneChip technology. GeneChip analysis proved the high expression level of ST6Gal-I, [beta]4Gal-TI, II and III, GalNacT-1, FT-III and showed that ST3Gal-IV was the most abundantly expressed enzyme in healthy tissue. The strong overexpression of FT-VI in healthy tissue has not been described so far, as well as the upregulation of FT-VIII and downregulation of GnT-I in carcinoma tissue. Quantitative RT-PCR confirmed that FT-VI expression was significantly enhanced in healthy tissue. On the other hand, GeneChip analysis failed to detect any expression of GnT-III and GnT-V as well as of ST3Gal-I and ST3Gal-II, although these sequences could be amplified from the samples used for microarray analysis. According to our restricted analysis of only those 39 glycosyltransferases present on the GeneChip U95A, alterations of sialyltransferases ST6Gal-I, ST3Gal-IV, of fucosyltransferases FT-VI, FT-III, and probably FT-VIII, of GalNacT-I, and of [beta]4GalTI-II seem to be of relevance for the aberrant biosynthesis of membrane-bound carbohydrates during colonic carcinogenesis and metastasis.


http://www.sciencedirect.com/science/article/B6T1W-3R38XW3-N/2/2536d759198610b95cd258fdeb28fbe2

Since there are conflict reports on the presence of -amino-acid oxidase in the mouse liver, this problem was examined. -Amino-acid oxidase activity was not detected in the homogenates of the mouse liver, lung, or heart, whereas it was detected in the homogenates of the mouse kidney and brain. Western blotting showed that a protein which reacted with the antisera against pig -amino-acid oxidase was present in the homogenates of the mouse kidney and brain but not in those of the liver or heart. Northern hybridization using a -amino-acid oxidase cDNA probe detected a hybridizing signal in poly(A)+ RNAs extracted from the mouse kidney and brain but not in those from the liver, heart, or lung. Reverse transcription-polymerase chain reaction using three primer pairs always amplified -amino-acid oxidase cDNA fragments of expected sizes in the mouse kidney and brain but very rarely did so in the liver, heart, or lung. The results indicate that -amino-acid oxidase is not present in the mouse liver in a measurable amount.

We cloned the feruloyl esterase A gene from Aspergillus awamori (AwFAEA) and engineered it to study substrate specificity and pH dependence of catalysis. Based on the crystal structures of two type-A feruloyl esterases (FAE-III and AnFAEA) from Aspergillus niger, residues located in the flap region of AwFAEA (Asp71, Thr72, Asp77, and Tyr80) were replaced with corresponding amino acid residues (Ile, Arg, Asn, and Phe), respectively, found in the lid of lipases from Rhizomucor miehei (RmLIP) and Humicola lanuginose (HlLIP). Furthermore, Asp77 of AwFAEA, which is conserved in Aspergillus FAEs and lipases, was replaced with a hydrophobic residue (Ile). Kinetic analysis of the mutant enzymes showed that the higher catalytic efficiency of the D77I and Y80F mutants toward [alpha]-naphthylbutyrate (C4) and [alpha]-naphthylcaprylate (C8), respectively, was due to a lower Km value. The higher catalytic efficiency of D77N toward C4 substrate was due to a combination of decreased Km and considerably increased kcat. The D71I and Y80F mutants showed some activity toward long-acyl chain esters. On the other hand, the D77I mutant had no detectable activity toward phenolic acid methyl esters and feruloylated arabinoxylan. Moreover, the pH optima of the D77I, D77N, and Y80F mutants increased from 5.0 to 7.0-8.0, 7.0, and 6.0, respectively.


Hemoglobins (Hbs) are heme proteins encountered in all five kingdoms of living organisms. In plants, two different classes of Hbs have been identified: nonsymbiotic (class I) from both monocot and dicot species and symbiotic (class II) Hbs from nitrogen-fixing plants. This work reports the cloning and analysis of three nonsymbiotic Hb genes from wheat (Triticum aestivum) and potato (Solanum tuberosum). The Hb cDNAs were amplified by reverse transcriptase polymerase chain reaction (RT-PCR) using consensus oligonucleotide primers for nonsymbiotic Hbs. A wheat Hb cDNA (TaHb1) was isolated and shows a very high similarity to nonsymbiotic Hbs from Hordeum vulgare (98%) and Zea mays (83%). Another wheat Hb cDNA, designated TaHb2, exhibited strong similarity to truncated bacterial Hbs, the so-called 2-on-2 Hbs. In addition, a third Hb was cloned from potato, StHb. Expression analysis by RT-PCR demonstrated a very high expression level of the TaHb1 gene only in wheat roots. In contrast, the other wheat hemoglobin gene, TaHb2, was demonstrated to be constitutively expressed although differences in expression level in different tissues were observed. The expression of the TaHb1 gene is induced in wheat roots exposed to microaerobic conditions. The potato Hb gene, StHb, was highly expressed in roots and also in tubers and stem tissue although at much reduced levels.

facilitate purification, have been raised against the herbicides paraquat and atrazine and expressed in transgenic Nicotiana tabacum cv. Samsun NN. Prior to purification, the anti-atrazine scAb is expressed as up to 0.014% of soluble leaf protein and has a binding profile in ELISA, against an atrazine-bovine serum albumin (BSA) conjugate, similar to that of the scAb produced in Escherichia coli. Competition ELISA has shown that the plant-derived scAb also recognises free atrazine. Following antibody affinity purification to isolate dimers, the affinity for immobilised antigen approaches that of the parental monoclonal antibody. This was confirmed by surface plasmon resonance analysis. The purified scAb also recognises related triazine herbicides. When isolated from cell-suspension cultures, the anti-paraquat scAb binds to a paraquat conjugate in a concentration-dependent manner, with a profile similar to the parental monoclonal antibody. This is the first demonstration that functional scAbs against organic pollutants can be produced in transgenic plants and that the scAbs may be appropriate for the development of immunoassay-based detection systems.


http://www.sciencedirect.com/science/article/B6T1W-3X3KGG6-9/2/66a4226176a1d020d9e82b798aa85470

Biosynthesis of carbohydrate structures is tissue-specific and developmentally regulated by glycosyltransferases like fucosyl-, sialyl- and N-acetylglucosaminyltransferases. During carcinogenesis, aberrant glycosylation leads to the development of tumor subpopulations with different adhesion properties. The aim of this contribution was to directly compare mRNA expression of several glycosyltransferases in surgical specimens of gastric carcinomas. Carcinoma specimens were classified and characterized according to the WHO/UICC system. In each case, the expression of 12 glycosyltransferase enzymes was studied simultaneously by RT-PCR. For semi-quantitative analysis, amplification of the sample sequence was compared with that of [beta]-actin, co-amplified within the same tube. Expression of N-acetylglucosaminyltransferase V in gastric carcinomas was significantly enhanced compared to normal tissue. Also, expression of sialyltransferase ST3Gal-IV and fucosyltransferase FT-IV was significantly enhanced in carcinoma tissue. No significant differences in glycosyltransferase expression were found in samples positive for Helicobacter pylori or between the different gastric regions. Thus, carcinogenesis is characterized by specific alterations in mRNA expression of several glycosyltransferases. Future studies will show whether RT-PCR detection of the expression of these enzymes could be helpful for prognostic purposes.


http://www.sciencedirect.com/science/article/B6T1W-3SXDXBS-7/2/26c072a5a940ffbdff335a30cfa1d37378

Solution hybridization is an essential step in sequencing and some point mutation detection methods. In practice, this hybridization is hampered resulting in the need of additional purification of the amplification products. The use of T7 gene 6 exonuclease may lead to efficient production of single-stranded DNA. In this study, the effect of pretreatment with exonuclease on direct cycle sequencing and point mutation detection was analyzed. Exonuclease-treated products were directly cycle sequenced without further purification. This resulted in highly efficient quality improvement for sequencing allowing detection of heterozygotes. Point mutation detection by Point-EXACCT (exonuclease-amplification coupled capture technique) demonstrated detection of
one cell containing a mutation in an excess of 75000 wild type cells. Exonuclease-enhanced
detection methods offer simple, rapid detection strategies that are easily adaptable for
widespread clinical laboratory use. With the use of exonuclease, the detection of heterozygosity
using fluorescent cycle sequencing is becoming more reliable. The high sensitivity of Point-
EXACT due to the use of exonuclease makes it a highly promising method for large-scale
screening of (pre)malignant changes in patients with a high risk for developing cancer.

jejunum." *Biochimica et Biophysica Acta (BBA) - General Subjects* 1243(2): 270.

http://www.sciencedirect.com/science/article/B6T1W-497C7TR-2P/2/dbb500c607ac2955714dd75a0752143b

We have previously demonstrated that intake of fat as well as carbohydrate affects the activity
and immunoreactive amount of sucrase-isomaltase (S-I) in rat jejunum. To examine whether diet-
related changes in sucrase and isomaltase activities are accompanied by the variations of
sucrase-isomaltase mRNA levels, 7-week-old rats were fed either a high-long-chain
triacylglycerols diet (73 energy% as corn oil), a high-medium-chain triacylglycerols (MCT) diet (66
energy% as MCT, 7 energy% as corn oil) or a high-carbohydrate diet (70 energy% as corn
starch) for 7 days. Northern blot analysis revealed that S-I mRNA levels were abundant in the
jejunum of rats fed the high-MCT diet; the levels were similar to those in the rats fed the high-
carbohydrate diet. Force-feeding a high-sucrose diet (40 energy% as sucrose) brought about a
parallel rise in both S-I mRNA and sodium-glucose cotransporter (SGLT1) mRNA levels within
12 h. Force-feeding the high-MCT diet also produced an elevation of S-I mRNA and SGLT1
mRNA. However, force-feeding a diet containing [alpha]-methylglucoside, a non-metabolizable
but actively transported sugar, did not increase S-I mRNA or SGLT1 mRNA level; sucrase activity
was nevertheless elevated by feeding [alpha]-methylglucoside diet. These results suggest that
not only carbohydrate intake but also MCT intake might influence S-I mRNA and SGLT1 mRNA
levels in the jejunum, presumably through common metabolite(s) of carbohydrates and MCT, and
that carbohydrate may play another role in enhancement of the sucrase activity through
modulation of translation and/or posttranslational modifications of the sucrase-isomaltase
complex.

phospholipase A2." *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism* 1346(2): 185.*

http://www.sciencedirect.com/science/article/B6T1X-3PH8VKP-9/2/1a1225c75d1c6fcc94fc65b2eda637ae

Mammalian pancreatic phospholipases A2 (PLA2) have recently been implicated in cell surface
receptor-mediated inflammation. As a first step toward understanding how human pancreatic
PLA2 (hp-PLA2) interacts with membranes and other biological targets including cell-surface
receptors, we constructed its bacterial expression vector which can be used for the mutagenesis
and protein over-expression. The expression vector (pSH-hp) was constructed using a synthetic
hp-PLA2 gene whose transcription is controlled by T7 promoter. hp-PLA2 was expressed as a mature protein in high concentration in Escherichia coli cells and formed inclusion body. The solubilization of inclusion body protein followed by the refolding and purification produced ca. 5 mg of pure protein from one liter of growth medium. Kinetic studies of recombinant human, bovine and porcine pancreatic PLA2s using polymerized mixed liposomes and micelles as substrates showed that despite their highly homologous structures these mammalian pancreatic PLA2s have distinct phospholipid head group specificity and different activity toward various lipid substrates.


While UDP-glucuronosyltransferases (UGTs) are known to be expressed at high levels in human liver, relatively little is known about extrahepatic expression. In the present study, UGT2B family isoforms involved in the glucuronidation of steroid hormones and bile acids have been characterized in microsomes prepared from jejunum, ileum and colon from six human subjects. Glucuronidation of androsterone and testosterone was highly significant and increased from proximal to distal intestine. In contrast, hyodeoxycholic acid was glucuronidated at a low level in jejunum and ileum and activity was barely detectable in colon. No significant glucuronidation of lithocholic acid was found. Small phenols were glucuronidated with much lower activity than found in liver. High levels of UGT protein were detected with polyclonal anti-rat androsterone- and testosterone-UGT antibodies, whereas UGT2B4, a major hepatic hyodeoxycholic acid-specific UGT, was undetectable using a highly specific anti-human UGT2B4 antibody. Screening for RNA expression by RT-PCR confirmed the absence of UGT2B4 and UGT1A6 and showed expression of UGT2B7, a hepatic isoform shown to glucuronidate androsterone, in all intestinal segments. To our knowledge, the presence of functional androsterone and testosterone directed isoforms in human intestine is a novel finding which supports the idea that the intestinal tract functions as a steroid-metabolizing organ and plays a significant role in steroid hormone biotransformation.


A cDNA fragment which encodes salmon peroxisome proliferator activated receptor [gamma] (sPPAR[gamma]) was amplified by PCR from the liver of Atlantic salmon (Salmo salar L.). The fragment was 627 bp long. The sequence of the amplified PCR product was similar to the PPAR[gamma] of mouse and hamster. 59% of the bases were identical. Northern blot analysis of salmon liver mRNA showed that the amplified sPPAR[gamma] fragment hybridised to three specific transcripts of lengths 1.6, 2.4 and 3.3 kb. Clofibrinic acid and bezafibrate, administered to salmon hepatocytes in culture, resulted in a 1.7-fold increase of the 1.6 kb sPPAR[gamma] transcript. The activity of acyl-CoA oxidase also increased approx. 1.7-fold after administration of fibrates. These results indicate that PPAR is an important factor in mediating enzymatic response to fibrates in fish.

http://www.sciencedirect.com/science/article/B6T1X-497C7XB-9/2/1389ac06fc1669f3ce935ee15328ae9a

The scavenger receptors type I and II are mediators for the binding and uptake of chemically modified lipoproteins and are restricted to cells of monocyte origin. These receptors are highly expressed during the process of monocyte to macrophage differentiation. Quantitative mRNA levels of scavenger receptors from peripheral blood mononuclear cells have been analyzed in 29 hyperlipidemic patients and 15 healthy controls. Macrophage scavenger receptor isoforms transcripts were studied in circulating peripheral blood mononuclear cells with a modified RT-PCR method based on the use of a non-modified internal standard and a mathematical logistic adjustment of the standard curve. This method makes it feasible to study the variation in the expression of the scavenger receptors gene in peripheral blood during different physiopathological conditions. We studied the expression of the scavenger receptors gene in different blood cell lines and was present in only those of monocytic origin. The results have shown evidence that levels of scavenger receptor type I transcripts were proportional to apoB/cholesterol levels whereas type II receptors did not show any transcriptional variability. These findings suggest that the cholesterol level exerts a selective up-regulation of the scavenger receptor type I which is detectable by the induced increment of circulating monocytes in the blood of hyperlipidemic patients.

Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids (12)


http://www.sciencedirect.com/science/article/B6VNN-44137SX-2/2/22c1be845d1e0ffcf7ec56d11fa3ff6a

The genome of the nematode Caenorhabditis elegans contains several genes that appear to encode proteins similar to CTP:phosphocholine cytidylyltransferase (CCT). We have isolated a 1044-nucleotide cDNA clone from a C. elegans cDNA library that encodes the 347-amino acid version of CCT that is most similar to previously-identified CCTs. Native and His-tagged forms were expressed and purified using a baculovirus expression system. The enzyme was maximally activated by 5 [mu]M phosphatidylcholine:oleate (50:50) vesicles with a kcat value in the presence of lipid 37-fold greater than the kcat value in the absence of lipid. To localize the region of C. elegans CCT critical for lipid activation, a series of C-terminal truncation mutants was analyzed. CCT truncated after amino acids 225 or 245 was quite active in the absence of lipids and not further activated in the presence of lipids, supporting the concept that the lipid-activation segment is inhibitory to catalysis in the absence of lipids. CCT truncated after amino acids 266, 281, or 319 was activated by lipid similar to wild-type enzyme. Kinetic analysis in the absence of lipid revealed the lipid-independent CCT truncated after amino acid 245 to have a kcat value 15-fold greater than either full-length CCT or CCT truncated after amino acid 266. We conclude that elements critical for activation of C. elegans CCT by lipids are contained within amino acids 246-
that this region is inhibitory in the absence of lipids, and that the inhibition is relieved by the association of the enzyme with lipid.


http://www.sciencedirect.com/science/article/B6VNN-423987T-4/2/81950b2f35c39252c37111ccee0263f31

The subcellular localisation of oligodeoxynucleotides (ODN) is a major limitation for their use against nuclear targets. In this study we demonstrate that an antisense ODN directed against cytosolic phospholipase A2 (cPLA2) mRNA is efficiently taken up and accumulates in the nuclei of endothelial cells (HUVEC), human monocytes and HeLa cells. Gel shift experiments and incubation of cells with oligonucleotide derivatives show that the anti-cPLA2 oligo binds a 37 kDa protein in nuclear extracts. The TAAAT sequence was identified as the major binding motif for the nuclear protein in competition experiments with mutated ODNs. Modification of the AAA triplet resulted in an ODN which failed to localise in the nucleus. Moreover, inserting a TAAAT motif into an ODN localising in the cytosol did not modify its localisation. The 37 kDa protein was purified and identified after peptide sequencing as glyceraldehyde-3-phosphate dehydrogenase (GAPDH). It was shown by confocal microscopy that GAPDH co-localises with anti-cPLA2 ODN in the nucleus and commercial GAPDH effectively binds the oligo. Competition experiments with increasing concentration of NAD+ co-factor indicate that the GAPDH Rossmann fold is a docking site for antisense oligonucleotides containing a TAAAT motif.


http://www.sciencedirect.com/science/article/B6VNN-44PKRN1-1/2/3026bf271ff85099a747bc2685f1cbe0

Both a 25-hydroxylation and a 1[alpha]-hydroxylation are necessary for the conversion of vitamin D3 into the calcium-regulating hormone 1[alpha],25-dihydroxyvitamin D3. According to current knowledge, the hepatic mitochondrial cytochrome P450 (CYP) 27A and microsomal CYP2D25 are able to catalyze the former bioactivation step. Substantial 25-hydroxylation activity has also been demonstrated in kidney. This paper describes the molecular cloning and characterization of a microsomal vitamin D3 25- and 1[alpha]-hydroxylase in kidney. The enzyme purified from pig kidney and the recombinant enzyme expressed in COS cells catalyzed 25-hydroxylation of vitamin D3 and 1[alpha]-hydroxysteroid D3 and, in addition, 1[alpha]-hydroxylation of 25-hydroxyvitamin D3. The cDNA encodes a protein of 500 amino acids. Both the DNA sequence and the deduced peptide sequence of the renal enzyme are homologous with those of the hepatic vitamin D3 25-hydroxylase CYP2D25. Genomic Southern blot analysis suggested the presence of a single gene for CYP2D25 in the pig. Immunohistochemistry experiments indicated that CYP2D25 is expressed almost exclusively in the cells of cortical proximal tubules. The expression of CYP2D25 in kidney, but not in liver, was much higher in the adult pig than in the newborn. These findings indicate a tissue-specific developmental regulation of CYP2D25. The results from the current and previous studies on renal vitamin D hydroxylations imply that CYP2D25 has a biological role in kidney.

http://www.sciencedirect.com/science/article/B6VNN-42VV8D4-7/2/7b3069ef6639015c81126d83d3e35a2b

Acyl-CoA:cholesterol acyltransferase (ACAT) catalyzes cholesterol esterification in mammalian cells. Two isoforms of ACAT have been reported to date (ACAT-1 and ACAT-2). ACAT-1 is ubiquitously expressed in tissues except the intestine. In contrast, ACAT-2 is expressed mainly in the intestine in humans. To investigate the relationship between ACAT-2 and dyslipidemia, we determined the structure of the human ACAT-2 gene and then studied the relationship between mutations of the ACAT-2 gene and dyslipidemia. To isolate human ACAT-2 genomic DNA, we designed primers based on the human ACAT-2 cDNA sequence: forward primer 5' - ACACCTCGATCTTGGTCCTGCCATA-3' and reverse primer 5' - GGAATGCAGACAGGGAGTCCT-3'. Using these primers, a human P1-derived artificial chromosome (PAC) library was screened by PCR-based procedures. Isolated PAC clones were completely digested with BamHI and subcloned into plasmid vector. Subclones that contained exons were screened by dot-blot hybridization using partial ACAT-2 cDNA fragments. The coding region of the ACAT-2 gene was encoded in 15 exons from 51 to 265 base pairs on a 21 kilobase span of genomic DNA. The exonic sequences coincided completely with that of ACAT-2 cDNA, and each exon-intron junction conserved splicing consensus sequences. Next, 187 (91 dyslipidemic and 96 normolipidemic) subjects were screened by PCR single-strand conformational polymorphism analysis of the ACAT-2 gene. Three mutations were identified by DNA sequencing: two missense mutations (E14G in exon 1 and T254I in exon 7) and a point mutation in intron 7 (-35G->A). Mutations in exon 1 and intron 7 were not associated with plasma concentrations of lipids and apolipoproteins (apo). However, plasma apoC-III levels in T254I heterozygotes were significantly higher than those in subjects without mutation. Plasma triglyceride (TG) levels in T254I heterozygotes were similar to those in subjects without mutation. Although further studies are needed, our data suggest that ACAT-2 may contribute to apoC-III gene expression and the assembly of apoC-III and TG, possibly in the intestine.


http://www.sciencedirect.com/science/article/B6VNN-42MN74W-B/2/34bbbe8ce713541919d4d2f1d5be0d1e

cDNAs encoding major plasma apolipoproteins (apo) were cloned from the eel Anguilla japonica liver and their nucleotide sequences determined. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that eel lipoproteins contain apolipoproteins of 28 kDa and 14 kDa as major components. Each of the two apolipoproteins showed two isoforms having different isoelectric points as demonstrated by two-dimensional electrophoresis. The two 28 kDa components had different N-terminal amino acid sequences, whereas the two 14 kDa components had an identical one. Then cDNA clones encoding these apolipoproteins were isolated from a cDNA library constructed from the eel liver. An acidic 28 kDa component (28 kDa-1) consisted of 259 amino acids including a putative signal peptide of 27 residues, whereas a basic 28 kDa component (28 kDa-2) was composed of 260 amino acids containing a putative signal peptide of 23 residues. The tandem repeating units, which are characteristic of apolipoproteins, for 28 kDa-1 showed 27.8% identity to that of porcine apoA-IV, although mammalian apoA-IV is about 40 kDa and much larger than 28 kDa-1. However, the repeating
units of 28 kDa-2 showed 52.5% identity to that of Atlantic salmon apoA-I. The 14 kDa apolipoprotein consisted of 142 amino acids containing a putative signal peptide of 20 residues. It has a novel sequence differing from apolipoproteins of other vertebrates. The transcriptional expressions of 28 kDa-1, 28 kDa-2, and 14 kDa components were all restricted to the liver, except for the transcripts of 28 kDa-2 which were also slightly expressed in the intestine.


http://www.sciencedirect.com/science/article/B6VNN-3VXS8YX-3/2/258814b7168aabf9c1739135aaf2f067

Bovine corneal epithelium contains arachidonate 12- and 15-lipoxygenase activity, while human corneal epithelium contains only 15-lipoxygenase activity. Our purpose was to identify the corneal 12- and 15-lipoxygenase isozymes. We used cDNA cloning to isolate the amino acid coding nucleotide sequences of two bovine lipoxygenases. The translated sequence of one lipoxygenase was 82% identical with human 15-lipoxygenase type 2 and 75% identical with mouse 8-lipoxygenase, whereas the other translated nucleotide sequence was 87% identical with human 12-lipoxygenase of the platelet type. Expression of 15-lipoxygenase type 2 and platelet type 12-lipoxygenase mRNAs were detected by Northern analysis. In addition to these two lipoxygenases, 12-lipoxygenase of leukocyte (tracheal) type was detected by polymerase chain reaction (PCR), sequencing, and Northern analysis. Finally, PCR and sequencing suggested that human corneal epithelium contains 15-lipoxygenase types 1 and 2.


http://www.sciencedirect.com/science/article/B6VNN-419BF60-J/2/9612452da9c8a2d61eba75c721ed8e1b

A shift from sialylation to fucosylation of mucosal glycoconjugates occurred in the mammalian digestive tract in the weaning period, but mice under germ-free conditions were found to express both fucosyl GM1 (FGM1) and fucosyl asialo GM1 (FGA1) in the stomach, cecum and colon, but not in the small intestine. By host-microbe interactions and administration of cycloheximide, FGA1 was quickly induced in the small intestine, but the concentrations of fucosylated glycolipids in the other regions were not altered significantly. Their expression coincided with the activity of GDP-fucose:GA1 [alpha]1,2-fucosyltransferase ([alpha]1,2-FT), and we isolated a cDNA with an open reading frame encoding the murine [alpha]1,2-FT (MFUT-II) of 347 amino acids with a predicted molecular mass of 39.21 kDa. The intraperitoneal injection of cycloheximide induced the mRNA and activity of [alpha]1,2-FT (MFUT-II) in the small intestine of germ-free mice, whereas no change in the mRNA or activity was observed in the stomach, cecum and colon, indicating that expression of FGA1 in response to microbial colonization or cycloheximide is transcriptionally regulated in a restricted region of the murine digestive tract. At 24 h after the administration of cycloheximide, FGA1 was preferentially produced in the upper half of the duodenal microvilli.

pathways for bile acid synthesis by hybrid WIF-B9 cells." Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1534(1): 45.

http://www.sciencedirect.com/science/article/B6VNN-44HXKJ2-2/2/3e65c6baf82f38d9c3bfec58ec6b3d5

The rat hepatoma-human fibroblast hybrid cell line WIF-B9 stably exhibits the structural and functional characteristics of normal differentiated hepatocytes. The abilities of these cells to synthesize bile acids and amitate them with glycine and taurine were investigated. The release of bile acids into the culture media over 72 h was assessed by gas chromatography-mass spectrometry. WIF-B9 cells were able to synthesize bile acids (1.10 +/- 0.17 nmol/mg protein) but less efficiently than rat hepatocytes in primary culture (2.19 +/- 0.19 nmol/mg protein; P<0.01). The patterns of major bile acid species produced by both types of cells were also different. Cholic acid (CA; 72%) and [beta]-muricholic acid (19%) were the major bile acids produced by rat hepatocytes, while chenodeoxycholic acid (CDCA) accounted for only 4.5% of total bile acids. In contrast, muricholic acids were absent, while CA (62%) and CDCA (34%) were the most abundant bile acids synthesized by WIF-B9 cells. Using reverse transcription-polymerase chain reaction and gene- and species-specific primers for key enzymes involved in bile acid synthesis, the expression of human, but not rat, orthologues of CYP7A1, CYP27, CYP8B and CYP7B1 was found in WIF-B9 cells. Induction of cell stress by serum deprivation did not change the amount of total bile acids synthesized by these cells, but an inversion of the CA-to-CDCA ratio from 1.8 to 0.3 together with a marked increase in the proportion of intermediate metabolites related to the acidic pathway was found. Using 500 [mu]M radiolabeled CA and 2 mM of taurine or glycine, the ability to amitate CA over 48 h was determined by high performance liquid chromatography. Rat hepatocytes conjugated more than 90% CA with either amino acid, whereas this ability was very poor (<2%) in WIF-B9 cells. Regarding the expression of enzymes and the products of bile acid synthesis, it may be concluded that the human phenotype predominates over that of the rat in WIF-B9 cells. Moreover, these cells are almost completely unable to further conjugate primary bile acids, which facilitates the manipulation of these steroids in analytical procedures. These characteristics make WIF-B9 cells a suitable in vitro model to carry out studies on bile acid synthesis by 'human-like' metabolic pathways.


http://www.sciencedirect.com/science/article/B6VNN-4BP3D26-1/2/a740bc0bbe84f3b740aaaf1ee977257

Background: Acyl-CoA:cholesterol acyltransferase (ACAT) plays important roles in cellular cholesterol homeostasis. Two isoforms of ACAT have been reported (ACAT-1 and ACAT-2). ACAT inhibitors cannot only prevent atherosclerosis formation, but may also induce its regression in animals. In humans, an ACAT inhibitor was shown to have a lipid-lowering effect. The present study was carried out to clarify the relationship between ACAT-1 gene variants and hyperlipidemia. Methods and results: To identify genetic variants, we screened 30 subjects with hyperlipidemia by direct sequencing. As a result, a missense variant (R526G) and a variant in the 5' untranslated region (-77G->A) were identified. The genotype frequencies of each variant were determined in 178 unrelated normolipidemic and 441 unrelated hyperlipidemic subjects. The alleles frequencies of the R526G variant in normolipidemic and hyperlipidemic subjects were 0.676 and 0.633, respectively. The alleles frequencies of the -77G->A variant in normolipidemic and hyperlipidemic subjects were 0.503 and 0.515, respectively. Differences in allele frequencies between normolipidemic and hyperlipidemic subjects were not significant in both variants. R526G variant did not affect plasma concentrations of lipids or apolipoproteins in subjects studied.
However, among hyperlipidemic subjects, plasma concentrations of HDL-C and apoA-I in subjects with -77G->A variant were significantly higher than those in subjects without variant. Conclusion: Two variants in ACAT-1 gene were identified in subjects with hyperlipidemia. -77G->A variant affects plasma HDL concentrations only in hyperlipidemic subjects. These data suggest that the intracellular FC concentration might modulate plasma HDL concentrations.


http://www.sciencedirect.com/science/article/B6VNN-4F00PNB-1/2/786fff02ce330100bed0de961c54088a

The membrane-bound acyl-CoA elongase complex is a key enzyme responsible for erucyl-CoA synthesis. Among the four putative genes encoding the four moieties of this complex in Brassica napus seeds, only one has been characterized, the Bn-fae1 gene, which encodes the 3-ketoacyl-CoA synthase. The genes encoding the other enzymes (3-ketoacyl-CoA reductase, 3-hydroxyacyl-CoA dehydratase and trans-2,3-enoyl-CoA reductase) have not been identified. We cloned two 3-ketoacyl-CoA reductase cDNA isoforms, Bn-kcr1 and Bn-kcr2, from B. napus seeds. Their function was identified by heterologous complementation in yeast by restoring elongase activities. The comparison of Bn-kcr mRNA expression in different B. napus tissues showed that the genes were preferentially expressed in seeds and roots. We also investigated the regulation of gene expression in High Erucic Acid Rapeseed (HEAR) and in Low Erucic Acid Rapeseed (LEAR) cultivars during seed development. The co-expression of Bn-fae1 and Bn-kcr observed in HEAR cultivar during seed development was different in LEAR cultivar, suggesting that expression of both genes was directly or indirectly linked.


http://www.sciencedirect.com/science/article/B6VNN-44137SX-7/2/e62aa8ccdf8ae991c4fd646ebd92410

The Bn-FAE1.1 and Bn-FAE1.2 genes encode the 3-ketoacyl-CoA synthase, a component of the elongation complex responsible for the synthesis of very long chain monounsaturated fatty acids (VLCMFA) in the seeds of Brassica napus. Bn-FAE1 gene expression was studied during seed development using two different cultivars: Gaspard, a high erucic acid rapeseed (HEAR), and ISLR4, a low erucic acid rapeseed (LEAR). The mRNA developmental profiles were similar for the two cultivars, the maximal expression levels being measured at 8 weeks after pollination (WAP) in HEAR and at 9 WAP in LEAR. Differential expression of Bn-FAE1.1 and Bn-FAE1.2 genes was also studied. In each cultivar the same expression profile was observed for both genes, but Bn-FAE1.2 was expressed at a lower level than Bn-FAE1.1. Secondly, VLCMFA synthesis was measured using particulate fractions prepared from maturing seeds harvested weekly after pollination. The oleoyl-CoA and ATP-dependent elongase activities increased from the 4th WAP in HEAR and reached the maximal level at 8 WAP, whereas both activities were absent in LEAR. In contrast, the 3-hydroxy dehydratase, a subunit of the elongase complex, had a similar activity in both cultivars and reached a maximum from 7 to 9 WAP. Finally, antibodies against the 3-ketoacyl-CoA synthase revealed a protein of 57 kDa present only in HEAR. Our results show: (i) that both genes are transcribed in HEAR and LEAR cultivars; (ii) that they are coordinately regulated; (iii) that Bn-FAE1.1 is quantitatively the major isoform expressed in seeds; (iv) that the Bn-FAE1 gene encodes a protein of 57 kDa responsible for the 3-ketoacyl-CoA
The expression of acetyl-CoA carboxylase (ACC) in mouse peripheral nervous system (PNS) was investigated. Both ACC 265 and ACC 280 isoforms were expressed in the sciatic nerve, although ACC 265 was predominant. ACC 265 transcripts originating from promoters P1 and P2 could be detected in the developing nerve, as well as the two splice products, which are characterized by the presence or the absence of a 24-base sequence before the codon serine-1200. The mRNA levels for ACC 265 parallel those of other lipogenic genes whose expression is linked to the myelination process. In addition, ACC 265 mRNA and protein levels in the nerves of the trembler mutant, which is a mouse model of PNS dysmyelination, represented around 30% of the normal values. The expression of the sterol regulatory element-binding proteins (SREBPs) was also studied. SREBP 1 mRNAs were expressed at a constant level during nerve development, and their quantities were normal in trembler. On the contrary, SREBP 2 mRNA quantities varied during the myelination period similarly to the lipogenic gene mRNAs, and the levels measured in trembler represented only 10% of the normal values. Taken together, these results suggest that the coordinate expression of several lipogenic genes, which occurs during PNS myelination, could possibly be regulated by SREBP 2.
The development of the polymerase chain reaction (PCR), which routinely can amplify specific target sequences more than one billion-fold, has made it possible to produce readily detectable amounts of DNA from a few copies of very rare sequences. We have begun a study of mitochondrial myopathies with the purpose of developing a diagnostic test using PCR to amplify appropriate mitochondrial DNA (mtDNA) target sequences from small amounts of sample. We have developed a 15-min procedure for recovering mtDNA which can be amplified by PCR to detectable levels, from as little as 30 [mu]l of blood or 5 [mu]l of amniotic fluid. We have microscopically selected HL60 cells, and have found that 28 cycles of PCR allows the detection of mitochondrial targets from a single cell. Using micromanipulation techniques, we utilized this approach to analyze mtDNA from a single cell isolated from an 8-cell stage mouse blastocyst. Finally, a single cell cultured from a patient with Leber's hereditary optic neuropathy, a mitochondrial myopathy, provided sufficient mtDNA for detection of the single base substitution that leads to loss of a restriction endonuclease recognition site for SfaNI and generation of a site for MaeIII.


In this study, we have evaluated the role of cytokine-induced neutrophil chemoattractant (CINC), in the upregulation of neutrophil Ca2+ signaling in neutrophils from thermally injured rats treated with anti-CINC antibody. Additionally, we have determined the effect of the treatment with CINC antibody on the accumulation of activated neutrophils in the intestinal wall, and the effect of such accumulation on gut bacterial translocation. Measurements of myeloperoxidase (MPO) activity and immunohistochemical localization of neutrophils determined neutrophil sequestration in the rat intestine. Agar culture analyses and a specific Escherichia coli [beta]-galactosidase gene polymerase chain reaction was carried out to detect gut indigenous bacterial invasion into intestinal wall and extraintestinal mesenteric lymph nodes (MLN). The results showed that pretreatment of rats with anti-CINC antibody attenuated the thermal injury-induced enhancement in [Ca2+]i responses in neutrophils both in the basal and Formyl-Met-Leu-Phe stimulated conditions. Moreover, treatment with the CINC antibody decreased neutrophil infiltration into the gut and attenuated thermal injury-caused translocation of bacteria into the MLN.


Mucopolysaccharidosis BID results from the deficiency of N-acetylglucosamine 6-sulfatase activity. A Nubian goat with this lysosomal storage disease has been identified. As a first step in developing this animal model for testing treatment methods, we cloned and sequenced the caprine N-acetylglucosamine 6-sulfatase cDNA coding region. Overall there is 88% nucleotide homology between the goat and human sequence and 94% homology of the deduced amino acid
sequence. The human and two ruminant species differ by the presence of an imperfect trinucleotide (CCG) repeat in the ruminant signal sequence.


http://www.sciencedirect.com/science/article/B6T1Y-47NVXDX-26/2/4c059e3df7774c329058ceaf8aa4f11a

Albumin Ortonovo is a slow moving variant of human serum albumin which has been found only in people coming from the small villages of Ortonovo and Nicola (Liguria, Italy) and reaches polymorphic frequency (>=1%) in the poorly admixed population group living in that area. This is the first report of a 'private' variant detected in a Caucasin population. It probably originated as a mutation in a founder individual many generations ago. Isoelectric focusing analysis of CNBr fragments from the purified variant localized the mutation in fragment CNBr (residues 447-548). This fragment was isolated on a preparative scale by reversed-phase HPLC and subjected to V8 proteinase digestion. Sequence analysis of the abnormal V8 peptide revealed that the variant arises from a previously unreported substitution at position 505 where glutamic acid has been replaced by lysine. The protein data were confirmed by DNA sequence analysis which indicated a single nucleotide change of in the corresponding codon of the structural gene. Since the amino acid substitution found in albumin Ortonovo accords with its electrophoretic mobility on cellulose acetate, residue 505 is probably exposed to the solvent. The clustering of the mutations in the intersubdomain connection linking subdomains IIIA and IIIB (residues 492-511) accords with the fact that this region lies on the molecular surface and is accessible to solvent.


http://www.sciencedirect.com/science/article/B6T1Y-47N6VM-23/2/823cfab9d5e5e042d8d5c6c98d54f028

LA Wistar rats have a deficiency of androsterone UDP-glucuronosyltransferase (UDPGT) and are present in Wistar rat colonies around the world. In order to clarify the molecular mechanism of the deficiency, androsterone UDPGT cDNA clone, pGT2 was isolated from rat liver cDNA library and was digested with restriction enzymes to afford three probes for Northern and Southern blot analyses in HA (normal), heterozygous LA and LA Wistar rats. In Northern blot analysis, androsterone UDPGT mRNA was totally absent in LA Wistar rat liver. Southern blot analysis suggested a large deletion of androsterone UDPGT gene in the rats. Genomic DNA amplifications with synthetic primers which have nucleotide sequences corresponding to the 5'-region of androsterone UDPGT cDNA, suggested that androsterone UDPGT gene has exon 1 with a length of some 700 bp and that this exon is deleted in LA Wistar rats. Based on these lines of evidence, it is concluded that the large portion of androsterone UDPGT gene is deleted in LA Wistar rats, which results in the absence of androsterone UDPGT mRNA and consequently the corresponding enzyme protein.

Mutations in the ATP-binding cassette transporter 1 (ABCA1) gene have been recently identified as the molecular defect in Tangier disease (TD) and familial high density lipoprotein deficiency (FHA). We here report novel mutations in the ABCA1 gene in two sisters from a Japanese family with TD who have been described previously (S. Ohtaki, H. Nakagawa, N. Kida, H. Nakamura, K. Tsuda, S. Yokoyama, T. Yamamura, S. Tajima, A. Yamamoto, Atherosclerosis 49 (1983)) and a family with FHA. Both probands of TD and FHA developed coronary heart disease sequence analysis of the ABCA1 gene from the patients with TD revealed a homozygous G to A transition at nucleotide 3805 of the cDNA resulting in the substitution of Asp 1229 with Asn in exon 27, and a C to T at nucleotide 6181 resulting in the substitution of Arg 2021 with Trp in exon 47. Sequence analysis of the ABCA1 gene from the FHA patient revealed a homozygous 4 bp CGCC deletion from nucleotide 3787 to 3790 resulting in premature termination by frameshift at codon 1224. These mutations were confirmed by restriction digestion analysis, and were not found in 141 control subjects. Our findings indicate that mutations in the ABCA1 gene are associated with TD as well as FHA.


Hypoxanthine phosphoribosyltransferases (HPRTs) are of biomedical interest because defects in the enzyme from humans can result in gouty arthritis or Lesch-Nyhan syndrome, and in parasites these enzymes are potential targets for antiparasite chemotherapy. In HPRTs, a long flexible loop (active site loop II) closes over the active site during the enzyme catalyzed reaction. Functional roles for this loop have been proposed but have yet to be substantiated. For the present study, seven amino acids were deleted from loop II of the HPRT from Trypanosoma cruzi to probe the functional role of this active site loop in catalysis. The mutant enzyme ([Delta]loop II) was expressed in bacteria, purified by affinity chromatography, and kinetic constants were determined for substrates of both forward (purine salvage) and reverse (pyrophosphorolysis) reactions catalyzed by the enzyme. Loop II deletion resulted in moderate (0.6-2.7-fold) changes in the Michaelis constants (Kms) for substrates other than pyrophosphate (PPi), for which there was a 5.8-fold increase. In contrast, kcat values were severely affected by loop deletion, with rates that were 240-840-fold below those for the wild-type enzyme. Together with previously reported structural data, these results are consistent with active site loop II participating in transition-state stabilization by precise positioning of the substrates for in line nucleophilic attack and in the liberation of PPI as a product of the salvage reaction.

Caudal type homeobox gene-1 and -2 (CDX-1 and CDX-2), homologues of the Drosophila homeobox gene caudal, encode transcription factors in endoderm derived tissues of the intestine. CDX genes control proliferation and differentiation of intestinal mucosal cells and colon cancer cells. Hirschsprung's Disease (HD) or congenital intestinal aganglionosis, a major developmental anomaly of intestine, which causes functional intestinal obstruction, is frequently associated with enterocolitis. Aetiology of HD-associated enterocolitis (HDEC) remains obscure. Reduction of gut mucosal enteroendocrine cells, and inefficient transfer of the secretory immunoglobulin A across the gut mucosal cell were shown to be associated with enterocolitis in HD patients suggesting that mucosa may directly involve in the pathophysiology of HDEC. This study aims to ascertain whether the CDX-1 and CDX-2 genes, that control the proliferation and differentiation of mucosal cells, play a role in HDEC. Using semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and in situ hybridisation, we analysed the expression of CDX-1 and CDX-2 genes in colon specimens of normal controls, necrotising enterocolitis (NEC) infants, and HD patients with and without enterocolitis. We showed for the first time that CDX-1 and CDX-2 genes were expressed in the colonic mucosal epithelium in normal, NEC and in HD infants. However, the expressions of both genes were reduced in patients with HDEC. Our findings suggest that reduced expression of CDX-1 and CDX-2 genes in mucosa may be associated with the development of HDEC.


Lysosomal [beta]-hexosaminidase (EC 3.2.1.52) occurs as two major isozymes hexosaminidase A ([alpha][beta]) and B ([beta][beta]). The [alpha] subunit is encoded by the HEXA gene and the [beta] subunit by HEXB gene. Defects in the [alpha] or [beta] subunits lead to Tay-Sachs or Sandhoff disease, respectively. While many HEXA gene mutations have been reported only three HEXB gene mutations are known. We report the characterization of two rare HEXB mutations present in genomic DNA from a single fibroblast cell line, GM203, taken from a patient with the infantile form of Sandhoff disease. The first is a single base pair deletion in exon 7 changing the codon for Gly-258, GGA, to GA and the second, a two base pair deletion in exon 11 changes the codons for Arg-435/Val-436, AGA/GTC, to AGTC. Each mutation produces a frame shift in the affected allele that results in a premature stop codon 17 or 20 codons downstream, respectively. These mutations also result in the inability to detect [beta]-mRNA by Northern blot analysis of total mRNA. These data are consistent with the idea that the severe infantile form of Tay-Sachs or Sandhoff disease is associated with a total lack of residual hexosaminidase A activity.


In Gaucher disease patients, over 100 disease-causing mutations have been identified. For identification of the 1504C->T (R463C) mutation it is common to use PCR-restriction fragmentation analysis using the restriction enzyme MspI. In the present study we investigated the reliability of this approach because accurate determination of genotypes is important in genotype-phenotype correlations. A simple modification, i.e. using the restriction enzyme Hphl
instead of MspI, revealed that type I and II Gaucher disease patients who had previously been identified as carrying the 1504C->T mutation in fact carried the 1505G->A (IVS10-1G->A) mutation. Sequencing of the appropriate fragment confirmed this. The PCR method easily differentiates between these two mutations in Gaucher disease patients, thus circumventing the need for sequencing procedures. The phenotypes of the patients found to be carrying the 1505G->A mutation are also described.


http://www.sciencedirect.com/science/article/B6T1Y-497H5B5-2/2/edf143727bab4f946b55d8d2c8f761cb

Subacute necrotising encephalomyopathy (Leigh syndrome) due to cytochrome c oxidase (COX) deficiency is often caused by mutations in the SURF1 gene, encoding the Surf1 protein essential for COX assembly. We have investigated five patients with different SURF1 mutations resulting in the absence of Surf1 protein. All of them presented with severe and generalised COX defect. Immunoelectrophoretic analysis of cultured fibroblasts revealed 85% decrease of the normal-size COX complexes and significant accumulation of incomplete COX assemblies of 90-120 kDa. Spectrophotometric assay of COX activity showed a 70-90% decrease in lauryl maltoside (LM)-solubilised fibroblasts. In contrast, oxygen consumption analysis in whole cells revealed only a 13-31% decrease of COX activity, which was completely inhibited by detergent in patient cells but not in controls. In patient fibroblasts ADP-stimulated respiration was 50% decreased and cytofluorometry showed a significant decrease of mitochondrial membrane potential [Delta][Psi]m in state 4, as well as a 2.4-fold higher sensitivity of [Delta][Psi]m to uncoupler. We conclude that the absence of the Surf1 protein leads to the formation of incomplete COX complexes, which in situ maintain rather high electron-transport activity, while their H+-pumping is impaired. Enzyme inactivation by the detergent in patient cells indicates instability of incomplete COX assemblies.


http://www.sciencedirect.com/science/article/B6T1Y-41JTMXY-7/2/f42305d489710cd9bf77501c1acf8f3

We have investigated the mRNA amounts of six lysosomal proteins ([beta]-hexosaminidase [alpha]- and [beta]-subunit, sphingolipid activator protein precursor, GM2 activator protein, lysosomal sialidase, [beta]-glucocerebrosidase) involved in the degradation of glycosphingolipids. We analyzed extracts from brain tissues of mouse models for lysosomal storage diseases, i.e., the GM2 gangliosidoses and the deficiency of the sphingolipid activator protein precursor (prosaposin). The mRNA levels were quantified by real-time reverse transcription-polymerase chain reaction. Although storage of the respective lysosomal proteins has been reported in human and mice, no increase of their mRNA amounts could be detected here. Our results indicate that there is no transcriptional upregulation of lysosomal proteins in the examined neuronal storage disorders.

Pro-inflammatory cytokine release after shock is central in the development of subsequent multiple organ dysfunction syndrome. Some studies suggest that interleukin-10 (IL-10) is an immunosuppressive mediator after injury or sepsis, while others suggest that IL-10 is an important regulator of the pro-inflammatory response. We hypothesized that in a model of trauma and hemorrhagic shock (TH), IL-10 regulates pro-inflammatory cytokine activity via an autocrine effect on cytokine mRNA transcription in Kupffer cells early after TH. To study this, male C3H/HeN mice were sham-operated or subjected to TH. Plasma levels of TNF-[alpha], IL-6 and PGE2 were elevated following TH. A sharp peak in IL-10 levels was observed at 2 h after the insult. Kupffer cell (KC) depletion prior to TH reduced plasma IL-6, IL-10 and TNF-[alpha] levels, whereas treatment with anti-IL-10 after TH increased IL-6 and TNF-[alpha] levels. Kupffer cell mRNA expression for IL-6, IL-10 and TNF-[alpha] was elevated in the TH group and further increased by anti-IL-10 treatment. These findings indicate that KC-dependent IL-10 regulates the early systemic inflammatory response after TH. Thus, while IL-10 is an important mediator of immunosuppression following traumatic injury, it also is beneficial with regard to its ability to counter-regulate the early inflammatory response under such conditions.


We applied RNA arbitrarily primed-PCR (RAP-PCR) to screen the genes differentially expressed between common congenital heart defects (CHD) [atrial septal defect, ventricular septal defect, Tetrology of Fallot (TOF)] and normal human heart samples. Three of these differentially amplified fragments matched cDNA sequences coding for proteins of unknown function in humans: hCALO (human homologue of calossin), NP79 (coding for a nuclear protein of 79KD) and SUN2 (Sad-1 unc-84 domain protein 2). The other four fragments were from known human genes: apolipoprotein J, titin, dystrophin and protein kinase C-delta. Northern blot analysis confirmed that all of these genes are expressed in the human heart. The results of RAP-PCR were reconfirmed by quantitative RT-PCR in TOF and control heart samples. Both techniques showed the levels of expression of hCALO, NP79 and SUN2 to be comparable in TOF and control samples and the level of expression of dystrophin and titin, both coding for cytoskeletal proteins, to be significantly upregulated in TOF samples. In summary, we have shown that the RAP-PCR technique is useful in the identification of differentially expressed gene from biopsy samples of human CHD tissues. In this manner, we have identified three novel genes implicated in the normal function of the human heart and two known genes upregulated in TOF samples.


We tested the hypothesis that presenilin-1 mutations associated with familial Alzheimer's disease do not disrupt protein transport from the endoplasmic reticulum to the Golgi apparatus. We used a system of immortalized mouse Kupffer cells expressing human presenilin-1 to address this question. The results of our studies indicate that presenilin-1 mutations do not disrupt protein transport from the endoplasmic reticulum to the Golgi apparatus. In conclusion, our findings suggest that presenilin-1 mutations associated with familial Alzheimer's disease do not disrupt protein transport from the endoplasmic reticulum to the Golgi apparatus.
Mutations in genes encoding presenilin-1 (PS1) and presenilin-2 (PS2) have been linked to familial forms of Alzheimer's disease (AD). Cells expressing mutant presenilins produce elevated levels of A[beta]42, the major amyloid peptide found in AD plaques. The mechanism whereby this occurs remains unknown, but the localization of presenilins to endoplasmic reticulum (ER) and Golgi compartments has suggested that they may function in intracellular trafficking pathways involved in processing [beta]-amyloid precursor proteins (APP). To test this possibility, we coexpressed PS1(wt), PS1(M146L), or PS1(L286V) in HEK293 cells together with the LDL receptor, a classic glycoprotein marker that undergoes post-translational O-glycosylation in the Golgi compartment. Pulse-chase analysis of the receptor indicated that mutant presenilins had no effect on ER->Golgi transport. Similar results were obtained when the studies were carried out with cells expressing the Swedish variant of APP (SWAPP751) instead of the LDL receptor. Moreover, secretion of the soluble exodomain polypeptide fragments of SWAPP751 that arise from [alpha]-secretase and [beta]-secretase cleavage was not markedly affected by the PS1 mutants. Despite the lack of discernible effect of the PS1 mutants on trafficking of proteins through the Golgi apparatus, they caused a substantial increase in the proportion of A[beta]42 relative to total A[beta] in the culture medium. The results suggest that mutant forms of PS1 cause elevated production of A[beta]42 by a mechanism that is independent of a major disruption of exocytic trafficking of APP.


http://www.sciencedirect.com/science/article/B6T1Y-4BH6H52-1/2/6740e6b32030fcfdcc307b5e52d8bb7

Most gastrointestinal stromal tumors (GISTs) contain activating mutations of the proto-oncogene c-kit. The GNNK- isoform of c-kit has a greater oncogenic potential than the GNNK+ isoform. We studied tumors from 29 patients with GIST, 19 of whom had c-kit mutations, and compared them to normal cells and HMC-1 mast cell line. c-kit transcripts were quantified by real-time PCR. The ratios of GNNK-/+ isoforms and of wild-type/mutant alleles were determined by RT-PCR and fluorometric quantification. On average, GISTs contained 1.9 times more c-kit transcripts than the HMC-1 cell line and GISTs with c-kit mutations contained 2.8 times more c-kit transcripts than those without (P=0.003). The median GNNK-/+ isoform ratios in GISTs with and without c-kit mutations were 4.4 and 4.1, respectively, and there was no difference in the GNNK-/+ ratios between the GISTs and the control samples. Both mutant and wild-type alleles of c-kit were expressed in similar amounts in 13/15 mutant GISTs. The oncogenic effects of KIT in GISTs are not related to the higher expression level of the GNNK- isoform. The high expression level of both mutated and wild-type allele transcripts of c-kit suggests that interactions between spontaneously activated and normal c-kit receptors are important in GIST tumorigenesis.


http://www.sciencedirect.com/science/article/B6T1Y-4BSWJX7-1/2/b41c12794fae92461767d6569eefc1b2

Inhalation of urban pollutants elevates the circulating levels of the vasoactive peptides endothelin (ET)-1 and ET-3 in rats. This effect could explain the association between episodic variations of urban pollutants and acute cardiopulmonary morbidity and mortality documented in epidemiological studies. Because the lungs are the primary source of circulating ET-1 and the
main site of clearance from circulation, we investigated the response of endothelin system genes in the lungs of Fischer-344 rats after 4-h nose-only inhalation of 0.8 ppm ozone plus 49 mg/m³ EHC-93 (Ottawa particles). The mRNA levels for preproET-1, preproET-3, endothelin-converting enzyme (ECE)-1, and ET receptor subtypes A and B were determined at 2 h, and 1, 2, 3, 7, and 14 days after exposure. The pollutants induced preproET-1 and ECE-1 (PP<0.05), and returned to control levels by 24 h, indicating that induction of ET-3 in the lungs is not responsible for the sustained elevation of ET-3 in plasma reported after inhalation of pollutants. Our results indicate that lung endothelin system genes respond rapidly and transiently to inhalation of urban pollutants, consistent with the dynamics of urban pollutant health effects in the human population.


http://www.sciencedirect.com/science/article/B6T1Y-3X88CR4-2/2/08f846c4dabaacc345c8aedde05eae19

Elevated plasma levels of homocysteine have been shown to interfere with normal cell function in a variety of tissues and organs, such as the vascular wall and the liver. However, the molecular mechanisms behind homocysteine effects are not completely understood. In order to better characterize the cellular effects of homocysteine, we have searched for changes in gene expression induced by this amino acid. Our results show that homocysteine is able to induce the expression and synthesis of the tissue inhibitor of metalloproteinases-1 (TIMP-1) in a variety of cell types ranging from vascular smooth muscle cells to hepatocytes, HepG2 cells and hepatic stellate cells. In this latter cell type, homocysteine also stimulated [alpha]1(I) procollagen mRNA expression. TIMP-1 induction by homocysteine appears to be mediated by its thiol group. Additionally, we demonstrate that homocysteine is able to promote activating protein-1 (AP-1) binding activity, which has been shown to be critical for TIMP-1 induction. Our findings suggest that homocysteine may alter extracellular matrix homeostasis on diverse tissue backgrounds besides the vascular wall. The liver could be considered as another target for such action of homocysteine. Consequently, the elevated plasma levels of this amino acid found in different pathological or nutritional circumstances may cooperate with other agents, such as ethanol, in the onset of liver fibrosis.


http://www.sciencedirect.com/science/article/B6T1Y-47NVXTM-5P/2/7e64b1ec564ec828e03c85dab2cb31fb

An aberrant ferrochelatase mRNA lacking exon 10 was found in a patient with erythropoietic protoporphyria (EPP). In her genomic DNA an A -> T transversion at position -3 of the donor site of intron 10 appeared to be responsible for the exon skipping. Both the patient and her sister were heterozygous for this mutation.

Wanner, R., A. Panteleyev, et al. (1996). "Retinoic acid affects the expression rate of the differentiation-related genes aryl hydrocarbon receptor, ARNT and keratin 4 in proliferative keratinocytes only." Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1317(2): 105.
The environmental contaminant dioxin exerts most of its effects by activating the aryl hydrocarbon receptor (AhR). The AhR is considered to play not only a role in the regulation of xenobiotic metabolism, but also for development, growth, and differentiation. The transcript levels of the AhR and its associated translocator protein (ARNT) were found to increase with ongoing differentiation in the human keratinocyte cell line HaCaT. Correspondingly, in situ hybridization studies in normal human skin revealed an absence of AhR-expression in proliferating basal cells and increasing transcript levels in upper cell layers, in dependence of keratinocyte differentiation. AhR expression in differentiation-deficient hyperproliferative psoriatic skin was markedly decreased. When keratinocytes were continuously treated with 1 μM retinoic acid (RA), the upregulation of AhR- and ARNT-mRNA levels was inhibited as was keratin 4-expression, a marker of HaCaT-keratinocyte differentiation. In contrast, treatment of already differentiated cells with RA did not down-regulate these transcript levels. The mRNA levels of the prevalent retinoic acid receptors in keratinocytes, RAR[gamma] and RXR[alpha], were not influenced by the process of differentiation or by addition of RA. Our data suggest that the regulation of AhR-, ARNT- and keratin 4-expression by RA is indirect and mediated by a yet to be identified factor.

Biochimica et Biophysica Acta (BBA) - Molecular Cell Research (15)


We produced recombinant human thrombin mutants to investigate the correlation between the thrombin enzyme and mitogenic activity. Single amino acid substitutions were introduced in the catalytic triad (H43N, D99N, S205A, S205T), in the oxy-anion binding site (G203A) and in the anion binding exosite-1 region (R73E). Proteins were produced as prethrombin-2 mutants secreted in the culture medium of DXB11-derived cell lines. All mutants were activated by ecarin to the corresponding thrombin mutants; the enzymatic activity was assayed on a chromogenic substrate and on the procoagulant substrate fibrinogen. Mutations S205A and G203A completely abolished the enzyme activity. Mutations H43N, D99N and S205T dramatically impaired the enzyme activity toward both substrates. The R73E mutation dissociated the amidolytic activity and the clotting activity of the protein. The ability of thrombin mutants to induce proliferation was investigated in NIH3T3 mouse fibroblasts and rat cortical astrocytes. The ability of the thrombin mutants to revert astrocyte stellation was also studied. The mitogenic activity and the effect on the astrocyte stellation of the thrombin mutants correlated with their enzymatic activity. Furthermore the receptor occupancy by the inactive S205A mutant prevented the thrombin effects providing strong evidence that a proteolytically activated receptor is involved in cellular responses to thrombin.

Denizot, Y., A. Besse, et al. (1999). "Interleukin-4 (IL-4), but not IL-10, regulates the synthesis of IL-6, IL-8
Leukemia inhibitory factor (LIF), interleukin 6 (IL-6) and IL-8 are important regulators of inflammation and hematopoiesis. Human bone marrow stromal cells regulate marrow hematopoiesis by secreting cytokines. By using reverse-transcriptase polymerase chain reaction (RT-PCR), we demonstrate that human bone marrow stromal cells constitutively express LIF, IL-6 and IL-8 transcripts. By using specific ELISAs, we found that their spontaneous productions of LIF, IL-6 and IL-8 are elevated in response to serum and after stimulation with the pro-inflammatory cytokines IL-1[alpha] and TNF-[alpha]. The anti-inflammatory cytokine IL-4 reduces their serum- and cytokine-induced LIF secretion. By contrast, IL-4 stimulates their serum- and IL-1[alpha]-induced IL-6 synthesis. IL-4 has no effect on the serum-induced IL-8 synthesis by marrow stromal cells, but stimulates their cytokine-induced IL-8 production. The anti-inflammatory cytokine IL-10 has no effect on the serum- and cytokine-induced LIF, IL-6 and IL-8 synthesis by bone marrow stromal cells. RT-PCR experiments reveal the presence of IL-4 receptor [alpha]-chain mRNA and IL-10 receptor mRNA in cultured bone marrow stromal cells. The differential regulation by IL-4 of two related cytokines, such as LIF and IL-6, and the enhanced effect of this 'anti-inflammatory' cytokine on IL-6 and IL-8 synthesis highlight the tightly controlled regulation and the complexity of the cytokine production within the human bone marrow.


http://www.sciencedirect.com/science/article/B6T20-497C80K-C/2/1e792c0b941fc69b6c1262c32e67bc3

Two phosphatidylinositol 4-kinase isozymes, type 3 and type 2, have been separated on hydroxylapatite after solubilizing bovine brain microsomes with Triton X-114. Employing a newly developed renaturation procedure following SDS-PAGE, we demonstrate that a 200 kDa polypeptide carries the enzymic activity of this type 3 isoform. Chromatography on hydroxylapatite, Heparin-Sepharose, Superdex 200 and finally SDS-PAGE results in an approximately 30000-fold purification. Tryptic peptides generated from the 200 kDa polypeptide after SDS-PAGE have been sequenced and the obtained data have been used for constructing and synthesizing degenerated oligonucleotides. Polymerase chain reaction as well as screening of cDNA libraries allowed several clones to be isolated from which a 4.7 kb contiguous sequence can be built up. The open reading frame covers 4.4 kb with a 0.3 kb untranslated 3’ end which yields a deduced amino acid sequence of 1,467 amino acids. The C-terminal part of ca. 300 amino acids represents the catalytic domain. Sequence alignment of this domain with the mammalian counterpart, the human type 2 phosphatidylinositol 4-kinase, the yeast kinases STT4 and PIK1, as well as with the catalytic domains of bovine, human, mouse and yeast phosphatidylinositol 3-kinases reveals a high degree of identity: 26 of these approximately 300 amino acids are invariable in all of these eight catalytic domains. Five motifs indicate nuclear localization and DNA binding properties of the enzyme. Two leucine zipper motifs (amino acids 358-386, 862-882) are detectable. Furthermore, a helix loop helix motif (amino acids 716-729) as well as two nuclear localization signals (amino acids 838-854, 345-349) indicate the presence of the type 3 isoform in the nucleus.

Inada, Y., T. Nakane, et al. (2003). "Relationship between ligand binding and YIPP motif in the C-terminal
The YIPP (tyrosine-isoleucine-proline-proline, amino acids 319-322) motif within the C-terminal part of the human AT1 receptor is associated with angiotensin II (AII)-induced activation of the Jak-STAT pathway and phospholipase C[gamma]1 phosphorylation. We report here that mutations of the YIPP motif strongly affect ligand-binding to the receptor. We analysed AT1 receptors of the wild type (WT) and 11 mutants with a FLAG-epitope-tag within their C-terminal portion. Mutations of the "P-P" amino acid sequence of this motif decreased both AII binding and the AII-induced intracellular Ca2+ transients. Mutant and WT receptors were expressed equally in the cell membrane and were localized within the plasma membrane. These results suggest that the "P-P" amino acid sequence within the YIPP motif is important for AII binding to the AT1 receptor.


Human tissue contents of gliostatin/platelet-derived endothelial cell growth factor (PD-ECGF) and its drug-induced expression in tumor cells were currently examined by a sandwich enzyme immunoassay (EIA) system and a reverse transcription-polymerase chain reaction (RT-PCR) method. Gliostatin/PD-ECGF was found to distribute in rather ubiquitous than specific human tissues and organs, with a relatively high levels in the tissues of digestive system (esophagus and rectum), brain, spleen, bladder and lung, but not in gall bladder, aorta, muscle, fat and kidney. Most of examined human tumor cell lines showed 4- or 5-fold higher contents (21.5 +/- 3.9 ng/mg protein) than normal tissue contents (4.4 +/- 1.1 ng/mg protein) on the average. While gliostatin/PD-ECGF is known to lack a signal sequence, some tumor cells (A431 and MKN74) appeared to release it into the conditioned medium. Expression of gliostatin/PD-ECGF in epidermoid carcinoma cell (A431) and stomach cancer cell (MKN45) was induced by dibutyryl cyclic AMP and phorbol ester, and uniquely in MKN45 by hydrocortisone. In particular, this hydrocortisone specifically caused an increase of the apparent secretion of MKN74 without its cytotoxic effects, suggesting a possible secretion of gliostatin/PD-ECGF in the restricted but not universal cell line. Biological significance on the chemical induction of gliostatin/PD-ECGF in tumor cells and on its extracellular secretion are discussed.

preparation of GLUT4-containing vesicles of rat adipocytes, and tryptic peptides were micro-
sequenced. From this information a cDNA encoding a single open reading frame for a protein of
22 kDa was cloned. This protein is homologous to known members of the reticulon protein family.

vp20 has two hydrophobic stretches of about 35 amino acids that could be membrane spanning
domains and an ER retention motif at its carboxy-terminus. vp20 was most abundant in the high
density microsome fraction of adipocytes, which is the fraction most enriched in ER. Only a small
fraction of vp20 was present in the GLUT4 vesicle population, and that fraction appears to be due
to ER vesicles that were non-specifically bound to the adsorbent. Analysis of tissue distribution of
vp20 in rats revealed that it is concentrated in muscle, fat and the brain.

folate receptor (FR-[alpha]) in the human placental syncytiotrophoblast and choriocarcinoma
cells." Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1223(1): 71.

http://www.sciencedirect.com/science/article/B6T20-47S6DP2-
FW/2/a9b70960ac6b1999931366df035ad89

The folate receptor (FR), an essential component in the process of folate uptake in various cells,
is known to exist in three isoforms, FR-[alpha], Fr-[beta] and FR-[gamma], with differential tissue
expression. Transfer of folate across the human placenta from mother to fetus involves
participation of a folate receptor expressed in the syncytiotrophoblast, but the isoform identity of
this receptor has not been established. Based on the tissue/cell type from which these isoforms
have been cloned, it is currently believed that FR-[alpha] is the isoform expressed in adult tissues
whereas FR-[beta] is the isoform expressed in fetal tissues including placenta. The present study,
undertaken primarily to establish the isoform identity of the FR expressed in the placental
syncytiotrophoblast, does not support this currently prevailing nomenclature. Reverse
transcription coupled with polymerase chain reaction (RT-PCR) of total/poly(A)+ RNA from
placenta, cultured trophoblast cells and JAR choriocarcinoma cells with primer pairs specific for
either FR-[alpha] or FR-[beta] reveals that while both isoforms are detectable in the whole
placental tissue, only FR-[alpha] is present in the normal trophoblast cells and in the
choriocarcinoma cells. Northern analysis with probes designed to distinguish between the mRNA
transcripts coding for these two isoforms corroborate the RT-PCR findings. Furthermore,
the nucleotide sequences of the PCR products obtained from the trophoblast cells and JAR choriocarcinoma cells with primer pairs specific for
either FR-[alpha] or FR-[beta] reveals that while both isoforms are detectable in the whole
placental tissue, only FR-[alpha] is present in the normal trophoblast cells and in the
choriocarcinoma cells. Northern analysis with probes designed to distinguish between the mRNA
transcripts coding for these two isoforms corroborate the RT-PCR findings. Furthermore,
the nucleotide sequences of the PCR products obtained from the trophoblast cells and JAR choriocarcinoma cells are identical to the nucleotide sequence of the FR-[alpha] cDNA. These studies establish that it is the
FR-[alpha] isoform, and not the FR-[beta] isoform, which is selectively expressed in the placental
trophoblast cells. FR-[beta], which is known to be present in the placenta, most likely arises from
the maternal decidua normally associated with this tissue.

Qu, X.-W., H. Wang, et al. (1999). "Type I nitric oxide synthase (NOS) is the predominant NOS in rat
small intestine. Regulation by platelet-activating factor." Biochimica et Biophysica Acta (BBA) -
Molecular Cell Research 1451(1): 211.

http://www.sciencedirect.com/science/article/B6T20-3X52KFM-
P/2/09b3a198df770046fecb2a5ab256b2de

Constitutive nitric oxide synthase (cNOS) may play an important protective role in the intestine,
since our previous study has shown that the degree of bowel injury induced by platelet-activating
factor (PAF), a potent inflammatory mediator, is inversely related to the cNOS content of the
intestine. This study aims to examine the composition of the cNOS system in rat small intestine,
and its regulation by PAF. We found that an approximately 120 kDa NOS I (neuronal NOS) is the
predominant NOS in rat intestine, as evidenced by the following: (a) immunoblotting with specific
antibodies detected a NOS I of approximately 120 kDa, but little NOS III; (b) the Ca2+-dependent,
constitutive NOS (cNOS) activity of the rat intestine was removed by immunoprecipitation with the anti-NOS I, but not anti-NOS II or anti-NOS III antibodies; (c) RT-PCR revealed constitutive expression of NOS I in the intestinal tissue, but only a minute amount of NOS III. Immunofluorescent staining with anti-NOS I located NOS in the Auerbach plexus and nerve fibers in the muscle layer. We also found that this 120 kDa NOS I is rapidly (within 1 h) down-regulated in response to PAF administration. The protein level, enzyme activity as well as mRNA of nNOS were all decreased in the intestine.


http://www.sciencedirect.com/science/article/B6T20-4DKD4XW-1/2/3c0b54bb55aabc8c47b7bb4f33a2ef8b

Differentiation of Drosophila Schneider cells caused by DNA double-strand break (DSB)-inducing topoisomerase II (topo II) inhibitors were attenuated by ICRF-193, a non-DNA-damaging topo II inhibitor. ICRF-193 did not inhibit differentiation induced by neocarzinostatin (NCS), a drug that causes DNA DSBs independent of topo II. Schneider cells differentiated upon treatment with [gamma]-ray. These results suggest that DNA DSBs induce myogenic differentiation of Schneider cells. We also found DNA replication inhibitors, hydroxyurea (HU), aphidicolin, and ethylmethanesulfonate (EMS) induced myogenic differentiation of Schneider cells. HU-induced differentiation was inhibited upon pretreatment of cells with chemical inhibitors of PP 1/2A, p38 MAPK, JNK, and proteasome. RT-PCR analysis revealed that the expressions of fusion-competent myoblast-specific genes lmd, sns, and del were induced in Schneider cells upon treatment with NCS or HU, whereas expressions of three founder cell-specific genes, duf, ants, and rols, were undetectable. These results indicate that the expression of fusion competent-myoblast-specific genes is induced during myogenic differentiation of Drosophila Schneider cells by DNA DSBs or replication inhibition.


The mechanism(s) involved in immortalization that constitute the first step during malignant transformation has been the subject of our interest. By the use of spontaneously immortalized mouse embryonic fibroblasts we have earlier identified two stages of immortalization which are characterized by growth characteristics of the cells, their conditioned medium and the protein markers such as p53, p81 and mortalin (Kaul et al. (1994) Biochim. Biophys. Acta, in press). The present study was planned to purify the mitogenic factors from the conditioned medium of stage II cells. Sequential purification by chromatography followed by peptide sequencing has characterized one of these as vascular endothelial growth factor (VEGF). Further analysis by RT-PCR suggests that the spontaneously immortalized stage II fibroblasts have enhanced synthesis and secretion of VEGF as compared to their mortal parent cells. Expression of a novel 304 bp long form of VEGF is identified in immortal fibroblasts in addition to the three known alternatively spliced forms. The study points to the involvement of VEGF function during spontaneous immortalization of mouse embryonic fibroblasts.
X-chromosome inactivation is a phenomenon by which one of the two X chromosomes in somatic cells of female mammals is inactivated for life. The inactivated X chromosomes are covered with Xist (X-inactive specific transcript) RNA, and also enriched with the histone H2A variant, macroH2A1.2. The N-terminal one-third of macroH2A1.2 is homologous to core histone H2A, but the function of the C-terminal two-thirds, which contains a basic, putative leucine zipper domain, remains unknown. In this study, we tried analyzing protein-protein interaction with a yeast two-hybrid system to interact with the nonhistone region of mouse macroH2A1.2. The results showed that macroH2A1.2 interacts with mouse nuclear speckled type protein Spop. The Spop protein has a unique composition: an N-terminal MATH, and a C-terminal BTB/POZ domain. Further binding domain mapping in a glutathione-S-transferase (GST) pull-down experiment revealed that macroH2A1.2 binds the MATH domain of Spop, which in turn binds to the putative leucine zipper domain of macroH2A1.2.
cleavage sites, suggesting that it could be processed to give rise to all members of the melanocortin family, including adrenocorticotropic hormone and [alpha]-, [beta]- and [gamma]-melanocyte-stimulating hormones, as well as the other POMC-derived peptides. RT-PCR analysis detected the POMC mRNA in the brain, adrenal gland, gonads, kidney, uropygial gland and adipose tissues, each of which has been demonstrated to express melanocortin receptors. These results suggest that melanocortins act in a paracrine and/or autocrine manner to control a variety of functions both in the brain and in the peripheral tissues in the chicken.


Agouti-related protein (AGRP) is a naturally occurring antagonist of melanocortin action. It is expressed mainly in the arcuate nucleus where it plays an important role in the hypothalamic control of feeding and energy homeostasis by antagonism of central melanocortin 4 receptors in mammals. Besides in the brain, the melanocortin 4 receptor is expressed in numerous peripheral tissues in the chicken. To examine whether or not the peripheral melanocortin 4 receptor signaling could be regulated by AGRP, we cloned and localized the expression of the AGRP gene in the chicken. The chicken AGRP gene was found to encode a 154 or 165 amino acid protein, depending on the usage of two alternative translation initiation sites. The coding sequence consisted of three exons, like that of mammalian species. The C-terminal cysteine-rich region of the predicted AGRP displayed high levels of identity to mammalian counterparts (78-84%) and all 10 cysteine residues conferring functional conformation of AGRP were conserved; however, other regions showed apparently no homology, suggesting that biological activities of AGRP are located in its C-terminal region. RT-PCR analysis detected the AGRP mRNA in all tissues examined: the brain, adrenal gland, heart, liver, spleen, gonads, kidney, uropygial gland, skeletal muscle and adipose tissues. Interestingly, the skin also expressed the AGRP mRNA, where Agouti, another melanocortin receptor antagonist regulating hair pigmentation, is expressed in rodents. Most of those AGRP-expressing tissues have been demonstrated to express melanocortin 4 receptors and/or other subtypes of melanocortin receptor whose mammalian counterparts can bind AGRP. These results imply the possibility that some peripheral melanocortin systems could be regulated by the functional interaction between melanocortins and AGRP at melanocortin receptors in the chicken.


Chemokine-like factor 1 (CKLF1) is a novel cytokine first cloned from U937 cells. It contains different splicing forms and has chemotactic effects on a wide spectrum of cells both in vitro and in vivo; it can also stimulate the regeneration of skeletal muscle cells in vivo, but the mechanism remains unclear. To probe the myogenesis function of CKLF2, which is the largest isoform of CKLFS, C2C12 murine myoblasts were stably transfected with human CKLF2 eukaryotic expression vector. Compared with control vector transfected C2C12 cells, CKLF2 overexpression causes accelerated myoblast proliferation as determined by cell counting and [3H]TdR incorporation assays. In addition, CKLF2 overexpression also promotes cell differentiation, which
was determined by higher expression levels of myogenin, creatine kinase, myosin and the accelerated myoblast fusion. Further analysis also indicates that CKLF2 could activate the transcription activity of the bHLH/MyoD and MEF2 families. Finally, DNA synthesis and myotube formation could also be promoted by growing C2C12 cells in conditioned media from CKLF2-transfected cells. These findings strongly suggest a role for human CKLF2 in regulation of skeletal muscle myogenesis.

Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology


http://www.sciencedirect.com/science/article/B6T21-3V5MPS1-B/2/7d1baf0a3b6d559b6f2a226f5b178a

Inositol(1,3,4,5)tetrakisphosphate (InsP4) and phosphatidylinositol(3,4,5)trisphosphate (PtdInsP3) are two potential second messengers with a still largely unknown mode of action. We recently cloned the 42 kDa protein p42IP4 previously purified from pig cerebellum, which binds InsP4 (Kd ~2 nM) and PtdInsP3 with comparable affinities (Stricker et al., FEBS Lett. 405 (1997) 229). The protein p42IP4 (pig) is highly homologous to centaurin-[alpha], a larger protein of 46 kDa, derived from a rat brain cDNA library clone (Hammonds-Odie et al., J. Biol. Chem. 271 (1996) 18859). Here we investigated whether also p42IP4 is expressed in rat brain and how it might be related to centaurin-[alpha]. When we carried out RT-PCR using mRNA from brain of rats of different ages we obtained several clones corresponding to p42IP4, but not to centaurin-[alpha]. The existence of p42IP4 in rat brain is supported by the following findings: (1) biochemical analysis of the purified rat brain protein shows inositol phosphate ligand affinities identical to those of the protein from other species; (2) Western blot analysis of rat brain membrane fractions using a peptide-specific antiserum revealed only the 42 kDa protein (p42IP4), but did not give evidence for the occurrence of a larger 46 kDa centaurin-[alpha]-like protein in rat brain; and (3) the amino acid sequences deduced from p42IP4 cDNA are highly homologous in several species and are confirmed by protein fragment microsequences. Thus, p42IP4 from rat brain which has two pleckstrin homology domains is a protein largely conserved between different species and most likely has an important function in inositol phosphate or inositol lipid signal transduction.


http://www.sciencedirect.com/science/article/B6T21-3W7XBPV-8/2/5def93440cd3f56d0e92aee8e5cee98

Cathepsin D was purified to homogeneity from the liver of Antarctic icefish by anion-exchange chromatography followed by affinity chromatography on concanavalin-A Sepharose. The purified enzyme showed a molecular mass of 40 kDa and displayed optimal activity at pH 3.0 with a synthetic chromogenic substrate. The N-terminal sequence of this proteinase was determined by
automated Edman degradation and was used to design a primer for use in reverse-transcriptase polymerase chain reaction. The open reading frame of the cloned cDNA encoded an aspartic proteinase, which contained the experimentally determined N-terminal sequence. The predicted sequence (396 residues) had a high similarity with those of cathepsin D from various vertebrate sources, but was considerably different from that of nothepsin, a distinct aspartic proteinase described previously from Antarctic fish [1]. Determination of kinetic parameters for substrate hydrolysis showed that, at temperatures between 8 and 50[deg]C, the icefish cathepsin D had a higher specificity constant (kcat/Km) than human cathepsin D. The stability of both enzymes was measured at 50[deg]C and half-lives of 55 and 3 min were derived for icefish and human cathepsin D, respectively.


http://www.sciencedirect.com/science/article/B6T21-47RSDDH-188/2/dec798cd2b55aadcb2047aec49f9454

NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) catalyzes the first step in the catabolic pathway of the prostaglandins. This enzyme oxidizes the 15-hydroxyl group of prostaglandins to produce 15-keto metabolites which are usually biologically inactive. In this study the cDNA for human placental 15-PGDH was expressed in Escherichia coli and the recombinant enzyme was purified to homogeneity and characterized. The N-terminus of the recombinant protein was sequenced and found to be identical with the known amino-acid sequence of 15-PGDH. Determinations of Km and Vax values for a number of the prostaglandins and NAD+ indicate that the recombinant enzyme does not appear to be kinetically different from the human placental enzyme. Site-directed mutagenesis was used to examine the importance of two residues which are highly conserved in the short-chain dehydrogenases which are known to be related to 15-PGDH. Tyrosine-151 was changed to phenylalanine and serine while lysine-155 was changed to glutamine and leucine. Western blot analysis indicated that the mutant and wild-type proteins were expressed at the similar levels. However, all of the mutant proteins were found to be inactive. These results indicate that both tyrosine-151 and lysine-155 are required for 15-PGDH activity.


http://www.sciencedirect.com/science/article/B6T21-3XX6VSX-31/2/272caac42310c24cf6f42c6df0c4962a

Factor XIIIa belongs to a family of ubiquitous transglutaminases, which catalyze formation of covalent bonds between the [epsilon]-amino group of specific lysines and the [gamma]-carboxyl group of glutamines. Factor XIII is synthesized as a zymogen and after activation, it participates in both the coagulation and fibrinolytic mechanisms. Most transglutaminases are intracellular, but factor XIII is both intracellular and extracellular. The biosynthesis of extracellular (plasma) factor XIII, with the structure of a noncovalent heterotetramer, A2B2, is complex. Here, evidence is presented from PCR analysis and Northern blotting that mRNAs for both A and B subunits are present in the liver. The distribution of mRNA, specific for factor XIII subunits, in various human tissues was also analyzed. Among the tissues examined, the only signal for B subunit was found in the liver. For subunit A, the signal was observed in placenta, liver, kidney, lung, skeletal muscle
and heart with varying intensities; in brain or pancreas there was no signal. With an immunoperoxidase method, factor XIII A subunit was identified in the PLC/PRF/5 cell line. By ELISA and reverse immunoblotting, with antibodies specific for the A-B complex, it was also shown that these cells produce and secrete factor XIII. From all of these results, we conclude that the liver is a source of plasma factor XIII, and that the complex A2B2 is secreted from these cells.


http://www.sciencedirect.com/science/article/B6T21-3X5GRHM-8/2/894a052d0d590cb63209194738699675

Fusion proteins of rat cytochrome P4501A1 with maize ferredoxin I (Fd) and pea ferredoxin NADP+ reductase (FNR), the last electron transfer proteins of the photosynthetic channel in plant chloroplasts, were obtained by gene fusion in the yeast expression vector pAAH5N. The encoded fusion proteins P4501A1-Fd, P4501A1-FNR, P4501A1-Fd-FNR and P4501A1-FNR-Fd were produced in microsomes of the yeast Saccharomyces cerevisiae AH22. Enzymatic assays were carried out in vitro with the isolated microsomes. P4501A1-Fd-FNR and P4501A1-FNR-Fd were found to catalyze P450-monooxygenase activities towards 7-ethoxycoumarin and the herbicide chlortoluron. P4501A1-Fd-FNR was the most efficient enzyme as measured in vitro in ferricyanide and cytochrome c reductions, as well as P450-monooxygenase assays. Apparent Km and kcat of P4501A1-Fd-FNR were 70 [mu]M and 7800 min-1 for NADPH, 13.2 [mu]M and 51.1 min-1 for 7-ethoxycoumarin, and 21.3 [mu]M and 23.8 min-1 for the herbicide chlortoluron, respectively. Fd in P4501A1-Fd-FNR fusion enzyme was found to be a limiting factor compared to P4501A1 fused to the yeast NADPH-cytochrome P450 reductase, an artificial enzyme described previously. The efficiency of electron transfer in the P4501A1 fusion proteins and a possible in vivo molecular coupling of Fd and FNR with microsomal cytochrome P4501A1 produced in plant chloroplasts are discussed.


http://www.sciencedirect.com/science/article/B6T21-47PNWW9-2X/2/df3497de3a8b0b27285861c32cd4fda

Transglutaminases (EC 2.3.2.13) catalyze an acyl-transfer reaction between peptide-bound glutamine residues and primary amines, including the [epsilon]-amino group of lysine residues in protein. Purified human erythrocyte transglutaminase was found to have another activity, i.e., GTP hydrolysis. Treatment of the enzyme with iodoacetamide, a cysteine-directed reagent, caused a 94% loss of TGase activity within 8 min, but no significant loss of GTPase activity. Cys-277, a known residue which is selectively modified by iodoacetamide, was replaced with Ser by site-directed mutagenesis to assess the role of the Cys-277 in the transglutaminase/GTPase activities. Wild-type cDNA, coding for human endothelial cell transglutaminase, and its C277S-mutated cDNA were cloned into a plasmid vector that contained a promotor from phage T7, and then expressed in Escherichia coli. The wild-type recombinant enzyme was indistinguishable from human erythrocyte transglutaminase in mobility on a SDS-polyacrylamide gel, immunoreactivity and catalytic activities for transglutaminase and GTPase. However, the recombinant enzyme was not blocked at the N-terminal alanine residue, as is the case in the naturally occurring erythrocyte enzyme. The C277S mutant enzyme showed no transglutaminase activity, but had Km and kcat
values for GTPase activity that were comparable to those of wild-type recombinant and natural erythrocyte enzymes. These results demonstrate that Cys-277 is essential for transglutaminase activity but not for GTPase activity, and that N-terminal blocking of tissue-type transglutaminase is not critical for either transglutaminase or GTPase activities.


We isolated cDNA clones for cytochromes b561 from sheep and porcine adrenal medullae using the RT-PCR technique. Comparison of the deduced amino acid sequences of various species showed that there are two fully-conserved regions in this cytochrome. In addition, one methionyl and six histidyl residues (potential heme ligands) are fully-conserved. Based on a plausible structural model in which a polypeptide spans the vesicle membranes six times and holds two heme B molecules, the first conserved sequence (69ALLVYRVFR77) is located on the extravesicular side of an [alpha]-helical segment and the second one (120SLHSW124) is located in an intravesicular loop connecting two [alpha]-helical segments, respectively. Consideration of the relative locations of the fully-conserved sequences, and the methionyl and histidyl residues in the model led to a proposal that the first and second conserved sequences are likely to form the binding sites for extravesicular ascorbic acid and intravesicular semidehydroascorbic acid, respectively. A mild alkaline-treatment of purified bovine cytochrome b561 in oxidized state led to a specific loss of an electron-accepting ability from ascorbic acid for a half of the heme center, suggesting a distinct role for each of the two hemes.


p-Aminobenzoic acid (PABA), an essential component of the vitamin folic acid, is derived from the aromatic branch-point precursor chorismate in two steps. 4-Amino-4-deoxychorismate (ADC) synthase converts chorismate and glutamine to ADC and glutamate, and is composed of two subunits, PabA and PabB. While various experiments have suggested that PabA and PabB act as a complex, attempts to isolate the intact complex have failed. We report here the first successful copurification of PabA and PabB by gel filtration chromatography. The association of PabA and PabB is greatly enhanced by the presence of 5 mM glutamine, and by preincubation at 37[deg]C. Conversely, the association is greatly reduced at cold temperatures. We also report the isolation and characterization of both chemically induced and site-directed mutations in PabB. Mutated PabB enzymes fall into three categories according to their properties: deficiency of chorismate amination coupled with failure to associate with PabA, deficiency of chorismate amination coupled with retention of PabA association, and competency of chorismate amination with failure of PabA association.
In this study we purified and investigated the catalytic properties of a manganese peroxidase isoenzyme produced by the fungus Pleurotus ostreatus in liquid medium with peptone as nitrogen source. The isoenzyme was purified to homogeneity by chromatography on Bio-Rad Q-cartridge, Sephacryl S-200 and Mono-Q with activity yield of 59% and a purification factor of 36. The P. ostreatus MnP obtained had the same pi (3.75) and N-terminal sequence as MnP-1 of Pleurotus eryngii produced in the same medium (both exhibiting Mn-independent activities on phenolic and non-phenolic substrates). However, the N-terminal sequence of this P. ostreatus isoenzyme differed from a previous published sequence of MnP from this fungus. The results obtained show the importance of media composition in the production of different isoenzymes within the same fungal species. We have also demonstrated by Southern blots that the different isoenzymes are probably encoded by different genes, and that the MnP genes in both Pleurotus species are similar but different to those of Phanerochaete chrysosporium.

Tumor-associated aldehyde dehydrogenase (T-ALDH) is strongly expressed in hepatocellular carcinoma (HCC) but undetectable in normal liver. In the present study, this enzyme from human HCC, HCC T-ALDH, was purified and the partial amino acid sequences (384 residues) determined by direct protein sequencing matched the amino acid sequence (453 residues) deduced from cloned HCC T-ALDH cDNAs with an open reading frame. The coding sequences of HCC T-ALDH cDNA, human stomach ALDH3A1 cDNA [Hsu et al., J. Biol. Chem. 267 (1992) 3030-3037] and human squamous cell carcinoma (SCC) T-ALDH cDNA (Schuuring et al., GenBank I.D. M74542) matched one another except for discrepancies at four positions, with consequent P12R, I27F and S134A substitutions. R and A were found in HCC and SCC T-ALDHs, whereas P and S were present in stomach ALDH3A1. To confirm that these discrepancies would have general occurrence, coding sequences of HCC T-ALDH cDNAs from six patients and stomach ALDH3A1 cDNAs from two individuals were examined and all were found to encode ALDH3A1 having R, I and A at protein positions 12, 27 and 134, respectively, indicating HCC T-ALDH to be variant ALDH3A1 which is common in human stomach tissues.

Although originally described in the male rodent genital tract, cysteine-rich secretory proteins (CRISPs) are expressed in a variety of mammalian tissue and cell types. The proteins of the male...
genital tract have been observed associated to spermatozoa and are believed to play a role in mammalian fertilization. Here we describe the identification and primary structure of the first equine member of the CRISP family. Equine CRISP-3 is transcribed and expressed in the stallion salivary gland, in the ampulla and the seminal vesicle. It displays all 16 conserved cysteine residues and shows 82% homology to human and 78% to guinea pig CRISP-2 (AA1, TPX 1) and 77% to human CRISP-3. In contrast to other mammals, in the horse CRISP-3 is synthesized in great amounts in the accessory sexual glands, ampulla and seminal vesicle, thus allowing the isolation of equine CRISP-3 in amounts suitable for biochemical, physiological and structural studies from stallion seminal plasma.


http://www.sciencedirect.com/science/article/B6T21-437XS03-P/2/d3c51317136776ecfabe3551292de247

We attempted to apply the directed evolution approach to enhancing enzyme properties in the presence of organic solvents, in which enzyme stability and activity were often drastically reduced. Stability and catalytic activity of phospholipase A1 in the presence of an organic solvent were enhanced by error-prone polymerase chain reaction (PCR) and DNA shuffling followed by a filter-based visual screening. Three mutants (SA8, SA17 and SA20) were isolated on indicator plates (i.e., 1% phosphatidylcholine gels containing 30% dimethyl sulfoxide (DMSO)) after a second mutant library was treated in 50% DMSO for 36 h. The half-life values of the three mutants exhibited an approximately 4-fold increase. The three mutants also exhibited increased stability in all organic solvents tested compared with the wild-type enzyme. Thus, an enzyme variant having superior catalytic efficiency in most of the organic solvents could be obtained by using any solvent suitable for designing the efficient screening system, regardless of the properties of the particular solvent.


http://www.sciencedirect.com/science/article/B6T21-3W8YRM1-J/2/6e5f28ea11c9f1e00f849d1c78a9d98c

A gene encoding a thermostable ascorbate oxidase (ASOM) was cloned from Acremonium sp. HI-25 and sequenced. The gene comprised 1709 bp and was interrupted by a single intron of 57 bp. ASOM consisted of 551 amino acids including a signal peptide with a molecular mass of 61200, and contained four histidine-rich regions with high sequence homology to the corresponding regions of other multicopper oxidases. The ASOM gene was expressed in Aspergillus nidulans under the Aspergillus oryzae Taka-amylase A gene promoter. The recombinant enzyme (An-ASOM) exhibited almost the same enzymatic properties as ASOM. The ASOM gene was mutated by site-directed mutagenesis with reference to the amino acid sequences of plant enzymes to generate enzymes with altered azide sensitivity. Site-directed mutagenesis at the trinuclear active copper site resulted in an increase in azide resistance; the Ala465Leu and Phe463Trp/Ala465Leu mutants exhibited approximately 10 and 20% increases in azide resistance, respectively.
Genes encoding 10914 Da and 58267 Da polypeptides homologous to groES and groEL of Escherichia coli were cloned and sequenced from a thermophilic cyanobacterium, Synechococcus vulcanus. The deduced amino acid sequence of the GroEL protein was much more homologous to GroELs of other cyanobacteria which accompany GroES than another GroEL homolog of S. vulcanus (GroEL2) reported previously (M. Furuki, N. Tanaka, T. Hiyama, and H. Nakamoto, Biochim. Biophys. Acta 1294 (1996) 106-110). We designate the gene as groEL1 to distinguish it from the non-operon forming groEL2 gene. A 9-base pair inverted repeat sequence (TTAGCACTC-N9-GAGTGCTAA) was located upstream of the promoter region of groEL1, which was absent in groEL2. Southern blot analysis indicated that only one groESL1 operon was present in the genomic DNA of S. vulcanus. The amount of the bicistronic, 2.3 kb transcript of groESL1 operon increased 30-fold within 30 min upon heat shock. The increase was completely inhibited by chloramphenicol, suggesting the involvement of heat-induced production of a polypeptide. Introduction of the cloned groEL1 gene into a groEL defective mutant of E. coli resulted in the complementation of heat sensitivity, which contrasted with the previous result with groEL2.

By microsequencing and cDNA cloning we have identified the transformation-sensitive protein No. IEF SSP 9302 as the human homologue of calumenin. The nucleotide sequence predicts a 315 amino acid protein with high identity to murine and rat calumenin. The deduced protein contains a 19 amino acid N-terminal signal sequence, 7 EF-hand domains and, at the C-terminus, a HDEF sequence which has been reported to function as retrieval signal to the ER. The calumenin transcript is ubiquitously expressed in human tissue, at high levels in heart, placenta and skeletal muscle, at lower levels in lung, kidney and pancreas and at very low levels in brain and liver. Calumenin belongs to a family of multiple EF-hand proteins that include the ER localized proteins reticulocalbin and ERC-55 and the Golgi localized Cab45. Since its Ca2+ binding may be important for the function of the protein we have used microdialysis experiments in order to analyse for the affinity and the capacity of recombinant human (rh) calumenin. All 7 EF-hands of the protein are functional and bind Ca2+, each with an affinity of 1.6 x 103 M-1. The relatively low affinity for the EF-hands may suggest a role for the protein in Ca2+-dependent processes in the ER.
Protein kinase recognition sequences and proteinase sites were engineered into the cDNA encoding firefly luciferase from Photinus pyralis in order to establish whether these modified proteins could be developed as bioluminescent indicators of covalent modification of proteins. Two key domains of the luciferase were modified in order to identify regions of the protein in which peptide sequences may be engineered whilst retaining bioluminescent activity; one between amino acids 209 and 227 and the other at the C-terminus, between amino acids 537 and 550. Mutation of amino acids between residues 209 and 227 reduced bioluminescent activity to less than 1% of wild-type recombinant. In contrast, engineering peptide sequences at the C-terminus resulted in specific activities ranging from 0.06-120% of the wild-type recombinant. Addition of cyclic AMP dependent protein kinase catalytic subunit, to a variant luciferase incorporating the kinase recognition sequence, LRRASLG, with a serine at amino-acid position 543 resulted in a 30% reduction in activity. Alkaline phosphatase treatment restored activity. The bioluminescent activity of a variant luciferase containing a thrombin recognition sequence, LVPRES, with the cleavage site positioned between amino acid 542 and 543, decreased by 50% when incubated in the presence of thrombin. The results indicate regions within luciferase where peptide sequences may be engineered while retaining bioluminescent activity and have shown changes in bioluminescent activity when these sites are subjected to covalent modification. Changes in secondary structure, charge and length at the C-terminus of luciferase disrupt the microenvironment of the active site, leading to alterations in light emission. This has important implications both in understanding the evolution of beetle bioluminescence and also in the development of bioluminescent indicators of the covalent modification of proteins.


http://www.sciencedirect.com/science/article/B73DJ-46Y507X-1/2/5495c71b76b5991e207dd1ba62a28fb3

S100A11 is a member of a multigenic family of Ca2+-modulated proteins of the EF-hand type. We studied the subcellular localization of S100A11 in developing and adult avian skeletal muscle cells by confocal laser scanning microscopy and immunogold cytochemistry to get information about possible functional roles of this protein. Analyses of [alpha]-actinin, S100A1 and S100B were done in parallel for comparison. Low levels of S100A11 were found in skeletal muscle cells at embryonic day (E) 8. At E12, S100A11 was found in myotubes in the form of fine dots located between Z-discs, and on the sarcolemma and its invaginations. At E15, S100A11 was found on the sarcolemma and internal membranes, likely longitudinal tubules, where the protein was co-localized in part with S100A1 and S100B. At E18 and afterwards, co-localization of the three S100 proteins on internal membranes was almost complete. No evidence for association of S100A11 with the contractile elements of the sarcomeres was obtained. Our data suggests that, like S100A1 and S100B, S100A11 might have a role in the regulation of membrane activities, probably in relation to Ca2+ fluxes in skeletal muscle cells.

Mycobacterium tuberculosis is a major global pathogen whose threat has increased with the emergence of multidrug-resistant strains. The cell wall of M. tuberculosis is thick, rigid, and hydrophobic, which serves to protect the organism from the environment and makes it highly impermeable to conventional antimicrobial agents. There is little known about cell wall autolysins (also referred to as peptidoglycan hydrolases) of mycobacteria. We identified an open reading frame (Rv3915) in the M. tuberculosis genome designated cwIM that appeared consistent with a peptidoglycan hydrolase. The 1218-bp gene was amplified by PCR, cloned and expressed in E. coli strain HMS174(DE-3), and its gene product, a 47-kDa recombinant protein, was purified and partially characterized. Purified CwIM was able to lyse whole mycobacteria, release peptidoglycan from the cell wall of Micrococcus luteus and Mycobacterium smegmatis, and cleave N-acetylmuramoyl-L-alanyl-D-isoglutamine, releasing free N-acetylmuramic acid. These results indicate that CwIM is a novel autolysin and identify cwIM as the first, to our knowledge, autolysin gene identified and cloned from M. tuberculosis. CwIM offers a new target for a unique class of drugs that could alter the permeability of the mycobacterial cell wall and enhance the effectiveness of treatments for tuberculosis.


Steady-state kinetic approaches were used to investigate the binding of a novel Penicillium funiculosum xylanase, XYNC, with three known xylanase inhibitor proteins from wheat (Triticum aestivum). The xylanase gene (xynC) was cloned from a P. funiculosum genomic library and the deduced amino acid sequence of XYNC exhibited high sequence similarity with fungal family 11 xylanases. xynC was overexpressed in P. funiculosum and the product (XYNC: Mr=23.6 kDa; pI=3.7) purified and shown to efficiently degrade birchwood xylan [Km=0.47% w/v, Vmax=2540 [][mu]mol xylose min-1 (mg protein)-1 at pH 5.5 and 30 [deg]C] and soluble wheat arabinoxylans [Km=1.45% w/v, Vmax=7190 [mu]mol xylose min-1 mg protein)-1 at pH 5.5 and 30 [deg]C]. The xylanase activity of XYNC was inhibited strongly by three xylanase inhibitor proteins from wheat; XIP-I, TAXI I and TAXI II. The inhibition for each was competitive, with very tight binding (Ki=3.4, 16 and 17 nM, respectively) equivalent to free energy changes ([Delta]G[deg]) of -49, -45 and -45 kJ mol-1. This is the first report describing a xylanase that is inhibited by all three wheat xylanase inhibitor proteins described to date.


Gluconobacter strains effectively produce -sorbose from -sorbitol because of strong activity of the -sorbitol dehydrogenase (SLDH). -sorbose is one of the important intermediates in the industrial
vitamin C production process. Two kinds of membrane-bound SLDHs, which consist of three subunits, were reportedly found in Gluconobacter strains [Agric. Biol. Chem. 46 (1982) 135, FEMS Microbiol. Lett. 125 (1995) 45]. We purified a one-subunit-type SLDH (80 kDa) from the membrane fraction of Gluconobacter suboxydans IFO 3255 solubilized with Triton X-100 in the presence of -sorbitol, but the cofactor could not be identified from the purified enzyme. The SLDH was active on mannitol, glycerol and other sugar alcohols as well as -sorbitol to produce respective keto-aldoses. Then, the SLDH gene (sldA) was cloned and sequenced. It encodes the polypeptide of 740 residues, which contains a signal sequence of 24 residues. SLDH had 35-37% identity to those of membrane-bound quinoprotein glucose dehydrogenases (GDHs) from Escherichia coli, Gluconobacter oxydans and Acinetobacter calcoaceticus except the N-terminal hydrophobic region of GDH. Additionally, the sldB gene located just upstream of sldA was found to encode the polypeptide consisting of 126 very hydrophobic residues that is similar to the one-sixth N-terminal region of the GDH. Development of the SLDH activity in E. coli required co-expression of the sldA and sldB genes and the presence of PQQ. The sldA gene disruptant showed undetectable oxidation activities on -sorbitol in growing culture, and resting-cell reaction (pH 4.5 and 7); in addition, they showed undetectable activities on -mannitol and glycerol. The disruption of the sldB gene by a gene cassette with a downward promoter to express the sldA gene resulted in formation of a larger size of the SLDH protein and in undetectable oxidation of the polyols. In conclusion, the SLDH of the strain 3255 functions as the main polyol dehydrogenase in vivo. The sldB polypeptide possibly has a chaperone-like function to process the SLDH polypeptide into a mature and active form.

http://www.sciencedirect.com/science/article/B73DJ-4DB4YJK-2/2/a0356b1a6be6e8fd049f56c34919148d

The molecular mechanism of action of presynaptically toxic secreted phospholipases A2 (sPLA2s) isolated from snake venoms is not completely understood. It has been proposed that the positive charge in the [beta]-structure region is important for their toxic activity. To test this hypothesis, we characterised several mutants of ammodytoxin A (AtxA) possessing substitution of all five basic residues in this region. The mutations had relatively little influence on the catalytic activity of AtxA, either on charge-neutral or anionic phospholipid vesicles. An exception was R72 when replaced by a hydrophobic (higher activity) or an acidic (lower activity) residue. Lethal potencies of the eight single site mutants were up to four times lower than that of the wild-type, whereas the triple mutant (K74S/H76S/R77L) was 13-fold less toxic. The substitutions also lowered the affinity of the toxin, slightly to moderately, for the neuronal receptors R25 and R180. Interaction with calmodulin was only slightly affected by substitutions of K86, more by those of the K74/H76/R77 cluster and most by those of R72 (up to 11-fold lower binding affinity). The results clearly indicate that the basic amino acid residues in the [beta]-region of AtxA contribute to, but are not necessary for, its neurotoxic effect.

http://www.sciencedirect.com/science/article/B73DJ-4CVR4MF-2/2/ace27cda631f575951f69b8cf4368f1e

The eukaryotic Melanoplus sanguinipes entomopoxvirus (MsEPV) genome reveals a homologous
sequence to eubacterial nicotinamide adenine dinucleotide (NAD+)-dependent DNA ligases [J.
Virol. 73 (1999) 533]. This 522-amino acid open reading frame (ORF) contains all conserved
nucleotidyl transferase motifs but lacks the zinc finger motif and BRCT domain found in
conventional eubacterial NAD+ ligases. Nevertheless, cloned MsEPV ligase seals DNA nicks in a
NAD+-dependent fashion, while adenosine 5'-monophosphate (ATP) cannot serve as an
adenylation cofactor. The ligation activity of MsEPV ligase requires Mg2+ or Mn2+. MsEPV ligase
seals sticky ends efficiently, but has little activity on 1-nucleotide gap or blunt-ended DNA
substrates even in the presence of polyethylene glycol. In comparison, bacterial NAD+-
dependent ligases seal blunt-ended DNA substrates in the presence of polyethylene glycol.
MsEPV DNA ligase readily joins DNA nicks with mismatches at either side of the nick junction,
except for mismatches at the nick junction containing an A base in the template strand (A/A, G/A,
and C/A). MsEPV NAD+-dependent DNA ligase can join DNA probes on RNA templates, a
unique property that distinguishes this enzyme from other conventional bacterial NAD+ DNA
ligases. T4 ATP-dependent DNA ligase shows no detectable mismatch ligation at the 3' side of
the nick but substantial 5' T/G mismatch ligation on an RNA template. In contrast, MsEPV ligase
joins mismatches at the 3' side of the nick more frequently than at the 5' side of the nick on an
RNA template. The complementary specificities of these two enzymes suggest alternative primer
design for genomic profiling approaches that use allele-specific detection directly from RNA
transcripts.

analysis of Leishmania hypoxanthine-guanine phosphoribosyltransferase." Biochimica et
http://www.sciencedirect.com/science/article/B73DJ-46DP2XDB/2/a5c3309e825d18913817b4c35bab754f

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (EC 2.4.2.8) is an important enzyme
involved in the recycling of purine nucleotides in all cells. Parasitic protozoa of the order
Kinetoplastida are unable to synthesize purines de novo and use the salvage pathway for the
synthesis of nucleotides; therefore, this pathway is an attractive target for antiparasitic drug
design. The hgprt gene was cloned from a Leishmania tarentolae genomic library and the
sequence determined. The L. tarentolae hgprt gene contains a 633-nucleotide open reading
frame that encodes a 23.4-kDa protein. A pairwise alignment of the different HGPRT's sequences
revealed a 26%-53% sequence identity with the Leishmania sequences and 87% identity to the
HGPRT of Leishmania donovani. A recombinant protein was expressed in Escherichia coli,
purified to homogeneity and found to retain enzymatic activity. The steady-state kinetic
parameters were determined for the recombinant enzyme and the enzyme is active as a
homodimer in solution. Single crystals were obtained for the L. tarentolae HGPRT representing
the first Leishmania HGPRT crystallized and initial crystallographic data were collected. The
crystals obtained belong to the orthorhombic space group (P212121) with unit cell parameters
a=58.104 Å, b=85.443 Å and c=87.598 Å and diffract to a resolution of 2.3 Å. The availability of
the HGPRT enzyme from Leishmania and its crystallization suitable for X-ray diffraction data
collection should provide the basis for a functional and structural analysis of this enzyme, which
has been proposed as a potential target for rational drug design, in a Leishmania model system.

Leishmania tarentolae: potential implications for APRT catalytic mechanism." Biochimica et
http://www.sciencedirect.com/science/article/B73DJ-49MD4SV-1/2/7f7c716ac41aef5b1b48e735777cedd0
The three-dimensional structure of Leishmania tarentolae adenine phosphoribosyltransferase (APRT) in complex with adenosine-5-monophosphate (AMP) and a phosphate ion has been solved. Refinement against X-ray diffraction data extending to 2.2-A resolution led to a final crystallographic R factor of 18.3%. Structural comparisons amongst this APRT enzyme and other 'type I' PRTases whose structures have been determined reveal several important features of the PRTases catalytic mechanism. Based on structural superpositions and molecular interaction potential calculations, it was possible to suggest that the PRPP is the first substrate to bind, while the AMP is the last product to leave the active site, in accordance to recent kinetic studies performed with the Leishmania donovani APRT.

Biochimie (3)


http://www.sciencedirect.com/science/article/B6VRJ-4DS4255-2/2/c5c38402b9b27dc4e6fd6b95faffd4d2b

In the human nuclear genome only a few copies coding for full-length 7SL RNA genes exist. The Hs7SL-1 gene has recently been classified as type 4 of RNA polymerase III (pol III)-transcribed genes as it was demonstrated that mutations in an external transcriptional activator (ATF) binding site and in an internal CG dinucleotide at positions +15/+16 reduced 7SL RNA expression in vivo and in vitro. We have extended the elucidation of external and internal promoter elements and have discovered two novel regulatory sequences: a TATA-like element in the upstream region and internal A and B box-like motifs. This study was greatly facilitated by the identification of a second, new functional human 7SL RNA gene which we called Hs7SL-3. Remarkably, Hs7SL-3 RNA is synthesized twice as efficiently as Hs7SL-1 in HeLa nuclear extract. Comparison of the upstream regions revealed the presence of two conserved elements in the two human 7SL RNA genes, an ATF/CRE binding site at -43 to -50 and a TATA-like box centered around position -25. Mutational analyses indicated that both external promoter elements are important for efficient transcription. In addition, two sequence motifs can be identified in Hs7SL-1 and Hs7SL-3 at positions 10-19 and 50-60, respectively, downstream of the transcription start site that resemble putative A and B boxes. Single and multiple nucleotide substitutions in these regions also influenced transcription activity to a great extent. The requirement of intragenic functional A and B boxes in combination with the external ATF/CRE and TATA-like promoter elements for the efficient transcription of human 7SL RNA genes is reminiscent of at least two other classes of pol III-transcribed genes in human cells, such as Epstein-Barr virus-encoded EBER and vault RNA genes.

Biochimie (3)


http://www.sciencedirect.com/science/article/B6VRJ-3Y6Y729-2/2/6b22293247205f69bca308b1da671db

Here we report the cloning of a cDNA encoding the first nucleoside diphosphate kinase (NDPK) isolated from plant mitochondria. Amplification of a 317 nt product was performed by PCR, using
oligonucleotide primers based on partial amino acid sequences of the pea mitochondria NDPK and other NDPK isoforms. By screening of a pea cDNA library with this PCR product, a full length clone was obtained. Northern analysis revealed the presence of a 1.1 kb single transcript, with high expression in young leaves and reproductive tissues. The clone encodes a precursor protein of 232 amino acids (26 kDa), including an N-terminal extension of 80 amino acids (9 kDa). Analysis of the deduced amino acid sequence confirmed its identity with the sequences obtained from the purified mature pea mitochondrial NDPK. In vitro import experiments carried out in isolated pea mitochondria showed targeting and processing of the 27 kDa precursor into a 16.5 kDa mature form. Phylogenetic analysis of some vertebrate and plant isoforms of NDPK showed that the pea mtNDPK groups together with the NDPK3 isoform from A. thaliana and the chloroplastic NDPK III from spinach. We suggest that it is possible to design a novel classification of the different NDPK isoforms according to their subcellular localisation and origin.


http://www.sciencedirect.com/science/article/B6VRJ-4F5S6CP-1/2/fe385b093c09be8fa92ef8e3e24c4a07

Lactose-binding proteins with molecular masses of 14-, 17-, 18-, 28-, and 34-kDa were identified in extracts from porcine small intestinal mucosa. Amino acid sequence analysis of peptides generated by CNBr cleavage of the 34-kDa protein, the most abundant of these proteins, identified this protein as porcine galectin-4. To determine if a porcine homolog of murine galectin-6 is expressed in small intestine, primers for a reverse transcriptase-polymerase chain reaction (RT-PCR) were developed that amplified across the linker region of galectin-4, which is the region that differs between murine galectins-4 and -6. Using these primers, this RT-PCR approach identified two galectin-4 isoforms that differed in the length of their linker region. The larger isoform, galectin-4.1, is nine amino acids longer in its linker region than the smaller isoform, galectin-4.2. Based on nucleotide sequence similarities, the two isoforms are likely splice variants of galectin-4 pre-mRNA and not products of separate genes like murine galectins-4 and -6.

Biological Psychiatry


http://www.sciencedirect.com/science/article/B6T4S-46WV648-3/2/81ed2b4d4175f4ca49186e7f1ef71187

Background Neurocognitive deficits are recognized as a cardinal feature of schizophrenia, but the determinants of these deficits remain unknown. Recent reports have suggested that a functional polymorphism, Val158Met in exon III of the catechol-O-methyltransferase gene, shares approximately 4% variance with performance on the Wisconsin Card Sorting Test. These findings led to suggestions that the catechol-O-methyltransferase polymorphism may exert its effects by modulating prefrontal dopamine function, but few other neurocognitive measures have been examined, leaving open questions about phenotypic specificity. Methods We examined the effects of the catechol-O-methyltransferase Val158Met polymorphism in 58 individuals with chronic
schizophrenia who completed a battery of 15 neurocognitive tests, which were reduced to four reliable neurocognitive domain scores. We examined the effects of genotype on these four domains and on global neurocognitive ability. Results The Met allele was associated with better performance in the Processing Speed and Attention domain, but not with other domain scores measuring executive and visuo-perceptual functions, declarative verbal learning and memory, simple motor ability, or global neurocognitive function. Genotype shared approximately 11% of variance with Processing Speed and Attention scores, and approximately 2% of variance with Wisconsin Card Sorting Test scores. Conclusions The findings provide independent support for the hypothesis that the catechol-O-methyltransferase Val158Met polymorphism influences neurocognitive function in schizophrenia, and suggest that the functional effects may be expressed on measures of Processing Speed and Attention. This information may prompt reconsideration of the "prefrontal dopamine" hypothesis and invites examination of a broader range of effects in efforts to refine the neurocognitive phenotype that is most relevant to variation in catechol-O-methyltransferase expression.


http://www.sciencedirect.com/science/article/B6T4S-484NBK4-1JJ/2/a85386f129250a2a18e2351af354ca3ff

Disturbances in dopaminergic transmission have been implicated in the etiology of psychotic disorders. Interindividual differences in deoxyribonucleic acid (DNA) sequences coding for dopamine receptor proteins might contribute to the genetic background of these diseases. We have identified a variation in exon 1 of the dopamine D4 receptor (DRD4) gene, which is characterized by a polymorphic 12 base pair (bp) repeat. This repeat codes for a sequence of four amino acids in the extracellular N-terminal part of the receptor, which borders the first putative transmembrane domain. The 12bp repeat occurs as a two-fold repeat in the more common variant (A1 allele) and is represented only once in the rarer one (A2 allele). The frequency of this DNA polymorphism was determined in a sample of 59 patients suffering from delusional disorder, in 79 schizophrenic patients, and in 75 control subjects. Sixteen (27%) of the 59 patients with delusional disorder carried the A2 allele compared with six (8%) of the controls. The observed difference in genotype frequencies between patients with delusional disorder and controls was highly significant. There were no significant differences in genotype frequencies between schizophrenics and controls. Our results strongly suggest the involvement of genetic variation in the DRD4 gene in conferring susceptibility to delusional disorder.


http://www.sciencedirect.com/science/article/B6T4S-3VXS9G6-8/2/87c784b1b6beef036206562de9d7dc9d

Background: Allelic variation at the CYP2D6 gene has been reported to be associated with Parkinson's disease (PD) and Lewy body dementia (LBD), but not with Alzheimer's disease (AD). AD has been associated with apolipoprotein E (apoE) [epsilons]4 allele loading. Methods: We examined CYP2D6 and apoE polymorphisms in a sample of 259 patients with dementia, 210 of whom had a diagnosis of AD, and 107 healthy controls. Results: We found that the allelic frequency in our AD sample did not vary from that in the controls. The debrisoquine hydroxylase poor metabolizer phenotype was not more prevalent among AD cases than among controls in contrast to that reported for PD and LBD. We also found that CYP2D6 status does not modify the
risk effect for AD conferred by apoE [eps]4 alleles.

Conclusions: These findings provide some support to the notion that, at a genetic level, at least at this locus, AD could be distinct from PD and LBD.


http://www.sciencedirect.com/science/article/B6T4S-4CRYB9X-2/2/3df5b4ecb6c4cad4cd78f6f327af032e

Background: Synapsin II encodes a neuron-specific phosphoprotein that selectively binds to small synaptic vesicles in the presynaptic nerve terminal. The expressions of messenger ribonucleic acid and protein of synapsin II have been reported to be significantly reduced in the brains of schizophrenia patients. The synapsin II gene is located on 3p25, a region that has been implicated to be associated with schizophrenia by genetic linkage. All these findings suggest synapsin II as a candidate gene for schizophrenia.

Methods: In this work, we studied four markers (two single nucleotide polymorphisms (SNPs): rs308963 and rs795009; and two insertion/deletion polymorphisms: rs2307981 and rs2308169) covering 144.2 kilobase pairs (kb) with an average interval of 38 kb in synapsin II in a sample of 654 schizophrenic patients and 628 normal control subjects to explore the mechanism underlying schizophrenia.

Results: We found significant differences in allele frequency distribution of SNP rs795009 (p = .000018, odds ratio 1.405, 95% confidence interval 1.202-1.641) between patients and control subjects. The T allele was significantly higher in patients than in control subjects. Moreover, the overall frequency of haplotype showed significant differences between patients and control subjects (p < .00001).

Conclusions: This study suggests a positive association between synapsin II and schizophrenia, implying that synapsin II is involved in the etiology of schizophrenia.


http://www.sciencedirect.com/science/article/B6T4S-3WJ6WN8-N/2/a50e6d353dcf1b2aa8da9c38510b500f

Background: As part of an ongoing, larger study, "Phenomenology and Course of Pediatric Bipolarity", a subset of prepubertal and early adolescent onset bipolar (PEA-BP) probands, on whom trio blood collection was complete, were used to study genetic transmission of the serotonin transporter linked promoter region (HTTLPR) short and long alleles using the transmission disequilibrium test (TDT). The HTTLPR alleles were selected based on postulated serotonergic mechanisms for PEA-BP and on the burgeoning number of HTTLPR allele studies in bipolar (BP) adults.

Methods: There were 46 complete trios of PEA-BP probands and both biological parents. Probands had a mean age of 11.1 +/- 3.0 years and a mean age of onset of PEA-BP of 8.1 +/- 4.0 years. Comprehensive diagnostic assessments included a semi-structured research interview, the WASH-U-KSADS, administered separately to mothers and to children by blind raters. Probands manifested severe impairment (CGAS 43.9 +/- 8.9), elated mood (84.8%), grandiosity (78.3%), rapid cycling (78.3%) and psychosis (63.0%). The HTTLPR length variant was genotyped using fluorescently labeled primers and automated capillary electrophoresis using laser-induced fluorescence.

Results: The TDT was not significant (TDT chi square = .020, df = 1, p = .89).

Conclusions: This negative result is consistent with the one negative TDT and two negative linkage studies of HTTLPR alleles in bipolar adults in the literature.

http://www.sciencedirect.com/science/article/B6T4S-3W4XK23-C/2/54785e1540e50c5a3e61bb81301a64d7

Background: The goal of this study was to evaluate the role of genetic variation in the coding sequence of tryptophan hydroxylase (TPH) in the pathogenesis of several psychiatric diseases in which altered serotonin function has been implicated: bipolar affective disorder (BP), obsessive-compulsive disorder (OCD), anorexia nervosa (AN), seasonal affective disorder (SAD), panic disorder (PD), and alcoholism (Alc). Methods: Ninety-three percent of the TPH coding sequence was screened by polymerase chain reaction single-strand conformation polymorphism (SSCP) for DNA sequence variations in 128 AN, 88 OCD, 72 SAD, 45 PD, and 36 BP patients and 142 normal volunteers. Also included in the screening were 61 Alc randomly selected from a Finnish alcoholic population in which an association of a TPH intron 7 polymorphism with suicidality was previously observed. Polymorphisms detected by SSCP were characterized by DNA sequencing and by allele-specific restriction enzyme digestion. Genotyping was then performed in 34 Finnish alcoholic suicide attempters. Results: A rare silent mutation was identified in exon 10 and is designated T1095C. The C1095 allele was found in 1 OCD and in 2 AN subjects; all 3 individuals were heterozygous (C1095/T1095) for the variant allele. No association was observed between this TPH T1095C variant with either OCD, AN, Alc, or suicidality. Conclusion: These results suggest that the coding sequence of the TPH gene does not contain abundant variants, and may not play a major role in vulnerability to several psychopathologies in which reduced serotonin turnover has been implicated.


http://www.sciencedirect.com/science/article/B6T4S-40NMS5D-7/2/cb3246537bc2f2dc3dd0b9f4603806da

Background: Substantial evidence indicates that lithium may exert its therapeutic effects through progressive adaptive changes at the level of gene expression; however, the study of lithium-regulated genes has been primarily undertaken with the "candidate gene" approach based on a specific testable hypothesis. The aim of our study was to identify lithium-regulated genes that would not be predicted a priori by the candidate gene approach. Methods: Differential display polymerase chain reaction was used to isolate and identify messenger RNAs (mRNAs) that are differentially expressed in the frontal cortex of rats given lithium for 5 weeks to achieve plasma lithium concentrations of 0.6 to 0.9 mmol/L. Results: A putative lithium-regulated complementary DNA fragment (LRG1) was identified. Northern blot analysis revealed that 5 weeks of lithium treatment, but not 1 week, significantly reduced LRG1 mRNA levels. LRG1 mRNA levels were similarly reduced by 5 weeks of carbamazepine, but not valproate administration. Sequence analysis and search of the GenBank database revealed that LRG1 is analogous to the sequence of the gene for rat aldolase A. Conclusions: These results demonstrate that chronic administration of lithium, but not short-term administration, downregulates the levels of aldolase A mRNA, suggesting this effect may play a role in mediating the therapeutic action of this agent.

Background: Obsessive-compulsive disorder (OCD) is a common and severe psychiatric illness that affects 1-3% of the population and presents a well-established co-morbidity with major depressive disorder (MDD). Twin and family studies have suggested a genetic component in the etiology of OCD, although the mode of inheritance is unknown. Pharmacotherapy of the disease implicates both serotonergic and dopaminergic pathways. Previously, guided by the 22q11 microdeletion-related psychiatric phenotype, we provided evidence for a sexually dimorphic association between OCD and the gene for catechol-O-methyltransferase (COMT). In this report, we use 110 nuclear OCD families to analyze the inheritance of variants of COMT and monoamine oxidase-A (MAOA), another gene modulating monoamine metabolism.

Methods: A sample of 110 nuclear OCD families was collected, and lifetime diagnoses were ascertained using the Diagnostic Interview for Genetic Studies (DIGS). DNA was genotyped for functional variants of the COMT and MAO genes, and allele inheritance was examined using the Transmission Disequilibrium Test (TDT) and Haplotype-based Haplotype Relative Risk (HHRR) test. Results: We provide evidence supporting the previously reported sexually dimorphic association between low COMT enzymatic activity and OCD. We also provide evidence for a similar sexually dimorphic association between OCD and an allele of the MAOA gene, previously linked to high MAO-A enzymatic activity. In agreement with the well-established action of MAO-A inhibitors as antidepressants, this association is particularly marked among male OCD probands with co-morbid MDD, who represent more than 50% of our male OCD sample. Conclusions: Our analysis indicates that variants of two genes modulating monoamine metabolism contribute significantly to OCD susceptibility. Most importantly, an unexpected sexually dimorphic pattern of genetic susceptibility to OCD is revealed and suggests the possibility that profound gender differences in genetic predisposition may exist not only for other OCD susceptibility genes, but for an array of other psychiatric disorders as well.


http://www.sciencedirect.com/science/article/B6T4S-47DPS5T-4/2/a31b4e6139c3befaea5beb2f67e468f4

Background: Based on the dopamine hypothesis, the dopamine D1 receptor gene (DRD1) is considered to be a good candidate gene for bipolar disorder (BP). Methods: In our study, three polymorphisms of the DRD1 gene, -800T/C, -48A/G, and 1403T/C, were analyzed in 286 BP trios. Both the transmission disequilibrium test (TDT) and haplotype TDT were performed on the genotype data to test for the presence of linkage disequilibrium between DRD1 and bipolar disorder. With the extended transmission disequilibrium test (ETDT), we also calculated the maternal transmission and paternal transmission for each allele. Results: Although no association was found for each individual polymorphism, there is a significant association between DRD1 and BP for haplotype TDT analysis ($\chi^2 = 16.068, df = 3, p = .0011$). Conclusions: These results indicate that DRD1 may play a role in the etiology of bipolar disorder.


http://www.sciencedirect.com/science/article/B6T4S-49MX2KJ-D/2/d70a2c88958f79dab632dca9cddffabf
Behavioral inhibition to the unfamiliar (BI), a heritable temperamental profile involving an avoidant response to novel situations, may be an intermediate phenotype in the development of anxiety disorders. Corticotropin-releasing hormone (CRH) is a key mediator of the stress response through its effects on the hypothalamic-pituitary-adrenal axis and limbic brain systems. Transgenic mice overexpressing CRH exhibit BI-like behaviors, implicating this gene in the development of the phenotype.

Methods

We genotyped a marker tightly linked to the CRH locus in 85 families of children who underwent laboratory-based behavioral assessments of BI and performed family-based association analyses.

Results

We observed an association between an allele of the CRH-linked locus and BI (p = .015). Among offspring of parents with panic disorder, this association was particularly marked (p = .0009). We further demonstrate linkage disequilibrium between this marker and single nucleotide polymorphisms encompassing the CRH gene.

Conclusions

These results are consistent with the possibility that variants in the CRH gene are associated with anxiety proneness.


http://www.sciencedirect.com/science/article/B6T4S-3WY9RW4-3/2/30326eaac93428eb85b32e02c206373c

Background: The goal of the current study was to explore the clinical, neuropathological, and neurochemical correlates of the DXS1047 202bp allele in a group of 50 autopsy-confirmed cases of Alzheimer's disease (AD) who lacked other concomitant brain diseases. We previously published the results of a genome survey for novel risk loci for typical-onset (>= 60 years) AD conducted at 10cM resolution (Zubenko et al 1998a, b). This survey detected associations of alleles at six microsatellite loci with AD, including the 202bp allele of the DXS1047 locus that resides within Xq25 on the human cytogenetic map.

Methods: Clinical assessments were performed as part of a longitudinal study of AD and related disorders. Autopsies were performed using standardized methods and the resulting diagnoses were made according to established criteria. Genotyping, morphometry, and neurochemical analyses were performed using postmortem brain tissue.

Results: Patients with AD who carried the DXS1047 202bp allele manifested cortical norepinephrine levels that ranged from 2.1 to 3.6 times the corresponding values for noncarriers (p = .002), controlling for the potential effects of gender, age at symptomatic onset or death, and postmortem interval. In contrast, carriers tended to have lower cortical levels of dopamine (p = .10).

Conclusions: These findings support the results of our previous genome survey and suggest that the DXS1047 locus, or a locus in close proximity, modulates biological variables relevant to the pathophysiology of AD. In addition to providing insights into the clinical biology of AD, the characterization of biologically meaningful subtypes, including genotypic subtypes associated with particular neurobiological derangements, may be important to the advancement of experimental therapeutics in AD.


http://www.sciencedirect.com/science/article/B6T4S-3XF07F8-3/2/a8dccc3b7ea136357220d0614167c1d43

Background: In a previous genome survey, we detected associations of alleles at six microsatellite loci with typical-onset AD, including the 234bp allele of the D10S1423 locus. The goal of the current study was to explore the clinical, neuropathological, and neurochemical correlates of the D10S1423 234bp allele in a group of 50 autopsy-confirmed cases of Alzheimer's disease (AD) who lacked other brain diseases.

Methods: Clinical assessments were performed as
part of a longitudinal study of AD and related disorders. Autopsies were performed using
standardized methods and diagnoses were made according to established criteria. Genotyping,
morphometry, and neurochemical analyses were performed using postmortem brain
tissue.

Results: Patients with AD who carried the D10S1423 234bp allele manifested substantial
reductions in dopamine levels in all six cortical regions examined. In contrast, carriers tended to
have higher concentrations of cortical norepinephrine and revealed a dosage effect of the
D10S1423 234bp allele.

Conclusions: These findings support the results of our genome survey
and suggest that a novel susceptibility gene for AD resides near the D10S1423 locus. The
characterization of biologically meaningful subtypes, including genotypic subtypes with particular
neurobiological derangements, may be important for the advancement of experimental
therapeutics in AD.

Biology of Blood and Marrow Transplantation  (2)

transcript levels in patients achieving a complete cytogenetic response after imatinib therapy for
posttransplantation chronic myelogenous leukemia relapse." Biology of Blood and Marrow
Transplantation 10(10): 718.

http://www.sciencedirect.com/science/article/B758K-4DCM2GK-
C/2/5f55d0f02201fe68c8fe59beb2f8d85

Imatinib induces a high complete cytogenetic response (CCR) rate in relapsed chronic
myelogenous leukemia. By analyzing minimal residual disease (MRD) under the levels of CCR,
we tried to assess the molecular response after imatinib therapy. By using real-time quantitative
reverse transcriptase-polymerase chain reaction (Q-RT-PCR), MRD was evaluated in 23 patients
(3 in cytogenetic relapse, 6 in chronic phase, 9 in accelerated phase, and 5 in blast crisis) who
were treated with standard-dose imatinib for relapsed chronic myelogenous leukemia after
allogeneic stem cell transplantation. With a median therapy time of 399 days (range, 35-817
days), 19 (83%) patients achieved a CCR. Meanwhile, 11 (58%) of them achieved a molecular
remission (MR), which was associated with improved survival. The Q-RT-PCR data were
compared according to the best response (MR, n = 11; CCR, n = 8) in the patients achieving a
CCR. The BCR-ABL/ABL ratios were similar in 2 groups at 3 months but were significantly
different at 6 months (median, 0.0000012 for MR and 0.00022 for CCR; P = .003). The probability
of a subsequent MR was significantly higher in patients with a lower BCR-ABL/ABL ratio at 6
months (100% for P = .006) or a greater reduction in the level between 3 and 6 months (log-
reduction [greater-than-or-equal]1.0, 100%; P = .003). Q-RT-PCR is a reliable method for
monitoring MRD: the early trends in the BCR-ABL/ABL ratio may be clinically useful in
discriminating patients who will achieve an MR from those who will remain in CCR.

lymphocyte infusion for hematologic or cytogenetic relapse of chronic myeloid leukemia induces
rapid and durable complete remissions and is associated with acceptable graft-versus-host
disease." Biology of Blood and Marrow Transplantation 10(3): 204.

http://www.sciencedirect.com/science/article/B758K-4BV4J4S-
7/2/9b6ccf39af1ca796e430d526f6db3001
Donor lymphocyte infusion (DLI) results in complete cytogenetic remission (CCR) of relapsed chronic-phase chronic myeloid leukemia (CML-CP) after allogeneic stem cell transplantation (SCT) in up to 80% of patients. The main complication of DLI is graft-versus-host disease (GVHD). Decreasing the dose of DLI is associated with less GVHD but also with a longer interval between treatment and CCR. We postulated that combining [alpha]-interferon ([alpha]-IFN) with DLI would enable us to decrease the dose of DLI, thereby limiting GVHD, and at the same time to decrease the interval between DLI and CCR for patients with either a hematologic or cytogenetic relapse. For molecular relapses, we hypothesized that because of a lower tumor load, very low doses of DLI without [alpha]-IFN could be an effective treatment. Two groups of CML-CP patients treated with DLI at a very low dose of 0.5 to 1.0 x 10^7 mononuclear cells per kilogram, containing 2 to 6 x 10^6 CD3+ T cells per kilogram, were analyzed: 13 patients with a cytogenetic or a hematologic relapse after allogeneic SCT (group A) were treated with additional [alpha]-IFN therapy at a dose of 3 x 10^6 U 5 d/wk, and 8 patients with a molecular relapse were treated without [alpha]-IFN (group B). Twelve patients from group A reached a CCR. The median interval between DLI and CCR was 7 weeks (range, 5-18 weeks) for group A. All patients with a CCR reached complete donor chimerism at a median of 10 weeks after DLI (range, 6-121 weeks). Eleven patients reached molecular remission at a median of 15 weeks after DLI (range, 8-34 weeks). In group B, all patients reached a molecular remission at a median of 14 weeks (range, 12-29 weeks). Five patients from group A developed acute GVHD grade II to IV and extensive chronic GVHD. In group B, 1 patient developed acute GVHD grade II to IV and subsequently developed extensive chronic GVHD. With a median follow-up of 62 months, 10 patients in group A are alive and in continuous CCR. One patient had a molecular relapse, for which she successfully received additional DLI; another patient reached molecular remission only after 5 doses of DLI. Two patients from group A died of a gram-negative sepsis, and 1 died of an acute myocardial infection. In group B, all patients are alive and in molecular remission with a median follow-up of 20 months. One patient's disease progressed but was successfully treated with DLI plus [alpha]-IFN. In conclusion, very-low-dose DLI in combination with [alpha]-IFN as treatment for cytogenetic or hematologic relapses of CML-CP after allogeneic SCT reduced the interval to obtain a CCR with acceptable GVHD when compared with the literature. Patients with a CCR also reached complete donor chimerism and complete molecular remissions. For patients with a molecular relapse, very-low-dose DLI alone is sufficient to induce molecular remissions in most patients and is associated with limited GVHD.

**Biology of the Cell** (1)


http://www.sciencedirect.com/science/article/B6VRK-4CPK7F0-1/2/09281d8ff5ac4b9e2e3d6f7bb3d52e57

We previously reported that when deprived of fibroblast growth factor, human umbilical vein endothelium-derived cells (HUVE-DCs) are capable of differentiating into smooth muscle-like cells through activin A-induced, Smad-dependent signaling, and that maintenance of the endothelial-cell phenotype and differentiation into smooth muscle-like cells are reciprocally controlled by fibroblast growth factor-1 and activin A (Ishisaki et al., 2003). Here, we examined how protein kinase C (PKC), which plays pivotal roles in the regulation of cellular proliferation and differentiation in numerous cell types, might affect the above differentiation. We found that phorbol-12-myristate-13-acetate-induced down-regulations of some PKCs accompany
suppressions of the expressions of smooth muscle cell markers in HUVE-DCs deprived of fibroblast growth factor. Moreover, the PKC-inhibitors Go6850 and Go6983 suppressed the differentiation of HUVE-DCs into smooth muscle-like cells. These results strongly suggest that activation of PKC is involved in the above differentiation.


http://www.sciencedirect.com/science/article/B6TF8-49FGJTF-B/2/315f6ba7ac71d8539962c90aabd121cd

Hairpin polyamides selectively recognize predetermined DNA sequences with affinities comparable to naturally occurring proteins. Internal side-by-side pairs of unsymmetrical aromatic rings within the minor groove of DNA distinguish each of the four Watson-Crick base pairs. In contrast, N-terminal ring pairs exhibit less specificity, with the exception of Im/Py targeting G.C base pairs. In an effort to explore the sequence specificity of new ring pairs, a series of hairpin polyamides containing 3-substituted-thiophene-2-carboxamide residues at the N-terminus was synthesized. An N-terminal 3-methoxy (or 3-chloro) thiophene residue paired opposite Py displayed 6- (and 3-) fold selectivity for T.A relative to A.T base pair, while disfavoring G,C base pairs by >200-fold. Our data suggests shape selective recognition with projection of the 3-thiophene substituent (methoxy or chloro) to the floor of the minor groove.


http://www.sciencedirect.com/science/article/B6TF8-48W2M9W-4/2/7086939f869a52fbd9c8a1804d2db3a6

Cloning of polyether polyketide synthase (PKS) genes for salinomycin biosynthesis was attempted from Streptomyces albus. Seven [beta]-ketoacyl synthase (KS) core regions were obtained by PCR amplification using primers designed based on the conserved KS domains of type I PKSs. Using the KS fragment as a probe, screening of an S. albus genomic DNA library was carried out by colony hybridization. From the positive cosmid clone isolated, a 4.5-kbBamHI fragment was subcloned and sequenced. It showed high homology with bacterial type I PKSs and was deduced to code for KS, malonyl transferase, and ketoreductase motifs. By gene disruption with this 4.5-kb BamHI fragment, the cloned gene was shown to be a part of the salinomycin biosynthetic gene cluster of S. albus.

http://www.sciencedirect.com/science/article/B6TF9-3YRV9TB-5/2/37f3684dc0916d1eb3b88f0b87dba3e9

We have succeeded in the acquisition of DNA aptamers that recognize chitin using in vitro selection. The obtained DNA aptamers have the stem-loop or bulge loop structures with guanine rich loop clusters and the clockwise B-form stems.


http://www.sciencedirect.com/science/article/B6TF9-3SFNS9Y-M/2/da7ebd62f77308773d5a5daddeec5b5f

The acyclic nucleoside triphosphates 1 and 2 were prepared and tested as substrates for several DNA replicating enzymes; AmpliTaq(R) FS and Taquenase(R) accepted these compounds as substrates leading to chain termination.

Bioresource Technology (2)


http://www.sciencedirect.com/science/article/B6V24-4BH67T9-3/2/0fb79c6b00649cb560460f783c96c574

A laboratory-scale continuously stirred anaerobic thermophilic batch digester was inoculated with cattle manure. Bacterial and archaeal communities, as well as digester performances, were analysed during reactor start-up for about 20 days. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) was used for overall detection and for study of the dynamics of microbial populations. Dominant bacteria and archaea 16S rDNAs were sequenced from the sample on day 12. Ten bacteria and 3 archaea OTUs (operational taxonomic units) were identified from the 52 clones sequenced.Sequences corresponding to the dominant bacterial SSCP peak were phylogenetically close to the 16S rDNA sequence of Bacillus thermoterrestris, whereas sequences corresponding to the two dominant archaeal SSCP peaks were phylogenetically close to the 16S rDNA sequence of Methanoculleus thermophilicus and Methanosarcina thermophila.

Miyatake, F. and K. Iwabuchi "Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost." Bioresource Technology In Press,
To clarify the characteristics of thermophilic bacteria in cattle manure compost, enzymatic activity and species diversity of cultivated bacteria were investigated at 54, 60, 63, 66 and 70 [°C], which were dependent on composting temperature. The highest level of thermophilic bacterial activity was observed at 54 [°C]. Following an increase in temperature to 63 [°C], a reduction in bacterial diversity was observed. At 66 [°C], bacterial diversity increased again, and diverse bacteria including Thermus spp. and thermophilic Bacillus spp. appeared to adapt to the higher temperature. At 70 [°C], bacterial activity measured as superoxide dismutase and catalase activity was significantly higher than at 66 [°C]. However, the decomposition rate of protein in the compost was lower than the rate at 66 [°C] due to the higher compost temperature.

**Blood (121)**


http://www.bloodjournal.org/cgi/content/abstract/102/9/3333

The degree of somatic mutation of immunoglobulin variable (Ig V) region genes is an important prognostic indicator of clinical course and outcome in B-cell chronic lymphocytic leukemia (B-CLL), although the reason for this association remains unclear. Furthermore, some B-CLL cells continue to acquire Ig V gene mutations after the transforming event. Because activation-induced cytidine deaminase (AID) is an essential component of the canonical somatic hypermutation process in healthy B cells, its expression in B-CLL is potentially relevant to the disease. We detected full-length AID transcripts and 3 splice variants by conventional reverse transcription polymerase chain reaction (RT-PCR) in approximately 40% of the cases examined. More sensitive real-time quantitative PCR detected AID transcripts in virtually all B-CLL samples tested, although the range of transcript levels was very large between different cases and varied within individual cases over time. Limiting dilution assays revealed that AID expression was restricted to a small fraction of the leukemic cells in the blood. However, this small fraction is not unique in its ability to express AID, because in vitro stimulation of B-CLL cells with appropriate stimuli significantly increased the fraction of AID-expressing cells. These data suggest that AID-mediated DNA alterations may occur in a variably sized, minor subset of B-CLL cells at any given time.


http://www.bloodjournal.org/cgi/content/abstract/100/3/791

Myelodysplastic syndrome (MDS) is a disease characterized by ineffective hematopoiesis. There are significant biologic and clinical differences between MDS and acute myeloid leukemia (AML). We studied a cohort of 802 patients, 279 (35%) with newly diagnosed MDS and 523 (65%) with newly diagnosed AML, and compared clinical and biologic characteristics of the 2 groups.
Complete clinical and cytogenetic data were available on all patients, and a subgroup of patients was studied for apoptosis, angiogenesis, proliferation, and growth factors. Our results demonstrate that MDS is a discrete entity that is different from AML and is characterized primarily by increased apoptosis in early and mature hematopoietic cells. Using cell sorting and loss of heterozygosity, we demonstrate that the leukemic cells from MDS patients are capable of differentiation into mature myeloid cells and monocytes. We also demonstrate that there is a significant overlap between AML and MDS when MDS is defined on the basis of an arbitrary percentage of blasts of 20% or 30%. These data suggest that despite similarities between AML and MDS in their responses to treatment and outcomes, MDS is biologically and clinically different from AML and should not be considered an early phase of AML. The data indicate that MDS must be better defined on the basis of its biology rather than the percentage of blasts; further, the data suggest that there is a need to develop therapeutic approaches that specifically address the biologic abnormalities of MDS.


http://www.bloodjournal.org/cgi/content/abstract/101/6/2419

The risk of hepatitis B virus (HBV) transmission by transfusion in sub-Saharan Africa is considered to be relatively low, and testing of blood donors is often not done or is done relatively poorly. To re-examine this attitude, we identified HBV chronically infected blood donors from a major hospital in Ghana with a range of hepatitis B surface antigen (HBsAg) assays. Test efficacy was estimated using HBV DNA as a gold standard, and the risk of HBV infection in blood recipients was estimated for different testing strategies. Particle agglutination, dipstick, and enzyme immunoassay (EIA) HBsAg screening detected 54%, 71%, and 97% of HBV infectious donors, respectively. The risk of HBV transmission to recipients less than 10 years old ranged between 1:11 and 1:326 with blood unscreened and screened by EIA, respectively. For older recipients, the risk decreased a further 4-fold because of the high frequency of natural exposure to HBV. A total of 98% of HBsAg-confirmed positive samples contained HBV DNA. HBV DNA load was less than 1 x 104 IU/mL in 75% of HBsAg-reactive samples, most of them anti-HBe reactive. Approximately 0.5% of HBsAg-negative but anti-HBc-positive samples contained HBV DNA. The use of sensitive HBsAg tests is critical to prevent transfusion transmission of HBV infection to young children in a population with a 15% prevalence of chronic HBV infection in blood donors. However, this will not have much effect on the prevalence of this infection unless other strategies to protect children from infection are also advanced in parallel.


http://www.bloodjournal.org/cgi/content/abstract/104/13/3872

The xeroderma pigmentosum group D (XPD) gene encodes a DNA helicase that functions in nucleotide excision repair of chemotherapy-induced DNA damage, the efficiency of which is predicted to be affected by a lysine to glutamine variant at codon 751. We hypothesized that this constitutive genetic variant may modify clinical response to chemotherapy, and we have examined its association with outcome following chemotherapy for acute myeloid leukemia (AML) in 341 elderly patients entered into the United Kingdom Medical Research Council AML 11 trial, and with the risk of developing chemotherapy-related AML. Among subjects treated for AML, disease-free survival at one year was 44% for lysine homozygotes, compared with 36% for heterozygotes and 16% for glutamine homozygotes (hazard ratio [HR], 1.30; 95% confidence interval [CI], 1.01-1.70; P =.04). Similarly, overall survival at one year was 38% for lysine...
homozygotes, 35% for heterozygotes, and 23% for glutamine homozygotes (HR, 1.18; 95% CI, 0.99-1.41; P = .07). Furthermore, homozygosity for the XPD codon 751 glutamine variant was associated with a significantly increased risk of developing AML after chemotherapy (odds ratio, 2.22 for Gln/Gln vs Lys/Lys; 95% CI, 1.04-4.74). These data suggest that the XPD codon 751 glutamine variant protects against myeloid cell death after chemotherapy. (Blood. 2004;104:3872-3877)


http://www.bloodjournal.org/cgi/content/abstract/99/4/1442

Regeneration of hematopoiesis after allogeneic hematopoietic cell transplantation (HCT) involves conversion of the recipient's immune system to donor type. It is likely that distinct cell lineages in the recipient reconstitute at different rates. Dendritic cells (DCs) are a subset of hematopoietic cells that function as a critical component of antigen-specific immune responses because they modulate T-cell activation, as well as induction of tolerance. Mature DCs are transferred with hematopoietic grafts and subsequently arise de novo. Little information exists about engraftment kinetics and turnover of this cell population in patients after allogeneic HCT. This study examined the kinetics of DC chimerism in patients who underwent matched sibling allogeneic HCT. T-cell, B-cell, and myelocytic and monocytic chimerism were also studied. Peripheral blood cells were analyzed at defined intervals after transplantation from 19 patients with various hematologic malignancies after treatment with myeloablative or nonmyeloablative preparatory regimens. Cell subsets were isolated before analysis of chimerism. Despite the heterogeneity of the patient population and preparatory regimens, all showed rapid and consistent development of DC chimerism. By day +14 after transplantation approximately 80% of DCs were of donor origin with steady increase to more than 95% by day +56. Earlier time points were examined in a subgroup of patients who had undergone nonmyeloablative conditioning and transplantation. These data suggest that a major proportion of blood DCs early after transplantation is donor-derived and that donor chimerism develops rapidly. This information has potential implications for manipulation of immune responses after allogeneic HCT.


http://www.bloodjournal.org/cgi/content/abstract/99/1/168

The messenger RNA (mRNA) from 5 of 69 patients with severe hemophilia A did not support amplification of complementary DNA containing the first few exons of the factor VIII (F8) gene but supported amplification of mRNA containing exon 1 of F8 plus exons of the VBP1 gene. This chimeric mRNA signals an inversion breaking intron 1 of the F8 gene. Using an inversion patient, one deleted for F8 exons 1 to 6, and cosmids mapped 70 to 100 kb telomeric of the F8 gene, this study shows that this break strictly affects a sequence (int1h-1) repeated (int1h-2) about 140 kb more telomerically, between the C6.1A and VBP1 genes. The 1041-base pair repeats differ at a single nucleotide (although int1h-2 also showed one polymorphism) and are in opposite orientation. The results demonstrate that they cause inversions by intrachromosome or intrachromatid homologous recombination. The genomic structure of the inversion region shows that transcription traverses intergenic spaces to produce the 2 chimeric mRNAs containing the F8 sequences and characteristic of the inversion. This observation prompts the suggestion that nature may use such extended transcription to test whether the addition of novel domains from neighboring genes creates desirable new genes. A rapid polymerase chain reaction test was developed for the inversion in both patients and carriers. This has identified 10 inversions,
affecting F8 genes with 5 different haplotypes for the BclI, introns 13 and 22 VNTR polymorphism, among 209 unrelated families with severe hemophilia A. This indicates a prevalence of 4.8% and frequent recurrence of the inversion. This should result in absence of F8, and one inversion patient is known to have inhibitors.


http://www.bloodjournal.org/cgi/content/abstract/102/5/1613

Cytogenetic aberrations are important prognostic factors in acute myeloid leukemia (AML). Of adults with de novo AML, 45% lack cytogenetic abnormalities, and identification of predictive molecular markers might improve therapy. We studied the prognostic impact of BAALC (Brain And Acute Leukemia, Cytoplasmic), a novel gene involved in leukemia, in 86 de novo AML patients with normal cytogenetics who were uniformly treated on Cancer and Leukemia Group B 9621. BAALC expression was determined by comparative real-time reverse transcriptase-polymerase chain reaction in pretreatment blood samples, and patients were dichotomized at BAALC's median expression into low and high expressers. Low expressers had higher white counts (P =.03) and more frequent French-American-British M5 morphology (P =.007). Compared to low expressers, high BAALC expressers showed significantly inferior overall survival (OS; median, 1.7 vs 5.8 years, P =.02), event-free survival (EFS; median, 0.8 vs 4.9 years, P =.03), and disease-free survival (DFS; median, 1.4 vs 7.3 years, P =.03). Multivariable analysis confirmed high BAALC expression as an independent risk factor. For high BAALC expressers the hazard ratio of an event for OS, EFS, and DFS was respectively 2.7, 2.6, and 2.2. We conclude that high BAALC expression predicts an adverse prognosis and may define an important risk factor in AML with normal cytogenetics.


http://www.bloodjournal.org/cgi/content/abstract/101/3/837

The proto-oncogene EVI1 encodes a DNA binding protein and is located on chromosome 3q26. The gene is aberrantly expressed in acute myeloid leukemia (AML) patients carrying 3q26 abnormalities. Two mRNAs are transcribed from this locus: EVI1 and a fusion of EVI1 with MDS1 (MDS1-EVI1), a gene located 5' of EVI1. The purpose of this study was to investigate which of the 2 gene products is involved in transformation in human AML. To discriminate between EVI1 and MDS1-EVI1 transcripts, distinct real-time quantitative polymerase chain reaction (PCR) assays were developed. Patients with 3q26 abnormalities often showed high EVI1 and MDS1-EVI1 expression. In a cohort of 319 AML patients, 4 subgroups could be distinguished: EVI1+ and MDS1-EVI1[-] (6 patients; group I), EVI1+ and MDS1-EVI1+ (26 patients; group II), EVI1[-] and MDS1-EVI1+ (12 patients; group III), and EVI1[-] and MDS1-EVI1[-] (275 patients; group IV). The only 4 patients with a 3q26 aberration belonged to groups I and II. Interestingly, high EVI1 and not MDS1-EVI1 expression was associated with unfavorable karyotypes (eg, [-7/7q-]) or complex karyotypes. Moreover, a significant correlation was observed between EVI1 expression and 11q23 aberrations (mixed lineage leukemia [MLL] gene involvement). Patients from groups I and II had significantly shorter overall and event-free survival than patients in groups III and IV. Our data demonstrate that high EVI1 expression is an independent poor prognostic marker within the intermediate-risk karyotypic group.

http://www.bloodjournal.org/cgi/content/abstract/102/10/3494

The chemokine receptor CX3CR1 (CX3C chemokine receptor 1) is expressed in mouse blood on natural killer (NK) cells and on monocytes. Because interleukin-15 (IL-15) is an essential cytokine for NK cell development and maintenance, we hypothesized that it may induce CX3CR1 expression on this cell type. In contrast, we found that in primary mouse bone marrow-derived NK cells IL-15 specifically inhibited CX3CR1 protein and mRNA accumulation, whereas the related cytokine IL-2 did not inhibit but instead increased CX3CR1 expression. Consistent with this finding, intravenous injection of a single dose of recombinant IL-15 into C57BL/6 mice decreased steady-state CX3CR1 levels 24 hours after injection in freshly isolated peripheral blood mononuclear cells (PBMCs), splenocytes, and bone marrow cells, and treatment of mouse PBMCs with IL-15 in vitro inhibited CX3CL1 (ligand for CX3CR1)-induced chemotaxis. These data suggest that IL-15 may be a negative regulator of innate immunity by inhibiting CX3CR1 expression. These data also suggest that IL-15 inhibition of CX3CR1 may subvert potential cell immunotherapy strategies in which IL-15 is used to expand NK cell populations in vivo or ex vivo. Finally, our results provide additional evidence for differential signaling by IL-2 and IL-15, despite usage of common {beta}-{gamma}c receptor chains. (Blood. 2003;102:3494-3503)


http://www.bloodjournal.org/cgi/content/abstract/99/12/4610

Reconstitution of T-cell immunity after bone marrow transplantation (BMT) is often delayed, resulting in a prolonged period of immunodeficiency. Donor lymphocyte infusion (DLI) has been used to enhance graft-versus-leukemia activity after BMT, but the effects of DLI on immune reconstitution have not been established. We studied 9 patients with multiple myeloma who received myeloablative therapy and T-cell-depleted allogeneic BMT followed 6 months later by infusion of lymphocytes from the same donor. DLI consisted of 3 x 10^7 CD4+ donor T cells per kilogram obtained after in vitro depletion of CD8+ cells. Cell surface phenotype of peripheral lymphocytes, T-cell receptor (TCR) V[beta] repertoire, TCR rearrangement excision circles (TRECs), and hematopoietic chimerism were studied in the first 6 months after BMT and for 1 year after DLI. These studies were also performed in 7 patients who received similar myeloablative therapy and BMT but without DLI. Phenotypic reconstitution of T and natural killer cells was similar in both groups, but patients who received CD4+ DLI developed increased numbers of CD20+ B cells. TCR V[beta] repertoire complexity was decreased at 3 and 6 months after BMT but improved more rapidly in patients who received DLI (P =.01). CD4+ DLI was also associated with increased numbers of TRECs in CD3+ T cells (P <.001) and with conversion to complete donor hematopoiesis (P =.05). These results provide evidence that prophylactic infusion of CD4+ donor lymphocytes 6 months after BMT enhances reconstitution of donor T cells and conversion to donor hematopoiesis as well as promoting antitumor immunity.


http://www.bloodjournal.org/cgi/content/abstract/101/2/476
The introduction of an inducible suicide gene has been proposed as a strategy to exploit the antitumor reactivity of donor T cells after allogeneic hematopoietic stem cell transplantation but permit control of graft-versus-host disease. However, there are several obstacles to this approach that may impair the ability of T cells to function and survive in vivo. These include the requirement for in vitro activation or long-term culture to introduce the transgene and obtain therapeutic cell numbers, the toxicity of drug selection to enrich transduced cells, and the immunogenicity of the transgene-encoded products. Here we have developed a transduction and selection strategy for generating large numbers of polyclonal T cells transduced with a retroviral vector encoding the human low-affinity nerve growth factor receptor (LNGFR) for selection and a Fas-based suicide construct (LV'VFas). Ligation of CD28 in conjunction with a T-cell receptor signal permitted efficient transduction, substantially promoted T-cell growth, and contributed to the generation of gene-modified T cells that retained clonal diversity, functional properties, and a homing receptor profile similar to untransduced peripheral blood lymphocytes. Microbeads conjugated directly to antibody specific to LNGFR significantly improved the immunomagnetic selection of LV'VFas-modified T cells and assisted in scaling of the selection procedure to therapeutic cell numbers. Thus, these studies identified a strategy that requires only a brief ex vivo culture and does not use drug selection to obtain large numbers of functional gene-modified polyclonal T cells that can be used for adoptive immunotherapy.


http://www.bloodjournal.org/cgi/content/abstract/101/12/4701

The BCR/ABL tyrosine kinase inhibitor imatinib mesylate (Gleevec, STI571; Novartis, Basel, Switzerland) has shown remarkable efficacy in the treatment of chronic myelogenous leukemia (CML), with a high proportion of patients achieving complete cytogenetic responses (CCRs). However, it is not clear whether remissions will be durable and whether imatinib mesylate can eliminate the malignant primitive progenitors in which the disease arises. We investigated whether residual BCR/ABL+ hematopoietic progenitors were present in patients who achieved CCRs with imatinib mesylate treatment. CD34+ progenitor cells were selected from bone marrow mononuclear cells (MNCs) and analyzed for the presence of the BCR/ABL fusion gene by fluorescence in situ hybridization (FISH). CD34+ cells were also plated in committed progenitor (colony-forming cell, or CFC) and primitive progenitor (long-term bone marrow culture-initiating cell, or LTCIC) cultures and resulting colonies analyzed for the presence of BCR/ABL+ cells by FISH. Using these assays, residual BCR/ABL+ progenitors were detected in all patients studied. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis demonstrated increased levels of BCR/ABL mRNA in CD34+ cells compared with total MNCs. Evaluation of samples collected at different time points demonstrated persistence of BCR/ABL+ progenitors despite continued treatment with imatinib mesylate. Our results indicate that inhibition of BCR/ABL tyrosine kinase activity by imatinib mesylate does not eliminate malignant primitive progenitors in CML patients. Patients in CCR with imatinib mesylate treatment need to be followed carefully to assess for risk of relapse.


http://www.bloodjournal.org/cgi/content/abstract/100/12/4001

Endoglin is an endothelial membrane glycoprotein involved in cardiovascular morphogenesis and
vascular remodeling. It associates with transforming growth factor-[beta] (TGF-[beta]) signaling receptors to bind TGF-[beta] family members, forming a functional receptor complex. Arterial injury leads to up-regulation of endoglin, but the underlying regulatory events are unknown. The transcription factor KLF6, an immediate-early response gene induced in endothelial cells during vascular injury, transactivates TGF-[beta], TGF-[beta] signaling receptors, and TGF-[beta]-stimulated genes. KLF6 and, subsequently, endoglin were colocalized to vascular endothelium (ie, expressed in the same cell type) following carotid balloon injury in rats. After endothelial denudation, KLF6 was induced and translocated to the nucleus; this was followed 6 hours later by increased endoglin expression. Transient overexpression of KLF6, but not Egr-1, stimulated endogenous endoglin mRNA and transactivated the endoglin promoter. This transactivation was dependent on a GC-rich tract required for basal activity of the endoglin promoter driven by the related GC box binding protein, Sp1. In cells lacking Sp1 and KLF6, transfected KLF6 and Sp1 cooperatively transactivated the endoglin promoter and those of collagen [alpha]1(I), urokinase-type plasminogen activator, TGF-[beta]1, and TGF-[beta] receptor type 1. Direct physical interaction between Sp1 and KLF6 was documented by coimmunoprecipitation, pull-down experiments, and the GAL4 one-hybrid system, mapping the KLF6 interaction to the C-terminal domain of Sp1. These data provide evidence that injury-induced KLF6 and preexisting Sp1 may cooperate in regulating the expression of endoglin and related members of the TGF-[beta] signaling complex in vascular repair.


http://www.bloodjournal.org/cgi/content/abstract/102/12/4090

Interleukin 21 (IL-21) is a newly described cytokine with homology to IL-4 and IL-15. They belong to a cytokine family that uses the common (gamma) chain for signaling but also have their private high-affinity receptors. Since it is well known that IL-4 modulates differentiation and activation of dendritic cells (DCs), we analyzed effects of IL-21 compared with IL-15 on DC differentiation, maturation, and function. Here we show that DCs generated with granulocyte-macrophage colony-stimulating factor (GMCSF) in the presence of IL-21 (IL-21DCs) differentiated into phenotypically and functionally altered DCs characterized by reduced major histocompatibility complex class II (MHCII) expression, high antigen uptake, and low stimulatory capacity for T-cell activation in vitro. Additionally, IL-21DCs completely failed to induce antigen (Ag)-specific T-cell mediated contact hypersensitivity. Furthermore, IL-21 blocked lipopolysaccharide (LPS)-induced activation and maturation of DCs, which was not mediated by release of the anti-inflammatory cytokine IL-10. In contrast, when supplementing GMCSF with IL-15, DCs differentiated into mature antigen-presenting cells (APCs) with low antigen uptake and highly significant increased capacities to stimulate T cells in vitro and in vivo. Taken together, these results identify a dichotomous action of these structurally related cytokines on DCs, establishing IL-21 as inhibitory cytokine on DC activation and IL-15 as potent stimulator of DC function, making both cytokines interesting targets for therapeutic manipulation of DC-induced immune reactions. (Blood. 2003;102: 4090-4098)


http://www.bloodjournal.org/cgi/content/abstract/102/4/1534

Interleukin-7 (IL-7) has been shown to enhance thymic output of newly developed T cells following bone marrow transplantation (BMT) in mice. In addition, IL-7 may affect peripheral
expansion of T cells. In order to study the relative contribution of thymopoiesis versus peripheral T-cell expansion in the setting of compromised thymopoiesis, we have applied IL-7 in an experimental stem cell transplantation model using T cell-deficient RAG-1-/- mice. C57BL/6 RAG-1-/- mice received transplants of syngeneic T-cell-depleted (TCD) bone marrow (Ly5.1) with or without supplemented T cells (Ly5.2). IL-7 was administered until day 63 after BMT. Peripheral blood T- and B-cell recovery was quantified by flow cytometry and thymopoiesis was studied by quantification of T-cell receptor rearrangement excision circles (TRECs). In mice receiving a T-cell-replete BMT, IL-7 selectively expanded mature CD45.2+ T cells without affecting the recovery of new bone marrow-derived CD45.1+ T cells. In contrast, IL-7 significantly enhanced the recovery of bone marrow-derived T cells after TCD BMT. Quantification of TRECs in mice receiving a TCD BMT revealed that enhanced T-cell recovery following IL-7 treatment resulted from a strong expansion of newly developed naive T cells. These results suggest that peripheral expansion of recent thymic emigrants or mature T cells may be a preferential mechanism by which IL-7 enhances T-cell recovery after BMT.


http://www.bloodjournal.org/cgi/content/abstract/99/7/2526

The identification of specific chromosome abnormalities in acute myeloid leukemia (AML) is important for the stratification of patients into the appropriate treatment protocols. However, a significant proportion of diagnostic bone marrow karyotypes in AML is reported as normal by conventional cytogenetic analysis and it is suspected that these karyotypes may conceal the presence of diagnostically significant chromosome rearrangements. To address this question, we have developed a novel 12-color fluorescence in situ hybridization (FISH) assay for telomeric rearrangements (termed M-TEL), which uses an optimized set of chromosome-specific subtelomeric probes. We report here the application of the M-TEL assay to 69 AML cases with apparently normal karyotypes or an isolated trisomy. Of the 69 cases examined, 3 abnormalities were identified, all in the normal karyotype group. The first was a t(11;19)(q23;p13), identified in an infant with AML-M4. In 2 other young patients with AML (< 19 years), an apparently identical t(5;11)(q35;p15.5) was identified. Breakpoint mapping by FISH and reverse transcriptase polymerase chain reaction (RT-PCR) analysis confirmed that this was the same t(5;11) as previously identified in 3 children with AML, associated with del(5q) and resulting in the NUP98-NSD1 gene fusion. The t(5;11) was not detected by 24-color karyotyping using multiplex FISH (M-FISH), emphasizing the value of screening with subtelomeric probes for subtle translocations. This is the first report of the t(5;11)(q35;p15.5) in association with an apparently normal karyotype, and highlights this as a new, potentially clinically significant chromosome rearrangement in childhood AML.


http://www.bloodjournal.org/cgi/content/abstract/99/2/443

The inv(16) cytogenetic subtype of acute myeloid leukemia (AML) has a relatively good prognosis. Many patients achieve complete remission (CR). The prognostic uncertainty of negative qualitative reverse transcription-polymerase chain reaction (RT-PCR) assays suggests the need to identify prognostically significant critical thresholds by real-time RT-PCR. A reliable and sensitive (10[-5]) real-time RT-PCR assay was set up for the evaluation of relevant
CBF[beta]-MYH11/ABL transcript ratios and was applied to the 21 patients with inv(16) AML routinely referred for cytogenetic and molecular monitoring in Seragnoli Institute (Bologna, Italy) since 1990. Among the 18 patients who underwent ablative chemotherapy, all achieved CR with a 3-year disease-free survival probability of 63% (95% CI, 40%-87%) and no recorded events after 26 months. Five patients had relapses; 2 died of disease and 3 entered second CR. Analysis of the 125 bone marrow (or peripheral blood) samples studied by real-time RT-PCR showed that transcript ratios of samples taken during CR at any time before a relapse were always greater than 0.12%, whereas those of samples taken during first or second CR from patients who did not subsequently have relapses were always less than 0.25%. This suggests that transcript ratios greater than 0.25% may correspond to high risk for relapse, whereas ratios below 0.12% might indicate the patient is in a curable state. If confirmed, such thresholds could open the way to a new phase in post-CR therapeutic decision making for patients with inv(16) AML.


Constitutive nuclear factor kappaB (NF-[kappa]B) activity protects quiescent mature immune cells from spontaneous apoptosis. Here, we examined whether NF-[kappa]B exerts its antiapoptotic function in these cells through the control of Bcl-2 family proteins. Specific pharmacologic inhibitors of NF-[kappa]B were used to achieve total NF-[kappa]B inactivation in quiescent human blood lymphocytes, granulocytes, and monocytes. NF-[kappa]B inhibition induced drastic lymphocyte and granulocyte apoptosis, but only moderate monocyte apoptosis. T- and B-cell apoptosis was slow and accompanied by a gradual down-regulation of the prosurvival Bcl-2 family proteins Bcl-xL and Bcl-2, respectively. By contrast, granulocyte apoptosis was fast and accompanied by a rapid cellular accumulation of Bcl-xS, the proapoptotic Bcl-x isoform that is generated from alternative splicing of the bcl-x pre-mRNA. Finally, antisense bcl-xL and bcl-2 knockdown in T and B cells, respectively, and induction of Bcl-xS expression in granulocytes through antisense oligonucleotide-mediated redirection of bcl-x pre-mRNA splicing were sufficient to induce significant apoptosis in these cells. Taken together, these results reveal that basal NF-[kappa]B activity preserves homeostasis of quiescent mature lymphocytes and granulocytes through regulation of distinct members of the Bcl-2 family. This study sheds light on the constitutive mechanisms by which NF-[kappa]B maintains defense integrity.


The human T-cell leukemia virus type 2 (HTLV-2), an oncogenic retrovirus closely related to HTLV-1, produces a lifelong infection whose possible association to certain human diseases is still debated. Although some viral products can influence the expression and action of cellular genes, very little is known about the molecular mechanisms involved. Here we show that the AIR-1-encoded human major histocompatibility complex (MHC) class II transactivator (CIITA) strongly inhibits viral replication, but not virus entry, in human B- and T-cell susceptible targets. This effect results from CIITA inhibiting the Tax-mediated transactivation of the HTLV-2 long-term repeat. Further molecular analysis shows that the N-terminal region of CIITA encompassing the first 321 amino acids is responsible for the inhibitory effect on viral replication. This region is crucial for the
transactivation of human MHC class II genes and includes the activation domain as well as domains interacting with coactivators that also are used by the viral transactivator Tax to modulate cellular functions. These results represent the first evidence that a cellular transcriptional activator, controlling the coordinate expression of the entire family of MHC class II antigen-presenting molecules, inhibits HTLV-2 viral replication by a distinct mechanism. In this new role CIITA may represent a new tool for therapeutic strategies aimed at counteracting HTLV-2 replication and spreading. (Blood. 2004;103:995-1001)


http://www.bloodjournal.org/cgi/content/abstract/99/1/180

Type Vicenza variant of von Willebrand disease (VWD) is characterized by a low plasma von Willebrand factor (VWF) level and supranormal VWF multimers. Two candidate mutations, G2470A and G3864A at exons 17 and 27, respectively, of the VWF gene were recently reported to be present in this disorder. Four additional families, originating from northeast Italy, with both mutations of type Vicenza VWD are now described. Like the original type Vicenza subjects, they showed a mild bleeding tendency and a significant decrease in plasma VWF antigen level and ristocetin cofactor activity but normal platelet VWF content. Unlike the original patients, ristocetin-induced platelet aggregation was found to be normal. Larger than normal VWF multimers were also demonstrated in the plasma. Desmopressin (DDAVP) administration increased factor VIII (FVIII) and VWF plasma levels, with the appearance of even larger multimers. However, these forms, and all VWF oligomers, disappeared rapidly from the circulation. The half-life of VWF antigen release and of elimination was significantly shorter than that in healthy counterparts, so that at 4 hours after DDAVP administration, VWF antigen levels were close to baseline. Similar behavior was demonstrated by VWF ristocetin cofactor activity and FVIII. According to these findings, it is presumed that the low plasma VWF levels of type Vicenza VWD are mainly attributed to reduced survival of the VWF molecule, which, on the other hand, is normally synthesized. In addition, because normal VWF-platelet GPIb interaction was observed before or after DDAVP administration, it is proposed that type Vicenza VWD not be considered a 2M subtype.


http://www.bloodjournal.org/cgi/content/abstract/101/1/151

We describe a von Willebrand disease (VWD) variant characterized by the persistence of von Willebrand factor (VWF) propeptide as a result of a C>T transition at nucleotide 2527 in exon 17 of the VWF gene. This mutation, which was present in the proband and his father, predicts the substitution of Cys for Arg at position 760 of pre-pro-VWF, 4 residues before the propeptide cleavage site belonging to a consensus sequence for substrate recognition by the processing enzyme paired dibasic amino acid-cleaving enzyme (PACE)/furin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) documented the presence of both processed and unprocessed VWF in the patient's plasma, with unprocessed VWF relatively less represented. The patient's hemostatic phenotype was characterized by a mild decrease in plasma factor VIII (FVIII) and VWF, a decrease in plasma VWF multimers, and a mild reduction in the FVIII binding capacity of VWF. The FVIII binding defect was more pronounced in the proband than in the father because he also inherited the type 2N Arg91Gln mutation from his mother. The persistence of VWF propeptide did not impair VWF synthesis because platelet VWF content was
normal, nor did it compromise VWF storage in endothelial cells, because of the normal post-1-deamino-8-D-arginine vasopressin (DDAVP) increase in plasma VWF. Coexpression of wild-type and Arg760Cys VWF into a Furin-producing BHK cell line resulted in decreased VWF secretion and a defect in the FVIII binding capacity of VWF, together with the persistence of VWF propeptide. These findings confirm that a normal consensus sequence for VWF propeptide cleavage and efficient cleavage are required in vivo for normal FVIII binding capacity of VWF.


http://www.bloodjournal.org/cgi/content/abstract/103/2/442

In a series of 153 children with T-cell malignancies enrolled in 2 consecutive European Organization for Research and Treatment of Cancer (EORTC) trials, we assessed the HOX11L2 expression and/or the presence of a t(5;14)(q35;q32). Additionally, in 138 of these patients, HOX11 expression and SIL-TAL rearrangement were also assessed. These alterations were mutually exclusive, and their frequency was 23% (n = 35), 7% (n = 10), and 12% (n = 17), respectively. HOX11L2/t(5;14) positivity was more frequent in acute lymphoblastic leukemia (ALL) with cortical T immunophenotype and in children aged between 6 and 9 years. In contrast with previously reported data, patients positive and negative for HOX11L2/t(5;14) were comparable with regard to clinical outcome as well as to the response to a 7-day prephase treatment or to residual disease at completion of induction therapy. The 3-year event-free survival (EFS) rate (±SE percentage) for patients positive and negative for HOX11L2/t(5;14) was 75.5% (±8.1%) and 68.3% (±5.0%), respectively; the hazard ratio was 0.84 (95% confidence interval, 0.40-1.80). Patients with HOX11-high expression and those with SIL-TAL fusion had low levels of residual disease at the end of induction and a favorable prognosis: the 3-year EFS rate was 83.3% (±8.5%) and 75.3% (±12.6%), respectively. The results obtained in HOX11L2/t(5;14) patients in this study do not confirm the unfavorable prognosis reported in previous studies.


http://www.bloodjournal.org/cgi/content/abstract/105/2/886

The extent and rapidity with which T cells are regenerated from graft-derived precursor cells directly influences the incidence of infection and the T-cell-based graft-versus-tumor effect. Measurement of T-cell receptor excision circles (TRECs) in peripheral blood is a means of quantifying recent thymic T-cell production and has been used after transplantation in many studies to estimate thymus-dependent T-cell reconstitution. We hypothesized that the quality of thymic function before transplantation affects thymus-dependent T-cell reconstitution after transplantation. We used real-time polymerase chain reaction (PCR) to quantify signal-joint TRECs (sjTRECs) before and after transplantation. T-cell reconstitution was evaluated by T-cell receptor {beta} (TCR{beta}) CDR3 size spectratyping. We tested 77 healthy sibling donors and 244 samples from 26 pediatric recipients of allogeneic hematopoietic stem cell transplantation (AHSCT). Blood from the healthy donors contained 1200 to 155 000 sjTREC copies/mL blood. Patients who had greater than 1200 copies/mL blood before transplantation showed early recovery of sjTREC numbers and TCR{beta} repertoire diversity. In contrast, patients who had fewer than 1200 copies/mL blood before transplantation demonstrated significantly slower restoration of thymus-dependent T cells. We conclude that the rate of reconstitution of thymus-
dependent T cells is dependent on the competence of thymic function in the recipients before transplantation. Therefore, pretransplantation measurement of sjTREC may provide an important tool for predicting thymus-dependent T-cell reconstitution after transplantation.


http://www.bloodjournal.org/cgi/content/abstract/103/12/4630

SOCS1 and SHP1 negatively regulate the Janus kinase/signal transducer and activator of transcription (Jak/STAT) signaling pathway. The role of promoter hypermethylation leading to epigenetic inactivation of SOCS1 and SHP1 in myeloma was investigated. The methylation-specific polymerase chain reaction (MSP) was used to define SOCS1 and SHP1 methylation in 34 diagnostic myeloma samples. For SOCS1, MSP primers 3' to the translation start site were unreliable and gave positive results in normal controls. However, primers in the 5' promoter region were specific, although no myeloma samples showed methylation. For SHP1, 27 of 34 (79.4%) myeloma samples showed SHP1 hypermethylation. The biologic significance of SHP1 methylation was investigated in the U266 human myeloma line. U266 contained completely methylated SHP1. Furthermore, there was constitutive STAT3 phosphorylation. Treatment with 5-azacytidine led to progressive demethylation of SHP1 on days 2 to 5, with consequent increasing reexpression of SHP1 as shown by reverse transcription-polymerase chain reaction (RT-PCR). Concomitant with increasing SHP1, a parallel down-regulation of phosphorylated STAT3 occurred, so that by day 5 phosphorylated STAT3 was barely detectable. The overall survivals of patients with and without SHP1 methylation were similar. SHP1 methylation leading to epigenetic activation of the Jak/STAT pathway might have a tentative role in the pathogenesis of myeloma, which should be further confirmed by functional studies in primary myeloma samples. (Blood. 2004;103:4630-4635)


http://www.bloodjournal.org/cgi/content/abstract/2004-04-1477v1

Perforin mutations have been demonstrated in a proportion of patients diagnosed with the familial form of hemophagocytic lymphohistiocytosis (HLH). In the present study, we evaluated whether some patients with lymphoma sharing clinical characteristics with HLH might harbor mutations of the perforin gene. We analysed 29 patients and we found that 4 patients, who developed either Hodgkin or non-Hodgkin lymphoma, had bi-allelic mutations of the perforin gene. One of these 4 patients, a 19-year-old female with T-cell lymphoma, had a brother, carrying the same mutations, who developed HLH. In 2 out of the 4 patients with bi-allelic mutations of the perforin gene, we evaluated perforin expression by flow-cytometry and natural killer (NK) activity, and both were found to be absent. Moreover, we documented the presence of mono-allelic mutations of the perforin gene in 4 more patients. One of these 4 latter patients also carried a mutation of the Fas gene. These data indicate that perforin deficiency, either alone or in combination with other mutations of genes involved in lymphocyte survival or functional activity, may be present in patients with lymphoma. These findings suggest that perforin plays a key role also in the mechanisms of immune-surveillance that prevent tumor growth and/or development.

Cook, G. A., C. M. Longhurst, et al. (2002). "Identification of CD9 extracellular domains important in
CD9, a 24-kDa member of the tetraspanin family, influences cellular growth and development, activation, adhesion, and motility. Our investigation focuses on the hypothesis that the CD9 second extracellular loop (EC2) is important in modulating cell adhesive events. Using a Chinese hamster ovary (CHO) cell expression system, we previously reported that CD9 expression inhibited cell adhesion to fibronectin and fibronectin matrix assembly. For the first time, a functional epitope on CD9 EC2 that regulates these processes is described. Binding of mAb7, an EC2-specific anti-CD9 monoclonal antibody, reversed the CD9 inhibitory activity on CHO cell adhesion and fibronectin matrix assembly. This reversal of cell phenotype also was observed in CHO cells expressing CD9 EC2 truncations. Furthermore, our data showed that the EC2 sequence 173LETFTVKSCPDAIKEVFDNK192 was largely responsible for the CD9-mediated CHO cell phenotype. Two peptides, 135K-V172 (peptide 5b) and 168P-I185 (peptide 6a), selectively blocked mAb7 binding to soluble CD9 and to CD9 on intact cells. These active peptides reversed the influence of CD9 expression on CHO cell adhesion to fibronectin. In addition, confocal microscopy revealed that CD9 colocalized with the integrin [alpha][5][beta]1 and cytoskeletal F-actin in punctate clusters on the cell surface, particularly at the cell margins. Immunoprecipitation studies confirmed CD9 association with [beta]1 integrin. The cellular distribution and colocalization of focal adhesion kinase and [alpha]-actinin with cytoskeletal actin was also influenced by CD9 expression. Thus, CD9 may exhibit its effect by modulating the composition of adhesive complexes important in facilitating cell adhesion and matrix assembly.
We report here 2 patients with chronic nonspherocytic hemolytic anemia (CNSHA) and severe red blood cell (RBC) adenylate kinase (AK) deficiency. One of these patients, a boy of Spanish origin, exhibited a neonatal icterus and splenomegaly and required blood transfusions until the age of 2 years. The other patient was a white, American infant born to parents who were first cousins; he also presented with neonatal icterus and anemia. In neither case was psychomotor impairment observed. The first patient was found to be a compound heterozygote for 2 different missense mutations, 118G>A(Gly40Arg) and 190G>A(Gly64Arg) (cDNA sequence first described by Matsuura et al, 1989). The second patient was homozygous for an in-frame deletion (GAC) from nucleotide (nt) 498 to 500 or nt 501 to 503 of the cDNA sequence, predicting deletion of either aspartic acid (Asp) 140 or 141. The crystal structure of porcine cytosolic AK was used as a molecular model to investigate how these mutations may affect enzyme structure and function.

Phosphoinositide 3-kinase (PI3-kinase)-dependent phosphorylation of the proapoptotic Bcl-2 family member Bad has been proposed as an important regulator of apoptotic cell death. To understand the importance of this pathway in nontransformed hematopoietic cells, we have examined the effect of survival cytokines on PI3-kinase activity and Bad expression and phosphorylation status in human neutrophils. Granulocyte macrophage-colony-stimulating factor (GM-CSF) and tumor necrosis factor-[alpha] (TNF-[alpha]) both reduced the rate of apoptosis in neutrophils cultured in vitro for 20 hours. Coincubation with the PI3-kinase inhibitor LY294002, which in parallel experiments abolished GM-CSF-primed, fMLP-stimulated superoxide anion production and GM-CSF-stimulated PtdIns(3,4,5)P3 accumulation, inhibited the GM-CSF and TNF-[alpha] survival effect. In contrast, the MAP kinase kinase (MEK1/2) inhibitor PD98059 and the protein kinase A inhibitor H-89 had only a marginal effect on GM-CSF-mediated neutrophil survival. GM-CSF substantially increased Bad phosphorylation at Ser112 and Ser136 and increased the cytosolic accumulation of Bad. GM-CSF also regulated Bad at a transcription level with a marked decrease in mRNA levels at 4 hours. TNF-[alpha] caused a biphasic effect on the rate of morphologic apoptosis, which corresponded to an early increase, and a late inhibition, of Bad mRNA levels. LY294002 inhibited GM-CSF- and TNF-[alpha]-mediated changes in Bad phosphorylation and mRNA levels. These data suggest that the survival effect of GM-CSF and TNF-[alpha] in neutrophils is caused by a PI3-kinase-dependent phosphorylation and cytosolic translocation of Bad, together with an inhibition of Bad mRNA levels. This has important implications for the regulation of neutrophil apoptosis in vivo.
CD14 but are distinguishable by the presence of CD2 on dendritic cells. CD2 is known to mediate the activation of T and natural killer (NK) cells through its interaction with CD58. CD2 epitopes recognized by anti-T111, -T112, and -T113 monoclonal antibodies (mAbs) are present on dendritic cells. Here we show that CD2 engagement significantly increases class II, costimulatory (CD40, CD80, CD86), adhesion (CD54, CD58), and CCR7 molecule expression on primary dendritic cells. Conversely, minimal or no change in the expression of the above antigens occurs on monocyte-derived dendritic cells, because these molecules are already maximally expressed. However, both kinds of dendritic cells release interleukin-1{beta} (IL-1{beta}) and IL-12 after CD2 engagement. Lastly, interference with dendritic cell CD2-T-cell CD58 engagement decreases naive CD4+CD45RA+ T-cell proliferation. Collectively, our results suggest another role of the CD2-CD58 pathway that allows nonimmune and immune cells to interact directly with dendritic cells and initiate innate and adaptive immune responses.


http://www.bloodjournal.org/cgi/content/abstract/104/3/744

The use of leukemia cells as antigen-presenting cells (APCs) in immunotherapy is critically dependent on their capacity to initiate and sustain an antitumor-specific immune response. Previous studies suggested that pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cells could be manipulated in vitro through the CD40-CD40L pathway to increase their immunostimulatory capacity. We extended the APC characterization of CD40L-activated BCP-ALL for their potential use in immunotherapy in a series of 19 patients. Engaging CD40 induced the up-regulation of CCR7 in 7 of 11 patients and then the migration to CCL19 in 2 of 5 patients. As accessory cells, CD40L-activated BCP-ALL induced a strong proliferation response of naive T lymphocytes. Leukemia cells, however, were unable to sustain proliferation over time, and T cells eventually became anergic. After CD40-activation, BCP-ALL cells released substantial amounts of interleukin-10 (IL-10) but were unable to produce bioactive IL-12 or to polarize TH1 effectors. Interestingly, adding exogenous IL-12 induced the generation of interferon-(gamma) (IFN-(gamma))-secreting TH1 effectors and reverted the anergic profile in a secondary response. Therefore, engaging CD40 on BCP-ALL cells is insufficient for the acquisition of full functional properties of immunostimulatory APCs. These results suggest caution against the potential use of CD40L-activated BCP-ALL cells as agents for immunotherapy unless additional stimuli, such as IL-12, are provided.


http://www.bloodjournal.org/cgi/content/abstract/100/8/2957

p16 and p15, 2 inhibitors of cyclin-dependent kinases, are frequently hypermethylated in hematologic neoplasias. Decitabine, or 5-Aza-2'-deoxycytidine, reverts hypermethylation of these genes in vitro, and low-dose decitabine treatment improves cytopenias and blast excess in ~50% of patients with high-risk myelodysplastic syndrome (MDS). We examined p15 and p16 methylation status in bone marrow mononuclear cells from patients with high-risk MDS during treatment with decitabine, using a methylation-sensitive primer extension assay (Ms-SNuPE) to quantitate methylation, and denaturing gradient gel electrophoresis (DGGE) and bisulfite-DNA sequencing to distinguish individually methylated alleles. p15 expression was serially examined in bone marrow biopsies by immunohistochemistry. Hypermethylation in the 5’p15 gene region was detected in 15 of 23 patients (65%), whereas the 5’p16 region was unmethylated in all patients.
Among 12 patients with hypermethylation sequentially analyzed after at least one course of decitabine treatment, a decrease in p15 methylation occurred in 9 and was associated with clinical response. DGGE and sequence analyses were indicative of hypomethylation induction at individual alleles. Immunohistochemical staining for p15 protein in bone marrow biopsies from 8 patients with p15 hypermethylation revealed low or absent expression in 4 patients, which was induced to normal levels during decitabine treatment. In conclusion, frequent, selective p15 hypermethylation was reversed in responding MDS patients following treatment with a methylation inhibitor. The emergence of partially demethylated epigenotypes and re-establishment of normal p15 protein expression following the initial decitabine courses implicate pharmacologic demethylation as a possible mechanism resulting in hematologic response in MDS.


http://www.bloodjournal.org/cgi/content/abstract/101/11/4583

BCR-ABL fusion oncogene is the molecular hallmark of chronic myelogenous leukemia (CML), a condition characterized by a progression from a chronic to acute phase leukemia because of secondary genetic events, the nature of which remains largely unknown. Here, we report that the expression of the p210 BCR-ABL fusion protein leads to a down-regulation of BRCA1 protein, a gene product involved in the maintenance of genome integrity. BRCA1 protein is nearly undetectable in leukemia cells from patients with CML, both during the chronic phase and in blast crisis. Similarly, stable transfection-enforced expression of p210 protein in established hematopoietic cell lines leads to severe BRCA1 depletion. The lack of significant change in BRCA1 mRNA level in cells expressing p210 supports the hypothesis that the regulation of BRCA1 protein level occurs after transcription. It is abolished on exposure of the cells to STI571 and by mutation in the adenosine triphosphate (ATP) pocket of p210 and thus seems to require the tyrosine kinase activity of BCR-ABL. Cell lines expressing high levels of BCR-ABL display an increased rate of sister chromatid exchange and chromosome aberrations after ionizing radiation. These findings reveal a novel link between the oncoprotein BCR-ABL and the tumor-suppressor protein BRCA1.


http://www.bloodjournal.org/cgi/content/abstract/2005-02-0555v1

Human erythrocyte R-type pyruvate kinase (RKP) deficiency is an autosomal recessive disorder produced by mutations in the PKLR gene causing chronic nonspherocytic haemolytic anaemia. Patient survival in severe RPK deficiency has been associated with the compensatory expression in the red blood cells of M2PK, an isoenzyme showing wide tissue distribution. We describe a novel homozygous null mutation of the PKLR gene found in a female patient diagnosed before birth as having a PK deficiency. The mutant PK gene revealed an 11-nt duplication at exon 8 causing frameshift of the PKLR transcript, predicting a truncated protein inferred to have no catalytic activity. Western blot analysis and qRT-PCR detected no M2PK expression in the peripheral blood red cell fraction. The expression of mutant RPK mRNA in the red blood cells was almost six times higher than that detected in a control patient with hereditary spherocytosis. This molecular phenotypic analysis of the null mutation in the PKLR gene provides evidence for a lack of M2PK in the mature red blood cells of this patient, and suggests that normal red cell functions and survival are achieved through a population of young erythroid cells released into the
circulation in response to anaemia.


http://www.bloodjournal.org/cgi/content/abstract/100/1/200

Because tumor-specific antigens have been identified in multiple myeloma (MM), immunotherapy might provide an additional treatment modality for the disease. Expression of CD40 ligand (CD40L) proximate to the MM cells might serve this purpose, either by increasing their capacity to present self-antigens by activation through their CD40 receptor or by the recruitment of professional antigen-presenting cells (APCs) able to take up and present tumor-associated antigens. To distinguish between these possibilities and predict whether human CD40[-] myeloma might respond to this approach, we examined 3 murine plasmacytoma cell lines, 2 (MPC-11 and S107) expressing the CD40 molecule and 1 (X-24) lacking such expression. Syngeneic BALB/cByJ mice were inoculated subcutaneously with tumor cells mixed with CL7.1 fibroblasts, retrovirally transduced to express either the mCD40L or the neo gene. For all 3 plasmacytoma cell lines, coinjection with CL7.1/mCD40L significantly reduced local tumor growth compared with controls. This effect was mediated by a systemic antitumor immune response, since mice immunized with tumor and CL7.1/mCD40L were resistant to subsequent challenge with tumor, and tumor growth inhibition was abolished when CD8+ or CD4+ lymphocytes were depleted. Because expression of CD40L gave equivalent protection from CD40+ and CD40[-] tumors and transgenic-CD40L failed to up-regulate costimulatory molecules in either tumor, the protective effects of CD40L probably resulted from recruitment/activation of professional APCs rather than from CD40 activation of plasmacytoma cells. As further support of this concept, we found that mice were also well protected if CL7.1 and CD40L were injected together with apoptotic plasmacytoma cells from these tumors. Hence, transgenic CD40L expression may produce an antitumor immune response against either CD40+ or CD40[-] tumors and may be of therapeutic value for both types of myeloma in humans.


http://www.bloodjournal.org/cgi/content/abstract/101/3/1111

The genetic defects underlying the pathogenesis of acute myeloid leukemia (AML) are still largely unknown. Retroviral insertion mutagenesis in mice has become a powerful tool to identify candidate genes involved in the development of leukemia and lymphoma. We have used this strategy with the 1.4 strain of Graffi murine leukemia virus (MuLV), which predominantly causes myeloid leukemias. Here, we report that Graffi-1.4-induced AML frequently harbors virus integrations in the gene encoding the transcription factor Yin Yang 1 (YY1). These integrations occurred in both orientations, and all were located in the 5’ promoter region of the gene, 0.5 to 1.5 kb upstream of the major transcriptional start site. Luciferase reporter assays showed that virus integration in this region increases promoter activity and renders it independent of a functional binding site for Sp1, a major transcriptional regulator of YY1. We used the murine 32D model to study the consequence of perturbed YY1 expression for myelopoiesis. YY1 protein levels were high in 32D parental cells maintained in interleukin-3-containing medium, but they dropped when the cells were induced to differentiate by granulocyte-colony-stimulating factor (G-CSF). Strikingly, G-CSF-induced neutrophilic differentiation was reduced in 32D cell transfectants ectopically expressing YY1. In similar experiments on primary bone marrow cells, enforced YY1 expression blocked the outgrowth of CFU-GM colonies. Increased YY1 expression was seen in
some cases of human AML. Collectively, these data imply a possible role of perturbed expression of YY1 in the development of AML through interference with the myeloid differentiation program in the leukemic progenitor cells.


http://www.bloodjournal.org/cgi/content/abstract/100/12/4082

Ataxia telangiectasia (A-T), a genetic disorder caused by the homozygous mutation of the ATM gene, frequently associates with variable degrees of cellular and humoral immunodeficiency. However, the immune defects occurring in patients with A-T are still poorly characterized. Here we show that the T-cell receptor (TCR) variable [beta] (BV)-chain repertoire of 9 A-T patients was restricted by diffuse expansions of some variable genes prevalently occurring within the CD4 subset and clustering to certain TCRBV genes (eg, 5.1, 11, 14, and 23). In addition, the study of the third complementarity-determining region (CDR3) showed, in all patients, significantly altered profiles in most BV genes examined suggesting diffuse oligoclonal expansions. The sequencing of TCR CDR3 regions revealed completely normal V(D)J coding joints and confirmed a reduced diversity of the antigen-receptor repertoire. The B-cell repertoire was similarly restricted and skewed by diffuse oligoclonal expansions with normal V(D)J joints. Thymic output, evaluated by measuring TCR rearrangement excision circles, was extremely low. The majority of peripheral T cells had the phenotype and the function of effector memory cells, indicating that in vivo they are able to respond normally by terminal differentiation to antigenic stimulation. These results indicate that ATM mutation limits the generation of a wide repertoire of normally functioning T and B cells.


http://www.bloodjournal.org/cgi/content/abstract/2004-11-4394v1

B-cell chronic lymphocytic leukemia (CLL) consists of two prognostic entities where cases with mutated immunoglobulin VH genes have better outcome than unmutated cases. VH mutated CLLs display longer telomeres compared to unmutated cases and telomere length has been indicated to predict outcome, although the prognostic value of telomere length has not been fully established in CLL. We analyzed telomere length, VH gene mutation status and clinical parameters in a large series of CLL. Telomere length was assessed by quantitative PCR, giving a very good correlation to telomere length estimated by Southern blotting (p< 0.001). The prognostic information given by mutation status (n=282) and telomere length (n=246) was significant (p< 0.001 respectively). Telomere length was a prognostic factor for stage A (p=0.021) and stage B/C (p=0.018) patients, whereas mutation status predicted outcome only in stage A patients (p< 0.001). Furthermore, mutated CLLs were subdivided by telomere length into two groups with different prognosis (p=0.003), a feature not seen for unmutated cases (p=0.232). Interestingly, the VH mutated group with short telomeres had an overall survival close to unmutated cases. Thus, by combining VH mutation status and telomere length an improved subclassification of CLL was achieved identifying previously unrecognized patient groups with different outcome.

Granziero, L., P. Circosta, et al. (2003). "CD100/Plexin-B1 interactions sustain proliferation and survival of

http://www.bloodjournal.org/cgi/content/abstract/101/5/1962

Growth and survival of chronic B-cell tumors are favored by the malignant cell's capacity to respond to selected microenvironmental stimuli provided by nontumoral bystander cells. To investigate which mechanisms operate in these crosstalks and whether they are malignancy-related or reproduce the mechanisms used by normal B cells we have studied the expression and functional role of semaphorin CD100 (now called Sema4D) in chronic lymphocytic leukemia (CLL) cells and normal CD5+ B cells. We demonstrate here that (1) leukemic and normal CD5+ B lymphocytes uniformly express CD100; (2) the CD100 high-affinity receptor Plexin-B1 is expressed by bone marrow stromal cells, follicular dendritic cells, and activated T lymphocytes, and is thus available to CD100+ lymphocytes in different specific microenvironments; and (3) upon interaction between CD100 and Plexin-B1 both CLL and normal CD5+ B cells increase their proliferative activity and extend their life span. These findings establish that Plexin-B1 is an easily accessible receptor for CD100 within the immune system. The encounter of CD100+ leukemic cells with Plexin-B1 may promote the proliferation and survival of malignant cells. The crosstalk operated by the CD100/Plexin-B1 interaction is not malignancy related but reproduces a mechanism used by normal CD5+ B cells.


http://www.bloodjournal.org/cgi/content/abstract/99/8/2997

Hepatocyte growth factor (HGF), a heparin-binding factor, is synthesized as a single-chain inactive precursor (pro-HGF), which is converted by proteolysis to an active heterodimer (mature HGF). HGF has pleiotropic activities and has been implicated in the regulation of mitogenesis, motogenesis, and morphogenesis of epithelial and endothelial cells. As polymorphonuclear neutrophils (PMNs) secrete numerous cytokines involved in the modulation of local inflammation, we investigated their ability to produce HGF. We found that HGF was stored in secretory vesicles and in gelatinase/specific granules. This intracellular stock was rapidly mobilized by degranulation when neutrophils were stimulated with phorbol myristate acetate or N-formylmethionyl-leucyl-phenylalanine. Cycloheximide did not affect the release of HGF. Moreover, HGF messenger RNA and protein expression was found in bone marrow myeloid cells, suggesting that HGF synthesis likely occurs during PMN maturation. In mature circulating PMNs, intracellular HGF was in the pro-HGF form, whereas the HGF secreted by degranulation was the mature form. Furthermore, PMNs pretreated with diisopropyl fluorophosphate only released the pro-HGF form, suggesting that PMN-derived serine protease(s) are involved in the proteolytic process. We also obtained evidence that secreted mature HGF binds PMN-derived glycosaminoglycans (probably heparan sulfate). These findings suggest that PMNs infiltrating damaged tissues may modulate local wound healing and repair through the production of HGF, a major mediator of tissue regeneration.


http://www.bloodjournal.org/cgi/content/abstract/100/4/1430

The ATM serine-threonine kinase plays a central role in the cellular response to DNA damage. Germ-line mutations in the ATM gene cause ataxia-telangiectasia (A-T), a multisystem disorder
associated with predisposition to lymphoma and acute leukemia. Moreover, somatic ATM mutations have been identified in T-cell prolymphocytic leukemia, mantle cell lymphoma, and B-cell chronic lymphocytic leukemia. In this study, the entire ATM coding sequence was examined in genomic DNA from 120 lymphoid neoplasms. Novel mutations and mutations implicated in cancer and/or A-T were found in 9 of 45 diffuse large B-cell lymphomas (DLBCLs), 2 of 24 follicular lymphomas, and 1 of 27 adult acute lymphoblastic leukemias, whereas no such mutations were detected among 24 peripheral T-cell lymphomas. The mutational spectrum consisted of 2 nonsense mutations, 1 mutation affecting RNA splicing, and 10 missense variants. Most of these mutations were associated with loss or mutation of the paired ATM allele, consistent with biallelic inactivation of ATM. Of the 9 DLBCLs with ATM mutations, 7 also carried TP53 mutations and/or deletions of the INK4a/ARF locus (P =.003). The ATM 735C>T substitution previously considered a rare normal variant was found to be 5.6 times more frequent in individuals with DLBCL than in random individuals (P =.026), suggesting that it may predispose to B-cell lymphoma. Our data suggest that ATM mutations contribute to the development of DLBCL, and that ATM and the ARF-p53 tumor suppressor pathway may cooperate in the pathogenesis of this malignancy.


http://www.bloodjournal.org/cgi/content/abstract/102/3/1035

Different biologic features have been associated with a more or less aggressive clinical course in chronic lymphocytic leukemia (CLL). In the present study, 20 patients with highly stable CLL observed at a single institution over a period of 10 to 23 years and who never required treatment were extensively characterized. The aim was to identify a distinct and reproducible biologic profile associated with disease stability that may be used to recognize at presentation CLL patients who are likely to have a very benign clinical course and for whom treatment is not indicated. The results obtained indicate that numerous parameters are closely associated with disease stability: a typical CLL morphology and immunophenotype, the lack of expression of the CD38 antigen, the mutated immunoglobulin (Ig) heavy (H) chain variable (V) pattern, the absence of p53 mutations, a CD4/CD8 ratio more than 1, the lack of 17p and 11q deletions and of complex karyotypic aberrations, and the occurrence of the 13q14 deletion. No case displayed the VH3-21 gene, linked in mutated CLL with a poor outcome. The VH1-69 gene associated with unmutated CLL cases was never detected. These biologic features were coupled with an indolent clinical course characterized by an unmodified clinical stage over time, and by lack of autoimmune phenomena and of major infections requiring parental antibiotics. At a time when aggressive therapeutic strategies are always more frequently used in the management of CLL, the distinctive features of patients with long-lived stable disease should be prospectively identified at presentation.


http://www.bloodjournal.org/cgi/content/abstract/103/6/2316

A high incidence of somatically acquired point mutations in the AML1/RUNX1 gene has been reported in poorly differentiated acute myeloid leukemia (AML, M0) and in radiation-associated and therapy-related myelodysplastic syndrome (MDS) or AML. The vast majority of AML1 mutations identified in these diseases were localized in the amino (N)-terminal region, especially in the DNA-binding Runt homology domain. In this report, we show that AML1 point mutations
were found in 26 (23.6%) of 110 patients with refractory anemia with excess blasts (RAEB), RAEB in transformation (RAEBt), and AML following MDS (defined these 3 disease categories as MDS/AML). Among them, 9 (8.2%) mutations occurred in the carboxy (C)-terminal region, which were exclusively found in MDS/AML and were strongly correlated with sporadic MDS/AML. All patients with MDS/AML with an AML1 mutation expressed wild-type AML1 protein and had a significantly worse prognosis than those without AML1 mutations. Most AML1 mutants lost trans-activation potential, regardless of their DNA binding potential. These data suggested that AML1 point mutation is one of the major driving forces of MDS/AML, and these mutations may represent a distinct clinicopathologic-genetic entity.


http://www.bloodjournal.org/cgi/content/abstract/101/2/673

Somatically acquired point mutations of AML1/RUNX1 gene have been recently identified in rare cases of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Moreover, germ line mutations of AML1 were found in an autosomal dominant disease, familial platelet disorder with predisposition to AML (FPD/AML), suggesting that AML1 mutants, as well as AML1 chimeras, contribute to the transformation of hematopoietic progenitors. In this report, we showed that AML1 point mutations were found in 6 (46%) of 13 MDS patients among atomic bomb (A-bomb) survivors in Hiroshima. Unlike acute or chronic leukemia patients among A-bomb survivors, MDS patients exposed relatively low-dose radiation and developed the disease after a long latency period. AML1 mutations also were found in 5 (38%) of 13 therapy-related AML/MDS patients who were treated with alkylating agents with or without local radiation therapy. In contrast, frequency of AML1 mutation in sporadic MDS patients was 2.7% (2 of 74). Among AML1 mutations identified in this study, truncated-type mutants lost DNA binding potential and trans-activation activity. All missense mutations with one exception (Gly42Arg) lacked DNA binding ability and down-regulated the trans-activation potential of wild-type AML1 in a dominant-negative fashion. The Gly42Arg mutation that was shared by 2 patients bound DNA even more avidly than wild-type AML1 and enhanced the trans-activation potential of normal AML1. These results suggest that AML1 point mutations are related to low-dose radiation or alkylating agents and play a role distinct from that of leukemogenic chimeras as a result of chromosomal translocations caused by sublethal radiation or topoisomerase II inhibitors.


http://www.bloodjournal.org/cgi/content/abstract/101/9/3509

Leukocytes are classified as myelocytic or lymphocytic, and each class of leukocytes consists of several types of cells that have different phenotypes and different roles. To define the gene expression in these cells, we have performed serial analysis of gene expression (SAGE) using human leukocytes and have provided the gene database for these cells not only at the resting stage but also at the activated stage. A total of 709 990 tags from 17 libraries were analyzed for the manifestation of gene expression profiles in various types of human leukocytes. Types of leukocytes analyzed were as follows: peripheral blood monocytes, colony-stimulating factor-induced macrophages, monocyte-derived immature dendritic cells, mature/activated dendritic cells, granulocytes, natural killer (NK) cells, resting B cells, activated B cells, naive T cells, CCR4[+/-] memory T cells (resting TH1 cells), CCR4+ memory T cells (resting TH2 cells), activated TH1 cells, and activated TH2 cells. Among 38 961 distinct tags that appeared more than once in
the combined total libraries, 27,323 tags were found to represent unique genes in certain type(s) of leukocytes. Using probability (P) and hierarchical clustering analysis, we identified the genes selectively expressed in each type of leukocytes. Identification of the genes specifically expressed in different types of leukocytes provides not only a novel molecular signature to define different subsets of resting and activated cells but also contributes to further understanding of the biologic function of leukocytes in the host defense system.


http://www.bloodjournal.org/cgi/content/abstract/100/5/1845

The p47-phox gene, NCF-1, has 2 nearly identical pseudogenes ([psi]NCF-1) in proximity at chromosomal locus 7q11.23. A dinucleotide deletion ([Delta]GT) at the beginning of exon 2 that leads to a frameshift and premature stop codon is considered the signature sequence of the pseudogenes. It is also the most prevalent mutation in p47-phox-deficient (A47{degrees}) chronic granulomatous disease (CGD) as a result of the insertion of a [Delta]GT-containing fragment of pseudogene into NCF-1. Extending our study of the relationship between NCF-1 and [psi] NCF-1 to 53 unaffected control individuals, we found that although in most (n = 44), the ratio of pseudogene ([Delta]GT) to functional gene (GTGT) sequence in amplicons spanning exon 2 was 2:1, as previously observed, surprisingly, in 7 persons the ratio was 1:1, and in 2 persons the ratio was 1:2. The lowered ratios are explained by the presence, in a heterozygous or homozygous state, respectively, of a pseudogene that contains GTGT rather than [Delta]GT. It is possible that this pseudogene has not undergone deletion of GT, but more likely, based on analysis of additional NCF-1/[psi]NCF-1 markers, it represents the previously unidentified product of the reciprocal crossover of DNA fragments between the functional gene and one of its pseudogenes. The mutated NCF-1 resulting from this event is the predominant A47{degrees}CGD allele. The existence of 2 extended haplotypes encompassing NCF-1/[psi]NCF-1 further complicates the detection of A47{degrees}CGD carriers. Although most have a [Delta]GT/GTGT ratio of 5:1, some have a ratio of 2:1 and are indistinguishable by this means from unaffected individuals.


http://www.bloodjournal.org/cgi/content/abstract/101/1/363

A major end point of nonmyeloablative hematopoietic stem cell transplantation is the attainment of either mixed chimerism or full donor hematopoiesis. Because the majority of human genetic disparity is generated by single nucleotide polymorphisms (SNPs), direct measurement of SNPs should provide a robust tool for the detection and quantitation of chimerism. Using pyrosequencing, a rapid quantitative sequencing technology, we developed a SNP-based assay for hematopoietic chimerism. Based on 14 SNPs with high allele frequencies, we were able to identify at least 1 informative SNP locus in 55 patients with HLA-identical donors. The median number of informative SNPs in related pairs was 5 and in unrelated pairs was 8 (P <.0001). Assessment of hematopoietic chimerism in posttransplantation DNA was shown to be quantitative, accurate, and highly reproducible. The presence of 5% donor cells was reliably detected in replicate assays. Compared with current measures of engraftment based on identification of short tandem repeats (STRs), variable number of tandem repeats (VNTRs), or microsatellite polymorphisms, this SNP-based method provides a more rapid and quantitative
assessment of chimerism. A large panel of SNPs enhances the ability to identify an informative marker in almost all patient/donor pairs and also facilitates the simultaneous use of multiple markers to improve the statistical validity of chimerism measurements. The inclusion of SNPs that encode minor histocompatibility antigens or other genetic polymorphisms that may influence graft-versus-host disease or other transplantation outcomes can provide additional clinically relevant data. SNP-based assessment of chimerism is a promising technique that will assist in the analysis of outcomes following transplantation.


http://www.bloodjournal.org/cgi/content/abstract/99/12/4547

Deletions of the derivative chromosome 9 have recently been reported in chronic myeloid leukemia. These deletions are large, occur at the time of the Philadelphia (Ph) translocation, span the translocation breakpoint, and represent a powerful prognostic indicator. However, the molecular mechanisms responsible for the poor prognosis associated with deletions are obscure, and several possible models are investigated here. First, we demonstrate that all derivative chromosome 9 deletions detected by fluorescence in situ hybridization were associated with an absence of ABL-BCR expression. However, loss of ABL-BCR expression also occurred without an overt deletion, suggesting the existence of other mechanisms by which ABL-BCR transcription can be abolished. Furthermore, analysis of survival in 160 patients demonstrated that loss of ABL-BCR expression, in contrast to deletion status, was not an indicator of poor prognosis. Second, we addressed the possibility that concomitant small deletions of the Ph chromosome modulate BCR-ABL transcription. Real-time reverse-transcription polymerase chain reaction was used to demonstrate that derivative chromosome 9 deletions were not accompanied by altered levels of BCR-ABL transcripts. Third, deletions may represent a consequence of genetic instability within the target cell at the time of the Ph translocation, with the poor prognosis reflecting a predisposition to subsequent additional genetic alterations. However, patients with deletions do not exhibit an increased frequency of secondary cytogenetic changes following disease progression. Taken together, these data support a model in which deletions of the derivative chromosome 9 result in rapid disease progression as a result of the loss of one or more genes within the deleted region.


http://www.bloodjournal.org/cgi/content/abstract/2004-12-4931v1

Multiple myeloma is a malignancy of plasma cells. Vaccine immunotherapy is among the novel therapeutic strategies under investigation for this disease. To identify myeloma-associated antigens as potential targets for vaccine immunotherapy, we surveyed a comprehensive panel of bone marrow specimens from patients with Monoclonal Gammopathy of Undetermined Significance (MGUS) and multiple myeloma for expression of Cancer-Testis (CT) antigens. Immunohistochemistry (IHC) demonstrated that 82% of stage III myeloma specimens expressed the CT antigen CT7 (also known as MAGE-C1), and 70% expressed MAGE-A3/6. Messenger RNA for CT7 and MAGE-A family members was detected in 87% and 100% of stage III samples, respectively. CT7 protein expression increased with advanced stage of disease. Higher levels of CT7 and MAGE-A3/6 proteins also correlated with elevated plasma cell proliferation. These results show that CT7 and MAGE-A3/6 are promising myeloma-associated antigens for
application in vaccine immunotherapy. Furthermore, the common expression and correlation with proliferation suggest a possible pathogenic role for these proteins in myeloma.


http://www.bloodjournal.org/cgi/content/abstract/2004-11-4458v1

Raf kinases play an integral role in the classic MAP kinase (Raf/MERK/ERK) intracellular signaling cascade, but their role in specific developmental processes is largely unknown. Using a genetic approach, we have identified a role for B-Raf during hematopoietic progenitor cell development and during megakaryocytopoiesis. Fetal liver and in vitro ES cell-derived myeloid progenitor development is quantitatively impaired in the absence of B-Raf. Biochemical data suggest that this phenotype is due to the loss of a normally occurring rise in B-Raf expression and associated ERK1/2 activation during hematopoietic progenitor cell formation. However, the presence of B-raf -/- ES cell-derived myeloid progenitors in the bone marrow of adult chimeric mice indicates the lack of an obligate cell-autonomous requirement for B-Raf in myeloid progenitor development. The lack of B-Raf also impairs megakaryocytopoiesis. Tpo-induced in vitro expansion of ES cell-derived megakaryocyte lineage cells fails to occur in the absence of B-Raf. Moreover, this quantitative in vitro defect in megakaryocyte lineage expansion is mirrored by chimeric mice data that show reduced B-raf -/- genotype contribution in megakaryocytes relative to its contribution in myeloid progenitors. Together, these data suggest that B-Raf plays a cell-autonomous role in megakaryocytopoiesis and a permissive role in myeloid progenitor development.


http://www.bloodjournal.org/cgi/content/abstract/104/8/2217

Tetraspanins are thought to facilitate the formation of multiprotein complexes at cell surfaces, but evidence illuminating the biologic importance of this role is sparse. Tetraspanin CD151 forms very stable laminin-binding complexes with integrins {alpha}3{beta}1 and {alpha}6{beta}1 in kidney and {alpha}3{beta}4 and {alpha}6{beta}4 in skin. It is encoded by a gene at the same position on chromosome 11p15.5 as the MER2 blood group gene. We show that CD151 expresses the MER2 blood group antigen and is located on erythrocytes. We examined CD151 in 3 MER2-negative patients (2 are sibs) of Indian Jewish origin with end-stage kidney disease. In addition to hereditary nephritis the sibs have sensorineural deafness, pretibial epidermolysis bullosa, and {beta}-thalassemia minor. The 3 patients are homozygous for a single nucleotide insertion (G383) in exon 5 of CD151, causing a frameshift and premature stop signal at codon 140. The resultant truncated protein would lack its integrin-binding domain. We conclude that CD151 is essential for the proper assembly of the glomerular and tubular basement membrane in kidney, has functional significance in the skin, is probably a component of the inner ear, and could play a role in erythropoiesis.

The NOD-LtSZ scid/scid (NOD/SCID) repopulation assay is the criterion for the study of self-renewal and multilineage differentiation of human hematopoietic stem cells. An important shortcoming of this model is the reported absence of T-cell development. We studied this aspect of the model and investigated how it could be optimized to support T-cell development. Occasionally, low-grade thymic engraftment was observed in NOD/SCID mice or Rag2[-/-]γc[-/-] mice. In contrast, the treatment of NOD/SCID mice with a monoclonal antibody against the murine interleukin-2Rβ (IL-2Rβ), (IL-2R[beta]) known to decrease natural killer cell activity, resulted in human thymopoiesis in up to 60% of the mice. T-cell development was phenotypically normal and resulted in polyclonal, mature, and functional CD1[-] TCRαβ+ CD4+ or CD8+ single-positive T cells. In mice with ongoing thymopoiesis, peripheral T cells were observed. TREC analysis showed that T cells with a naive phenotype (CD45RA+) emerged from the thymus. In approximately half of these mice, the peripheral T cells included a pauciclonal outgrowth of CD45RO+ cells. These data suggest that all elements of a functional immune system were present in these animals.


The novel immunosuppressant FTY720 activates sphingosine 1-phosphate receptors (S1PRs) that affect responsiveness of lymphocytes to chemokines such as stromal cell-derived factor 1 (SDF-1), resulting in increased lymphocyte homing to secondary lymphoid organs. Since SDF-1 and its receptor CXCR4 are also involved in bone marrow (BM) homing of hematopoietic stem and progenitor cells (HPCs), we analyzed expression of S1PRs and the influence of FTY720 on SDF-1/CXCR4-mediated effects in human HPCs. By reverse transcriptase-polymerase chain reaction (RT-PCR), S1PRs were expressed in mobilized CD34+ HPCs, particularly in primitive CD34+/CD38- cells. Incubation of HPCs with FTY720 resulted in prolonged SDF-1-induced calcium mobilization and actin polymerization, and substantially increased SDF-1-dependent in vitro transendothelial migration, without affecting VLA-4, VLA-5, and CXCR4 expression. In nonobese diabetic-severe combined immunodeficient (NOD/SCID) mice, the number of CD34+/CD38- cells that homed to the BM after 18 hours was significantly raised by pretreatment of animals and cells with FTY720, tending to result in improved engraftment. In addition, in vitro growth of HPCs (week-5 cobblestone area-forming cells [CAFCs]) was 2.4-fold increased. We conclude that activation of S1PRs by FTY720 increases CXCR4 function in HPCs both in vitro and in vivo, supporting homing and proliferation of HPCs. In the hematopoietic microenvironment, S1PRs are involved in migration and maintenance of HPCs by modulating the effects of SDF-1.


Thrombopoietin (TPO), the primary regulator of thrombopoiesis, is also an important, non-redundant mediator of hematopoietic stem cell (HSC) development. For example, following transplantation, HSC expansion is approximately 15-fold more robust in normal than in Tpo-/- mice. Vascular endothelial growth factor (VEGF) also plays an important role in HSC
development, where it acts in an intracellular autocrine fashion to promote cell survival. Thus, we tested the hypothesis that TPO affects the autocrine production of VEGF to account for its favorable effects on HSCs. We found that VEGF transcripts are reduced in purified sca-1+/c-kit+/Gr-1- marrow cells derived from Tpo-/- mice, and that TPO induces VEGF transcripts in these primitive hematopoietic cells. Additional studies determined that TPO induces VEGF expression by increasing the level of its primary transcription factor, HIF-1α, by enhancing its stability. Moreover, VEGF expression was important for the TPO effect on primitive hematopoietic cells, as blockade of the VEGF receptor with a specific inhibitor substantially blunted TPO induced growth of single sca-1+/c-kit+/Gr-1- marrow cells in serum-free cultures. Along with previous findings that TPO affects Hox transcription factors that regulate HSC proliferation, these data contribute to our growing understanding of the mechanisms by which a hormone can influence stem cell development.


http://www.bloodjournal.org/cgi/content/abstract/99/7/2586

Graft-versus-host disease (GVHD), a major complication after allogeneic transplantation, can be abrogated by the Campath (anti-CD52) monoclonal antibody. The induction of acute GVHD requires host antigens to be presented to donor T cells by antigen-presenting cells (APCs). Recent evidence has suggested that only host APCs can interact with donor T cells in the induction of GVHD. Because CD52 has been reported to be expressed on DCs, we reasoned that pretransplant Campath-1G might have a direct effect on circulating DCs in addition to any effects on donor T cells. Using direct immunostaining, we demonstrated expression of CD52 on DCs and that Campath-1G killed purified DCs in vitro. In vivo Campath also depleted DCs. Twenty-four hours after the first dose of Campath-1G, circulating DCs were reduced by a mean of 79% (range, 44%-96%). By day 0 after 5 doses of Campath-1G and chemoradiotherapy conditioning, DCs became undetectable in 7 of 9 cases, whereas in 6 of 7 patients receiving conditioning therapy without Campath-1G, host DCs were still detectable. The reconstitution of circulating DCs after transplantation was not affected by Campath-1G and in both groups DC1 (CD11c+) recovered more rapidly than DC2 (CD11c-). Analysis of chimerism confirmed that the DCs recovering after transplantation in patients receiving Campath-1G were of donor origin. We conclude that in vivo Campath-1G causes a rapid depletion of host circulating DCs and that this may, in part, explain the low incidence of acute GVHD. The reconstitution of donor DCs was not delayed, which may be important in preserving immune reconstitution.


http://www.bloodjournal.org/cgi/content/abstract/102/9/3136

Erythroid progenitors undergo renewal (proliferation without apparent differentiation) in response to erythropoietin (Epo), stem cell factor (SCF), and glucocorticoids (dexamethasone) (Dex). SCF and Dex cooperate with Epo to promote proliferation and inhibit differentiation of erythroid progenitors, while Epo alone is required to protect erythroid cells from apoptosis during terminal red cell maturation. To examine the mechanism of the synergistic interactions of Epo, SCF, and Dex, we analyzed gene expression patterns using DNA chip-based large-scale comparative gene profiling using microarrays enriched in hematopoietic transcripts or containing randomly selected genes. Differentially regulated genes were validated by real-time reverse transcription-
polymerase chain reaction (RT-PCR). The results reveal cooperative regulation of gene expression by glucocorticoids and Epo/SCF on a number of genes, such as CIS, BTG1, VDUP1, CXCR4, GILZ, and RIKEN29300106B05. While Epo and SCF never showed opposite effects on gene expression, Dex either enhanced or attenuated the effect of Epo and/or SCF. Several glucocorticoid receptor (GR)-target genes were regulated by Dex only in the presence of Epo and/or SCF, suggesting that the GR functions in the context of a larger transactivation complex to regulate these genes. The data also suggest that modulation of cytokine-induced signals by the GR is an important mechanism in erythroid progenitor renewal.


http://www.bloodjournal.org/cgi/content/abstract/101/9/3635

TEL/AML1-positive childhood acute lymphoblastic leukemias (ALLs) generally have low-risk features, but still about 20% of patients relapse. Our initial molecular genetic analyses in 2 off-treatment relapses suggested that the initial and relapse clones represent different subclones that evolved from a common TEL/AML1-positive, treatment-resistant precursor. In order to further elaborate on this hypothesis, we studied 2 patients with late systemic relapses of their TEL/AML1-positive ALL (41 months and 49 months after initial diagnosis, respectively) who had distinct clonal antigen receptor gene rearrangements at diagnosis and relapse. These clone-specific markers enabled us to determine the responsiveness of the individual clones to treatment. The matching genomic TEL/AML1 breakpoints of the initial and the relapse clones in these patients confirmed their origin from a common progenitor cell. This proof was especially important in one of these 2 leukemias without a common antigen receptor gene rearrangement. Our retrospective analysis revealed that in both cases the relapse clone was already present at diagnosis. Despite their small sizes (5 x 10\(^{-3}\) and 1 x 10\(^{-4}\), respectively), we were able to detect their much slower responses to therapy compared with the dominant leukemic clone. Moreover, in all instances, these initially slow-responding clones, after they had developed into the relapse leukemia, were rapidly eradicated by the relapse treatment, underlining their different biology at the 2 time points of leukemia manifestation. We thus hypothesize that the minor clone was not fully malignant at initial diagnosis but acquired further mutations that may be necessary for the manifestation of relapse.


http://www.bloodjournal.org/cgi/content/abstract/103/11/4317

Juvenile or type 2 hemochromatosis (JH) is transmitted as a recessive trait that leads to severe iron overload and organ damage typically before age 30 years. Linkage to a locus on chromosome 1q has been found in most patients with JH. The recently identified causal gene encodes hemojuvelin, a protein with a proposed crucial role in iron metabolism. A second, rare type of JH, with clinical expression identical to the 1q-linked form, is due to inactivation of hepcidin, the key regulator of iron homeostasis. Here we report the spectrum of mutations of the hemojuvelin gene (HJV) in 34 patients who did not show hepcidin mutations. This represents the largest cohort of patients with JH collected worldwide. We identified 17 different (16 novel) mutations of HJV, both at the homozygous and at the compound heterozygous state. Mutations either generate premature termination codons or were missense substitutions, affecting highly conserved residues, relevant to the protein structure and/or function. (Blood. 2004;103:4317-4321)

http://www.bloodjournal.org/cgi/content/abstract/102/6/2198

MLL rearrangements in acute myeloid leukemia (AML) include translocations and intragenic abnormalities such as internal duplication and breakage induced by topoisomerase II inhibitors. In adult AML, FLT3 internal tandem duplications (ITDs) are more common in cases with MLL intragenic abnormalities (33%) than those with MLL translocation (8%). Mutation/deletion involving FLT3 D835 are found in more than 20% of cases with MLL intragenic abnormalities compared with 10% of AML with MLL translocation and 5% of adult AML with normal MLL status. Real-time quantification of FLT3 in 141 cases of AML showed that all cases with FLT3 D835 express high level transcripts, whereas FLT3-ITD AML can be divided into cases with high-level FLT3 expression, which belong essentially to the monocytic lineage, and those with relatively low-level expression, which predominantly demonstrate PML-RARA and DEK-CAN. FLT3 abnormalities in CBF leukemias with AML1-ETO or CBF(beta)-MYH11 were virtually restricted to cases with variant CBF(beta)-MYH11 fusion transcripts and/or atypical morphology. These data suggest that the FLT3 and MLL loci demonstrate similar susceptibility to agents that modify chromatin configuration, including topoisomerase II inhibitors and abnormalities involving PML and DEK, with consequent errors in DNA repair. Variant CBF(beta)-MYH11 fusions and bcr3 PML-RARA may also be initiated by similar mechanisms.


http://www.bloodjournal.org/cgi/content/abstract/103/6/2325

The PTPN11 gene encodes SHP-2 (Src homology 2 domain-containing protein tyrosine Phosphatase), a nonreceptor tyrosine protein tyrosine phosphatase (PTPase) that relays signals from activated growth factor receptors to p21Ras (Ras) and other signaling molecules. Mutations in PTPN11 cause Noonan syndrome (NS), a developmental disorder characterized by cardiac and skeletal defects. NS is also associated with a spectrum of hematologic disorders, including juvenile myelomonocytic leukemia (JMML). To test the hypothesis that PTPN11 mutations might contribute to myeloid leukemogenesis, we screened the entire coding region for mutations in 51 JMML specimens and in selected exons from 60 patients with other myeloid malignancies. Missense mutations in PTPN11 were detected in 16 of 49 JMML specimens from patients without NS, but they were less common in other myeloid malignancies. RAS, NF1, and PTPN11 mutations are largely mutually exclusive in JMML, which suggests that mutant SHP-2 proteins deregulate myeloid growth through Ras. However, although Ba/F3 cells engineered to express leukemia-associated SHP-2 proteins cells showed enhanced growth factor-independent survival, biochemical analysis failed to demonstrate hyperactivation of the Ras effectors extracellular-regulated kinase (ERK) or Akt. We conclude that SHP-2 is an important cellular PTPase that is mutated in myeloid malignancies. Further investigation is required to clarify how these mutant proteins interact with Ras and other effectors to deregulate myeloid growth.


http://www.bloodjournal.org/cgi/content/abstract/101/2/433
We have cloned and characterized a novel human gene, HGAL (human germinal center-associated lymphoma), which predicts outcome in patients with diffuse large B-cell lymphoma (DLBCL). The HGAL gene comprises 6 exons and encodes a cytoplasmic protein of 178 amino acids that contains an immunoreceptor tyrosine-based activation motif (ITAM). It is highly expressed in germinal center (GC) lymphocytes and GC-derived lymphomas and is homologous to the mouse GC-specific gene M17. Expression of the HGAL gene is specifically induced in B cells by interleukin-4 (IL-4). Patients with DLBCL expressing high levels of HGAL mRNA demonstrate significantly longer overall survival than do patients with low HGAL expression. This association was independent of the clinical international prognostic index. High HGAL mRNA expression should be used as a prognostic factor in DLBCL.


http://www.bloodjournal.org/cgi/content/abstract/99/4/1398

Two recurrent translocations have been associated with mucosa-associated lymphoid tissue (MALT)-type lymphoma, t(11;18)(q21;q21) and t(1;14)(p22;q32). The first, t(11;18)(q21;q21), results in the fusion protein API2-MLT (API2-MALT1). Through t(1;14)(p22;q32), the BCL10 gene is entirely transferred to the IgH gene, resulting in its overexpression. Wild-type BCL10 is implicated in apoptosis, and it has been suggested that mutated forms gain oncogenic activity. The occurrence of genomic BCL10 mutations in 35 gastric MALT-type lymphomas with or without t(11;18)(q21;q21) (10 and 25 cases, respectively) was investigated. DNA extracted from either whole tissue sections or microdissected clusters of tumor cells was used. Five polymerase chain reactions amplifying the coding exons were performed and were followed by direct sequencing of the products. Twenty differences with the published BCL10 sequence, all single nucleotide substitutions, were detected in 16 cases. Of these, 12 represented known polymorphisms, either at codon 8, 213, or 5. Of the remaining 8 substitutions, 2 were silent and 6 resulted in amino acid substitutions. Mutation analysis results were correlated with the BCL10 expression pattern. Aberrant nuclear BCL10 expression was detected in 14 cases. No association could be demonstrated between the latter and the presence of BCL10 mutations. In contrast, all 10 cases carrying t(11;18)(q21;q21) showed nuclear expression, whereas this staining pattern was absent in 21 of 25 cases without t(11;18)(q21;q21). These results demonstrate that BCL10 mutations are rare in gastric MALT-type lymphoma and are not related to the aberrant nuclear expression of BCL10. In contrast, they indicate that the presence of the API2-MLT fusion protein is associated with aberrant nuclear BCL10 expression.


http://www.bloodjournal.org/cgi/content/abstract/101/12/4998

Although multiple myeloma (MM) is a unique entity, a marked heterogeneity is actually observed among the patients, which has been first related to immunoglobulin (Ig) types and light chain subtypes and more recently to chromosomal abnormalities. To further investigate this genetic heterogeneity, we analyzed gene expression profiles of 92 primary tumors according to their Ig types and light chain subtypes with DNA microarrays. Several clusters of genes involved in various biologic functions such as immune response, cell cycle control, signaling, apoptosis, cell adhesion, and structure significantly discriminated IgA- from IgG-MM. Genes associated with inhibition of differentiation and apoptosis induction were up-regulated while genes associated with immune response, cell cycle control, and apoptosis were down-regulated in IgA-MM. According to
the expression of the 61 most discriminating genes, BJ-MM represented a separate subgroup that did not express either the genes characteristic of IgG-MM or those of IgA-MM at a high level. This suggests that transcriptional programs associated to the switch could be maintained up to plasma cell differentiation. Several genes whose products are known to stimulate bone remodeling discriminate between {kappa}- and {lambda}-MM. One of these genes, Mip-1{alpha}, was overexpressed in the {kappa} subgroup. In addition, we established a strong association (P = .0001) between {kappa} subgroup expressing high levels of Mip-1{alpha} and active myeloma bone disease. This study shows that DNA microarrays enable us to perform a molecular dissection of the bioclinical diversity of MM and provide new molecular tools to investigate the pathogenesis of malignant plasma cells.


http://www.bloodjournal.org/cgi/content/abstract/105/7/2802

Human immunodeficiency virus-1 (HIV-1) Tat, a nuclear transactivator of viral gene expression, has the unusual property of being released by infected cells. Recent studies suggest that extracellular Tat is partially sequestered by heparan sulfate proteoglycans. As a consequence, Tat is concentrated on the cell surface and protected from proteolytic degradation, thus remaining in a biologically active form. We show that Tat binds the surfaces of both HIV-1-infected and surrounding uninfected cells. We provide evidence for a specific interaction between Tat and the HIV-1 glycoprotein 120 (gp120) envelope protein, which enhances virus attachment and entry into cells. We map the interacting sites of both Tat and gp120 and show that synthetic peptides mimicking the gp120 site inhibit HIV-1 infection. Our data demonstrate that membrane-associated Tat is a novel modulator of virus entry and suggest that the Tat-gp120 interaction represents a critical step in HIV-1 spreading during the course of infection.


http://www.bloodjournal.org/cgi/content/abstract/100/2/383

We describe a new B220+ subpopulation of immaturelike dendritic cells (B220+ DCs) with low levels of expression of major histocompatibility complex (MHC) and costimulatory molecules and markedly reduced T-cell stimulatory potential, located in the thymus, bone marrow, spleen, and lymph nodes. B220+ DCs display ultrastructural characteristics resembling those of human plasmacytoid cells and accordingly produce interferon-[alpha] after virus stimulation. B220+ DCs acquired a strong antigen-presenting cell capacity on incubation with CpG oligodeoxynucleotides, concomitant with a remarkable up-regulation of MHC and costimulatory molecules and the production of interleukin-12 (IL-12) and IL-10. Importantly, our data suggest that nonstimulated B220+ DCs represent a subset of physiological tolerogenic DCs endowed with the capacity to induce a nonanergic state of T-cell unresponsiveness, involving the differentiation of T regulatory cells capable of suppressing antigen-specific T-cell proliferation. In conclusion, our data support the hypothesis that B220+ DCs represent a lymphoid organ subset of immature DCs with a dual role in the immune system[---]exerting a tolerogenic function in steady state but differentiating on microbial stimulation into potent antigen-presenting cells with type 1 interferon production capacity.

http://www.bloodjournal.org/cgi/content/abstract/99/4/1282

Despite the information dealing with the differential phenotype and function of the main mouse dendritic cell (DC) subpopulations, namely, CD8[alpha][+] and CD8[alpha][+] DCs, their origin and involvement in antiviral immune responses in vivo are still largely unknown. To address these issues, this study used the changes occurring in DC subpopulations during the experimental infection by the Swiss (SW) strain of the mouse mammary tumor virus (MMTV). MMTV(SW) induced an 18-fold increase in lymph node DCs, which can be blocked by anti-CD62L treatment, concomitant with the presence of high numbers of DCs in the outer cortex, in close association with high endothelial venules. These data suggest that the DC increase caused by MMTV(SW) infection results from the recruitment of blood-borne DCs via high endothelial venules, by a CD62L-dependent mechanism. In addition, skin sensitization assays indicate that MMTV(SW) infection inhibits epidermal Langerhans cell migration to the draining lymph node. Moreover, data on the kinetics of MMTV(SW)-induced expansion of the different DC subsets support the hypothesis that CD8[+] and CD8[+] DCs represent different maturation stages of the same DC population, rather than myeloid- and lymphoid-derived DCs, respectively, as previously proposed. Finally, the fact that DCs were infected by MMTV(SW) suggests their participation in the early phases of infection.


http://www.bloodjournal.org/cgi/content/abstract/100/3/774

We describe data on a 7-year-old girl with congenital dyserythropoietic anemia (CDA), who also had familial Mediterranean fever (FMF). Repeated transfusions required since the age of 6 months to treat her CDA led to iron overload and a persistently high ferritin level. Her relapsing FMF made effective iron chelation therapy very difficult. Consequently, at the age of 4 years, she underwent allogeneic, sibling bone marrow transplantation (BMT). During conditioning for her BMT, symptoms of FMF, including splenomegaly, arthritis, and recurrent abdominal pain, began to resolve and she was gradually weaned off colchicine. Now, 2 years after the transplantation, she remains free from FMF symptomatology and is off all immunosuppressants. This case demonstrates that symptoms of FMF can be alleviated by the therapy used during allogeneic BMT. In this patient it is likely that the missing factor in FMF is now being provided by granulocytes derived from the stem cells within transplanted bone marrow.

Mirandola, P., C. Ponti, et al. (2004). "Activated human NK and CD8+ T cells express both TNF-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors but are resistant to TRAIL-mediated cytotoxicity." Blood 104(8): 2418-2424.

http://www.bloodjournal.org/cgi/content/abstract/104/8/2418

The expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and TRAIL receptors was investigated in resting and cytokine-activated purified primary human natural killer (NK) and CD8+ T cells. Resting NK and CD8+ T cells expressed the mRNA for all
TRAIL receptors, but TRAIL-R4 was the only receptor clearly detectable on the surface of both cell types. NK cells were activated by interleukin 2 (IL-2) or IL-15, whereas CD8+ T cells were activated by phytohemagglutinin (PHA) + IL-2 followed by IL-2 alone for up to 10 days. On activation, both cell types rapidly expressed TRAIL-R2 and TRAIL-R3, whose expression peaked at day 10 of culture. TRAIL-R1, however, was never expressed at any time point examined, whereas the expression of TRAIL-R4, which showed a progressive increase in CD8+ T cells, remained constant in NK cells. Notwithstanding the expression of TRAIL-R2, recombinant TRAIL did not show any cytotoxic activity on either NK or CD8+ T cells. Both resting and activated NK and CD8+ T cells were found to express high levels of the 2 isoforms of c-FLIP (cellular Fas-associated death domain protein [FADD]-like IL-1-converting enzyme [FLICE]-inhibitory protein). Small interference RNA-mediated inhibition of c-FLIP expression in NK cells abrogated their resistance to the apoptotic effect of soluble TRAIL. Thus, once activated the major cytotoxic effector cells are potentially sensitive to TRAIL but are physiologically protected from its apoptotic action by intracellular level of c-FLIP.


The recently published crystal structure of the external domains of [alpha]V[beta]3 confirms the prediction that the aminoterminal portion of [alpha]V, which shares 40% homology with [alpha]IIb, folds into a [beta]-propeller structure and that the 4 calcium-binding domains are positioned on the bottom of the propeller. To gain insight into the role of the calcium-binding domains in [alpha]IIb biogenesis, we characterized mutations in the second and third calcium-binding domains of [alpha]IIb in 2 patients with Glanzmann thrombasthenia. One patient inherited a Val298Phe mutation in the second domain, and the other patient inherited an Ile374Thr mutation in the third domain. Mammalian cell expression studies were performed with normal and mutant [alpha]IIb and [beta]3 cDNA constructs. By flow cytometry, expression of [alpha]IIb Val298Phe/[beta]3 in transfected cells was 28% of control, and expression of [alpha]IIbIle374Thr/[beta]3 was 11% of control. Pulse-chase analyses showed that both mutant pro-[alpha]IIb subunits are retained in the endoplasmic reticulum and degraded. Mutagenesis studies of the Val298 and Ile374 residues showed that these highly conserved, branch-chained hydrophobic residues are essential at these positions and that biogenesis and expression of [alpha]IIb[beta]3 is dramatically affected by structural variations in these regions of the calcium-binding domains. Energy calculations derived from a new model of the [alpha]IIb [beta]-propeller indicate that these mutations interfere with calcium binding. These data suggest that the [alpha]IIb calcium-binding domains play a key structural role in the [beta]-propeller, and that the structural integrity of the calcium-binding domains is critical for integrin biogenesis.


DNA damage activates the monoubiquitination of the Fanconi anemia (FA) protein, FANCD2, resulting in the assembly of FANCD2 nuclear foci. In the current study, we characterize structural features of FANCD2 required for this intranuclear translocation. We have previously identified 2 normal mRNA splice variants of FANCD2, one containing exon 44 sequence at the 3' end (FANCD2-44) and one containing exon 43 sequence (FANCD2-43). The 2 predicted FANCD2 proteins differ in their carboxy terminal 24 amino acids. In stably transfected FANCD2-/-- fibroblasts, FANCD2-44 and FANCD2-43 proteins were monoubiquitinated on K561. Only
FANCD2-44 corrected the mitomycin C (MMC) sensitivity of the transfected cells. We find that monoubiquitinated FANCD2-44 was translocated from the soluble nuclear compartment into chromatin. A mutant form of FANCD2-44 (FANCD2-K561R) was not monoubiquitinated and failed to bind chromatin. A truncated FANCD2 protein (Exon44-T), lacking the carboxy terminal 24 amino acids encoded by exon 44 but retaining K561, and another mutant FANCD2 protein, with a single amino acid substitution at a conserved residue within the C-terminal 24 amino acids (D1428A), were monoubiquitinated. Both mutants were targeted to chromatin but failed to correct MMC sensitivity. Taken together, our results indicate that monoubiquitination of FANCD2 regulates chromatin binding and that D1428 within the carboxy terminal acidic sequence encoded by exon 44 is independently required for functional complementation of FA-D2 cells. We hypothesize that the carboxy terminus of FANCD2-44 plays a critical role in sensing or repairing DNA damage.


http://www.bloodjournal.org/cgi/content/abstract/101/7/2833

Paroxysmal nocturnal hemoglobinuria (PNH) may arise during long-term follow-up of aplastic anemia (AA), and many AA patients have minor glycosylphosphatidylinositol (GPI) anchor-deficient clones, even at presentation. PIG-A gene mutations in AA/PNH and hemolytic PNH are thought to be similar, but studies on AA/PNH have been limited to individual cases and a few small series. We have studied a large series of AA patients with a GPI anchor-deficient clone (AA/PNH), including patients with minor clones, to determine whether their pattern of PIG-A mutations was identical to the reported spectrum in hemolytic PNH. AA patients with GPI anchor-deficient clones were identified by flow cytometry and minor clones were enriched by immunomagnetic selection. A variety of methods was used to analyze PIG-A mutations, and 57 mutations were identified in 40 patients. The majority were similar to those commonly reported, but insertions in the range of 30 to 88 bp, due to tandem duplication of PIG-A sequences, and deletions of more than 10 bp were also seen. In 3 patients we identified identical 5-bp deletions by conventional methods. This prompted the design of mutation-specific polymerase chain reaction (PCR) primers, which were used to demonstrate the presence of the same mutation in an additional 12 patients, identifying this as a mutational hot spot in the PIG-A gene. Multiple PIG-A mutations have been reported in 10% to 20% of PNH patients. Our results suggest that the large majority of AA/PNH patients have multiple mutations. These data may suggest a process of hypermutation in the PIG-A gene in AA stem cells.


http://www.bloodjournal.org/cgi/content/abstract/100/3/998

The transcription factor PU.1 is required for normal blood cell development. PU.1 regulates the expression of a number of crucial myeloid genes, such as the macrophage colony-stimulating factor (M-CSF) receptor, the granulocyte colony-stimulating factor (G-CSF) receptor, and the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor. Myeloid cells derived from PU.1-/-/[?] mice are blocked at the earliest stage of myeloid differentiation, similar to the blast cells that are the hallmark of human acute myeloid leukemia (AML). These facts led us to hypothesize that molecular abnormalities involving the PU.1 gene could contribute to the development of AML. We identified 10 mutant alleles of the PU.1 gene in 9 of 126 AML patients. The PU.1 mutations comprised 5 deletions affecting the DNA-binding domain, and 5 point mutations in 1) the DNA-
binding domain (2 patients), 2) the PEST domain (2 patients), and 3) the transactivation domain (one patient). DNA binding to and transactivation of the M-CSF receptor promoter, a direct PU.1 target gene, were deficient in the 7 PU.1 mutants that affected the DNA-binding domain. In addition, these mutations decreased the ability of PU.1 to synergize with PU.1-interacting proteins such as AML1 or c-Jun in the activation of PU.1 target genes. This is the first report of mutations in the PU.1 gene in human neoplasia and suggests that disruption of PU.1 function contributes to the block in differentiation found in AML patients.


http://www.bloodjournal.org/cgi/content/abstract/102/9/3323

We have identified a novel gene MEL1 (MDS1/EVI1-like gene 1) encoding a zinc finger protein near the breakpoint of t(1;3)(p36;q21)-positive human acute myeloid leukemia (AML) cells. Here, we studied the structure, expression pattern, and function of MEL1 in leukemia cells. In this study, we have identified 3 transcription start sites, 1 in exon 1 and 2 in exon 2, and 2 kinds of translation products, 170 kDa (MEL1) and 150 kDa (MEL1S). Notably, the 150-kDa band of MEL1S was detected mainly in the t(1;3)(p36;q21)-positive AML cells. By immunoblot analysis and proteolytic mapping, it is suggested that the 150-kDa band of MEL1S in the leukemia cells is translated from the internal initiation codon ATG597 in exon 4 and is mostly lacking the amino-terminal PR domain of MEL1. By the cyclic amplification and selection of targets (CASTing) method for identifying consensus sequences, it was shown that the consensus sequences of MEL1 were included in 2 different consensus sequences for DNA-binding domain 1 and 2 (D1-CONS and D2-CONS) of EVI1. In reporter gene assays, MEL1S activated transcription via binding to D2-CONS; however, the fusion of MEL1 or MEL1S to GAL4 DNA-binding domain (DBD) made them GAL4 binding site-dependent transcriptional repressors. Moreover, overexpression of MEL1S blocked granulocytic differentiation induced by granulocyte colony-stimulating factor (G-CSF) in interleukin-3 (IL-3)-dependent murine myeloid L-G3 cells, while MEL1 could not block the differentiation. Thus, it is likely that overexpression of the zinc finger protein lacking the PR domain (EVI1 and MEL1S) in the leukemia cells is one of the causative factors in the pathogenesis of myeloid leukemia.


http://www.bloodjournal.org/cgi/content/abstract/100/5/1787

The most frequent chromosomal aberrations in B-cell chronic lymphocytic leukemia (B-CLL) are deletions on 13q, 11q, and 17p, and trisomy 12, all of which are of prognostic significance. Conventional cytogenetic analysis and fluorescence in situ hybridization (FISH) are used for their detection, but cytogenetic analysis is hampered by the low mitotic index of B-CLL cells, and FISH depends on accurate information about candidate regions. We used a set of 400 highly informative microsatellite markers covering all chromosomal arms (allelotyping) and automated polymerase chain reaction (PCR) protocols to screen 46 patients with typical B-CLL for chromosomal aberrations. For validation, we compared data with our conventional karyotype results and fine mapping with conventional single-site PCR. All clonal cytogenetic abnormalities potentially detectable by our microsatellite PCR (eg, del13q14 and trisomy 12) were picked up. Allelotyping revealed additional complex aberrations in patients with both normal and abnormal B-CLL karyotypes. Aberrations detectable in the samples with our microsatellite panel were found on almost all chromosomal arms. We detected new aberrant loci in typical B-CLL, such as allelic
losses on 1q, 9q, and 22q in up to 25% of our patients, and allelic imbalances mirroring chromosomal duplications, amplifications, or aneuploidies on 2q, 10p, and 22q in up to 27% of our patients. We conclude that allelotyping with our battery of informative microsatellites is suitable for molecular screening of B-CLL. The technique is well suited for analyses in clinical trials, it provides a comprehensive view of genetic alterations, and it may identify new loci with candidate genes relevant in the molecular biology of B-CLL.


http://www.bloodjournal.org/cgi/content/abstract/102/9/3349

Ras gene mutations occur in 30% to 40% of patients with multiple myeloma (MM), and farnesylation is the first and most important step in the posttranslational modification of Ras proteins. R115777 is a newly synthesized potent farnesyl transferase inhibitor (FTI) and has recently demonstrated significant antitumor activities in vitro and in vivo. Therefore, we examined the effect of R115777 on the growth of fresh and cloned myeloma cells in vitro. R115777 inhibited the growth of fresh and cloned myeloma cells dose dependently, and effects were not dependent on the status of N-Ras mutation in fresh myeloma cells. Flow cytometric analysis using annexin V and 7-aminoactinomycin D (7AAD) showed that R115777 induced apoptosis of 2 of 3 myeloma cell lines at a concentration of 1.0 x 10^-8 M. R115777 appears to be a potent inducer of apoptosis, and its effects depend on the status of Ras mutation in cloned myeloma cells but not on the status of N-Ras mutation in fresh myeloma cells. This is the first report that demonstrates the relationship between the N-Ras mutation in fresh myeloma cells and the effect of R115777. R115777 might have some benefit in the treatment of myeloma patients.


http://www.bloodjournal.org/cgi/content/abstract/101/9/3622

The possible involvement of germline mutation of the ataxia telangiectasia mutated (ATM) gene in childhood acute leukemia with mixed lineage leukemia (MLL) gene rearrangement (MLL+) was investigated. Of the 7 patients studied, 1 showed a germline missense ATM mutation (8921C>T; Pro2974Leu), located in the phosphatidylinositol-3 (PI-3) kinase domain. In reconstitution assays, the ATM mutant 8921T could only partially rescue the radiosensitive phenotype of AT fibroblasts, and in an in vitro kinase assay, it showed a defective phosphorylation of p53-Ser15. Furthermore, the introduction of 8921T in U2OS cells, characterized by a normal ATM/p53 signal transduction, caused a significant reduction of in vivo p53-Ser15 phosphorylation, suggesting a dominant-negative effect of the mutant ATM over the wild-type protein. Our finding in this patient suggests that altered function of ATM plays some pathogenic roles in the development of MLL+ leukemia.


http://www.bloodjournal.org/cgi/content/abstract/101/6/2246

The first adult-repopulating hematopoietic stem cells (HSCs) emerge in the mouse aorta-gonad-mesonephros (AGM) region at embryonic day 10.5 prior to their appearance in the yolk sac and
fetal liver. Although several genes are implicated in the regulation of HSCs, there are gaps in our understanding of the processes taking place in the AGM at the time of HSC emergence. To identify genes involved in AGM HSC emergence, we performed differential display reverse transcriptase-polymerase chain reaction (DD RT-PCR). Differentially expressed genes included β-catenin and homologs of human TM9SF2 and TAB2. We characterized the expression pattern of Wnt/β-catenin signaling, mTM9SF2, and mTAB2 in the embryo and adult. Interestingly, the expression of mouse TAB2 (mTAB2) in the E11 dorsal aorta endothelium suggests a role for mTAB2 in HSC emergence and/or regulation. The identification of differentially expressed genes in the AGM region should yield further insights into the development of this tissue and into the emergence and regulation of HSCs.


http://www.bloodjournal.org/cgi/content/abstract/103/11/4084

Apoptosis is an essential process in embryonic tissue remodeling and adult tissue homeostasis. Within the adult hematopoietic system, it allows for tight regulation of hematopoietic cell subsets. Previously, it was shown that B-cell leukemia 2 (Bcl-2) overexpression in the adult increases the viability and activity of hematopoietic cells under normal and/or stressful conditions. However, a role for apoptosis in the embryonic hematopoietic system has not yet been established. Since the first hematopoietic stem cells (HSCs) are generated within the aortagonad-mesonephros (AGM; an actively remodeling tissue) region beginning at embryonic day 10.5, we examined this tissue for expression of apoptosis-related genes and ongoing apoptosis. Here, we show expression of several proapoptotic and antiapoptotic genes in the AGM. We also generated transgenic mice overexpressing Bcl-2 under the control of the transcriptional regulatory elements of the HSC marker stem cell antigen-1 (Sca-1), to test for the role of cell survival in the regulation of AGM HSCs. We provide evidence for increased numbers and viability of Sca-1+ cells in the AGM and subdissected midgestation aortas, the site where HSCs are localized. Most important, our in vivo transplantation data show that Bcl-2 overexpression increases AGM and fetal liver HSC activity, strongly suggesting that apoptosis plays a role in HSC development.


http://www.bloodjournal.org/cgi/content/abstract/2004-07-2656v1

Mast cells are known to play an important role in host defense against various pathogens, but their role in viral infection has not been clarified in detail. Double-stranded RNA, synthesised by various types of viruses and mimicked by poly(I:C), is recognized by Toll-like receptor 3 (TLR3). In this study, we demonstrate that poly(I:C) injection in vivo potently stimulates peritoneal mast cells to upregulate a number of different co-stimulatory molecules. Therefore, we examined the expression and the functional significance of TLR3 activation in mast cells. Mast cells express TLR3 on the cell surface and intracellularly. After stimulation of mast cells with poly(I:C) and Newcastle disease virus (NDV), TLR3 is phosphorylated and the expression of key antiviral response cytokines (IFN(β), ISG15) and chemokines (IP10, RANTES) is upregulated. Interestingly, mast cells activated via TLR3-poly(I:C) potently stimulate CD8+ T cell recruitment. Indeed, mast cell deficient mice (KitW/KitW-v) intraperitoneally injected with poly(I:C) show a decreased CD8+ T cell recruitment, whereas granulocytes normally migrate to the peritoneal cavity. Mast cell-reconstitution of KitW/KitW-v mice normalizes the CD8+ T cell influx. Thus, mast cells stimulated through engagement of TLR3 are potent regulators of CD8+ T cell activities in vitro and in vivo.

http://www.bloodjournal.org/cgi/content/abstract/101/9/3391

Imatinib mesylate (Gleevec), a small molecule inhibitor of abl, kit, and platelet-derived growth factor receptor (PDGFR) tyrosine kinases, has been reported to be effective in the treatment of hypereosinophilic syndrome (HES) and a rare eosinophilia-associated chronic myeloid disorder (eos-CMD) characterized by the t(5;12)(q33;p13) cytogenetic abnormality. In the current study, we sought to confirm the preliminary observations in HES as well as evaluate the therapeutic value of imatinib in eos-CMD that is not associated with t(5;12)(q33;p13). Five patients with HES (all men, median age = 46 years) and 2 with eos-CMD (both men, aged 45 and 58 years) were treated with imatinib at a starting dose of 100 to 400 mg/day. Cytogenetic studies showed no evidence of either the bcr-abl translocation or t(5;12)(q33;p13) in any patient. Screening of exons encoding the intracellular catalytic domains and extracellular ligand binding domains of PDGFRβ (exons 2-23) and c-kit (exons 1-21) in 6 patients demonstrated mostly previously known polymorphisms. At a median follow-up of 17 weeks (range, 10-33 weeks), 2 patients with HES and 1 with eos-CMD have achieved complete clinical remission and 1 additional patient with HES has achieved a partial remission. In contrast to previous observations, all 4 responding patients had elevated serum interleukin-5 levels. Although the drug was well tolerated in most patients, a previously unrecognized treatment toxicity of acute left ventricular dysfunction occurred in a responding patient with HES within the first week of treatment. Myocardial biopsy revealed eosinophilic infiltration and degranulation, and the cardiogenic shock was reversed with the prompt institution of corticosteroid therapy.


http://www.bloodjournal.org/cgi/content/abstract/99/1/213

Under conditions of impaired T-cell immunity, human cytomegalovirus (HCMV) can reactivate from lifelong latency, resulting in potentially fatal disease. A crucial role for CD8+ T cells has been demonstrated in control of viral replication, and high levels of HCMV-specific cytotoxic T-lymphocytes are seen in immunocompetent HCMV-seropositive individuals despite very low viral loads. Elucidation of the minimum portion of the anti-HCMV T-cell repertoire that is required to suppress viral replication requires further study of clonal composition. The ability of dendritic cells to take up and process exogenous viral antigen by constitutive macropinocytosis was used to study HCMV-specific T-cell memory in the absence of viral replication. The specificity and clonal composition of the CD8+ T-cell responses were evaluated using HLA tetrameric complexes and T-cell receptor [β] chain (TCRBV) spectratypic analyses. There was a skewed reactivity toward the matrix protein pp65, with up to 40-fold expansion of CD8+ T cells directed toward a single peptide-MHC combination. Individual expansions detected on TCRBV spectratype analysis were HCMV-specific and composed of single or highly restricted numbers of clones. There was preferential TCRBV gene usage (BV6.1/6.2, BV8, and BV13 in HLA-A*0201+ individuals) but lack of conservation of CDR3 length and junctional motifs between donors. While there was a spectrum of TCR repertoire diversity directed toward individual MHC-peptide combinations between donors, a relatively small number of clones appeared to predominate the response in each case. These data provide further insight into the range of anti-HCMV responses and will aid the design and monitoring of adoptive immunotherapy protocols.

http://www.bloodjournal.org/cgi/content/abstract/101/3/937

Drug-dependent antibodies (DDAbs) can cause the precipitous destruction of platelets if a patient is exposed to the drug for which the antibodies are specific. The molecular character of the epitopes recognized is poorly understood, and the mechanism by which drugs promote tight binding of these antibodies to platelet glycoproteins without linking covalently to protein or antibody is not yet known. We studied a group of quinine-dependent antibodies that react with human glycoprotein IIIa (GPIIIa; [beta]3-integrin subunit) but fail to recognize rat GPIIIa, despite close homology between the 2 proteins. By characterizing reactions of these antibodies with human/rat GPIIIa chimeras and selected GPIIIa mutants, we found that each of 3 quinine-dependent antibodies requires a 17-amino acid sequence in the newly recognized "hybrid" and PSI homology domains of GPIIIa for drug-dependent binding. Disulfide bonds are required to stabilize the target epitope. Monoclonal antibody AP3, which blocks the binding of these DDAbs to GPIIIa, was found to require a more limited stretch of the same peptide for its reaction with the glycoprotein. The findings suggest this region of GPIIIa may be a favored target for quinine-dependent antibodies and may provide a basis for further studies to elucidate the molecular basis of glycoprotein-drug-antibody interaction.


http://www.bloodjournal.org/cgi/content/abstract/105/3/931

The mechanisms by which intraperitoneal injection of peripheral blood mono-nuclear cells (PBMCs) from Epstein-Barr virus (EBV)-seropositive donors into severe combined immunodeficient (SCID) mice gives rise to lymphomas (hu/SCID tumors) are far from clear. This study addressed whether chemokine receptors and their ligands could be implicated in this experimental model. CXCR4 was found to be highly expressed in hu/SCID tumors; surface expression of CXCR4 was prevalently limited to a tumor cell subset poorly expressing CD23, whereas the CXCR4 ligand, CXCL12, was predominantly expressed by the tumor subpopulation expressing CD23. In vitro inhibition of this autocrine/paracrine CXCL12/CXCR4 axis significantly inhibited lymphoma proliferation and survival. Furthermore, CXCL12 was expressed in cells recovered from the mouse peritoneal cavity early after PBMC transfer as well as by EBV-transformed B cells but not by resting or activated B lymphocytes; also, lymphoma development was associated with a dramatic increase in the levels of murine CXCL12 present in the peritoneal cavity. Finally, antagonizing the CXCL12/CXCR4 axis in vivo strongly counteracted lymphoma development. These studies demonstrate that CXCL12 expression may be associated with EBV infection and suggest that the CXCR4/CXCL12 axis may participate in the EBV-associated lymphomagenesis process in immunodeficient hosts.


http://www.bloodjournal.org/cgi/content/abstract/104/13/4157

Natural killer (NK) cells are a component of the innate immunity against viral infections through
their rapid cytotoxic activity and cytokine production. Although the synthetic double-stranded (ds) RNA polyinosinic-polycytidylic acid (poly I:C), a mimic of a common product of viral infections, is known to rapidly up-regulate their in vivo functions, NK cell ability to directly respond to dsRNA is still mostly unknown. Our results show that treatment with poly I:C significantly up-regulates both natural and CD16-mediated cytotoxicity of highly purified human NK cells. Poly I:C also induces the novel capability of producing CXCL10 chemokine in human NK cells and synergistically enhances interferon-(gamma) (IFN-(gamma)) production induced by either adaptive or innate cytokines. In accordance with the expression of Toll-like receptor-3 (TLR3) and of TRIF/TICAM-1 adaptor, poly I:C stimulation induces the activation of interferon regulatory factor-3 (IRF-3) transcription factor and of p38 mitogen-activated protein kinase (MAPK) in human NK cells. Finally, we demonstrate that p38 MAPK activity is required for the dsRNA-dependent enhancement of cytotoxicity and CXCL10 production. The occurrence of dsRNA-induced signaling and functional events closely correlates with the TLR3 mRNA profile in different NK cell populations. Taken together, these data identify p38 as a central component of NK cell ability to directly respond to dsRNA pathogen-associated molecular pattern (PAMP).


The transcription factor C/EBP[alpha] is crucial for differentiation of mature granulocytes. Recently, different CEBPA gene mutations likely to induce differentiation arrest have been described in nearly 10% of patients with acute myeloid leukemia (AML). In the present study, we retrospectively analyzed the prognostic significance of CEBPA mutations in 135 AML patients (French-American-British [FAB]-M3 excluded). All patients were prospectively enrolled between 1990 and 1996 in a multicenter trial of the ALFA (Acute Leukemia French Association) Group (median age 45 years, median follow-up 5.7 years). Mutations were assessed using direct sequencing of the CEBPA gene. Twenty-two mutations were found in 15 (11%) of 135 patients tested. Twelve patients had at least one mutation located in the N-terminal part of the protein leading to the lack of expression of the full-length C/EBP[alpha] protein. CEBPA mutations were present only in patients belonging to the intermediate cytogenetic risk subgroup and associated with the FAB-M1 subtype ($P = .02$). FLT3 internal tandem duplication (ITD) was found in 5 of 15 CEBPA-mutated as compared with 30 of 119 CEBPA-nonmutated cases tested ($P = .54$). Presence of CEBPA mutations was identified as an independent good prognosis factor for outcome even after adjustment on cytogenetics and FLT3 status (estimated 5-year overall survival 53% vs 25%, $P = .04$). FLT3-ITD appeared to act as a major bad prognosis factor in patients with CEBPA-mutated AML. We thus propose a risk classification that includes in the favorable subgroup all patients from the intermediate subgroup displaying CEBPA mutations when not associated with FLT3-ITD.


Thrombotic microangiopathy (TM) is associated with abnormalities of von Willebrand factor-cleaving protease (VWCP) and other hemostatic factors. This study hypothesized that TM patients might have genetically determined thrombotic risk factors that predispose them to aberrant microvascular thrombosis. DNA samples from 30 white and 12 African American adult
TM patients were analyzed for genetic alleles associated with vascular thrombosis, and plasma samples were analyzed for levels of VWCP activity. DNA was analyzed by using allele-specific polymerase chain reaction for factor V 1691A (Leiden), factor II 20 210A, methylenetetrahydrofolate reductase 667T, type 1 plasminogen activator inhibitor 4G/5G, and platelet GPIa 807T. Patients were segregated by race (white or African American) and plasma level of VWCP activity (normal or deficient). The prevalence of factor V Leiden was significantly increased among the white TM patients that had normal VWCP activity: 4 (36%) of 11 patients compared with 6 (3%) of 186 white control subjects possessed the factor V Leiden allele (P < .001; odds ratio, 17.1; 95% confidence interval, 5.4-54.0). No factor V Leiden alleles were detected in 19 white TM patients with intermediate or deficient levels of VWCP activity or in any of 12 African American patients. The prevalence of other thrombosis-associated alleles did not differ between TM patients and control subjects. These findings suggest that factor V Leiden may be a pathogenic risk factor in TM patients that have normal VWCP activity.


http://www.bloodjournal.org/cgi/content/abstract/105/1/317

To assess a possible role in tumor progression, the occurrence and type of K- and N-RAS mutations were determined in purified tumor cells, including samples from patients with premalignant monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), and extramedullary plasma cell (PC) tumors (ExPCTs). Immunophenotypic aberrant PCs were flow sorted from 20 MGUS, 58 MM, and 13 ExPCT patients. One RAS mutation was identified in 20 MGUS tumors (5%), in contrast to a much higher prevalence of RAS mutations in all stages of MM (about 31%). Further, oncogene analyses showed that RAS mutations are not evenly distributed among different molecular subclasses of MM, with the prevalence being increased in MM-expressing cyclin D1 (P = .015) and decreased in MM with t(4;14) (P = .055). We conclude that RAS mutations often provide a genetic marker if not a causal event in the evolution of MGUS to MM. Surprisingly, RAS mutations were absent in bone marrow tumor cells from all patients with ExPCT, a result significantly different from intramedullary MM (P = .001). From 3 of 6 patients with paired intramedullary and extramedullary PCs and identical immunoglobulin heavy chain gene (IgH) sequences, RAS mutations were identified only in extramedullary PCs, suggesting a role for RAS mutations in the transition from intramedullary to extramedullary tumor. (Blood. 2005;105:317-323)


http://www.bloodjournal.org/cgi/content/abstract/101/3/1063

T-cell receptor-B-variable (TCR-BV) gene usage and the CDR3 size distribution pattern were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) in patients with B-cell chronic lymphocytic leukemia (B-CLL) to assess the T-cell repertoire. The use of TCR-BV families in CD4 and CD8 T cells stimulated with autologous activated leukemic cells was compared with that of freshly obtained blood T cells. Overexpression of individual TCR-BV families was found in freshly isolated CD4 and CD8 T cells. Polyclonal, oligoclonal, and monoclonal TCR-CDR3 patterns were seen within such overexpressed native CD4 and CD8 TCR-BV families. In nonoverexpressed TCR-BV families, monoclonal and oligoclonal populations were noted only within the CD8 subset. After in vitro stimulation of T cells with autologous leukemic B cells, analyses of the CDR3 length patterns showed that in expanded TCR-BV populations, polyclonal
patterns frequently shifted toward a monoclonal/oligoclonal profile, whereas largely monoclonal patterns in native overexpressed TCR-BV subsets remained monoclonal. Seventy-five percent of CD8 expansions found in freshly obtained CD8 T cells further expanded on in vitro stimulation with autologous leukemic B cells. This suggests a memory status of such cells. In contrast, the unusually high frequency of CD4 T-cell expansions found in freshly isolated peripheral blood cells did not correlate positively to in vitro stimulation as only 1 of 9 expansions continued to expand. Our data suggest that leukemia cell-specific memory CD4 and CD8 T cells are present in vivo of patients with CLL and that several leukemia cell-associated antigens/epitopes are present by the patients' immune system, indicating that whole leukemia cells might be of preference for vaccine development.

Roche-Lestienne, C., V. Soenen-Cornu, et al. (2002). "Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment." Blood 100(3): 1014-1018.

http://www.bloodjournal.org/cgi/content/abstract/100/3/1014

Targeting the tyrosine kinase activity of BCR-ABL represents a very promising therapeutic strategy in chronic myeloid leukemia (CML). Despite strong efficacy of the tyrosine kinase inhibitor STI571, resistance has been observed in a significant proportion of patients in advanced CML stage or in Ph-positive acute lymphoid leukemia (ALL). We investigated in this study the mechanism of resistance to STI571 through point mutations in the tyrosine kinase domain and/or BCR-ABL gene amplification in 24 patients (16 in chronic phase and 8 in accelerated phase of the disease) who obtained no cytogenetic response to STI571 treatment. Screening for the already-described Thr315Ile point mutation in the ABL domain using a reverse transcription polymerase chain reaction restriction fragment length polymorphism (RT-PCR-RFLP) technique, 3 patients showed a proportion of mutated transcript at the time of resistance. The same technique failed to detect mutation at diagnosis, but a specific allele-specific oligonucleotide (ASO)-PCR on DNA for the Thr315Ile mutation and, after sequencing, for 2 newly described Phe311Leu and Met351Thr substitutions, showed the presence of rare mutated cells prior to STI571 therapy. Furthermore, the increased proportion of mutated cells during treatment detected by ASO-PCR strongly suggested clonal selection by the functional inhibiting effect of these mutations. Finally, no BCR-ABL gene amplification was detected by fluorescent in situ hybridization (FISH) in the 24 STI571-resistant patients. Our data support that in CML patients treated with STI571, ABL mutations are not restricted to the accelerated phase of the disease and that, at least in some cases, mutations seem to occur prior to STI571 therapy, probably as second mutational events during the course of CML.

Roesler, J., J.-M. Izquierdo, et al. (2005). "Haploinsufficiency rather than the effect of an excessive production of soluble CD95 (CD95(Delta)TM) is the basis for ALPS Ia in a family with duplicated 3' splice site AG in CD95 intron 5 on one allele." Blood: 2004-08-3104.

http://www.bloodjournal.org/cgi/content/abstract/2004-08-3104v1

Autoimmune lymphoproliferative syndrome type Ia (ALPS Ia) is caused by mutations in the CD95/Apo-1/Fas (TNFRSF6) gene, which lead to a defective CD95 ligand (CD95L)-induced apoptosis. Soluble CD95 (sCD95) has been suggested to play an important role in the pathogenesis of diverse autoimmune and malignant diseases by antagonizing CD95L. Here we evaluate a family with four in five of its members harboring an ex-6 -3C->G mutation that affects the splice cis regulatory region (cctagag/ex-6 - >cctagag/ex-6) of the CD95 gene. The mutation causes skipping of exon-6, which encodes the transmembrane region of CD95, and thereby leads to an excessive production of sCD95 in all four affected individuals. The mutation is associated
with a low penetrance of disease phenotype and caused mild and transient ALPS in one male patient whereas all other family members are completely healthy. In all family members with the mutation we found that the cell surface expression of CD95 was low and the activated T cells were resistant to CD95-induced apoptosis. Unexpectedly, excessive production or addition of sCD95 had no effect on the CD95-induced apoptosis in diverse cells. In contrast, increasing the surface expression of CD95 was able to correct the defect in apoptosis. Thus we conclude that the ALPS in the patient was caused by haploinsufficiency of membrane CD95 expression. Our data challenges the hypothesis that sCD95 causes autoimmunity.


http://www.bloodjournal.org/cgi/content/abstract/100/7/2562

Granulocyte colony-stimulating factor (G-CSF) may affect T-cell homeostasis by multiple mechanisms, inducing polarization of cytokine secretion, inhibition of T-cell proliferation, and enhancement of T-cell apoptosis. We analyzed the production of interleukin-10 (IL-10) and transforming growth factor-[beta]1 (TGF-[beta]1) by T cells from healthy volunteer donors treated with recombinant human G-CSF. Highly purified CD4+ T cells obtained before and after G-CSF administration (pre-G and post-G, respectively) were activated using the allogeneic mixed leukocyte reaction. Post-G CD4+ T cells produced high levels of IL-10 but undetectable levels of IL-2 and IL-4, whereas the level of TGF-[beta]1 release was comparable to that of pre-G CD4+ T cells. Notably, post-G CD4+ T cells proliferated poorly in response to alloantigens and to recall antigens and suppressed the proliferation of autologous CD4+ T cells in a cell contact-independent and an antigen-nonspecific manner. TGF-[beta]1 and IL-10 were not dispensable for post-G CD4+ T cells to mediate suppression, as shown by neutralization studies. Compared with pre-G CD4+ T cells, alloantigen-activated post-G CD4+ T cells preferentially expressed markers associated with memory T cells, in conjunction with reduced levels of CD28 and CD62L. Collectively, these data demonstrate that CD4+ T cells exposed to G-CSF in vivo acquire the properties of T regulatory (Tr) cells once triggered in vitro through the T-cell receptor, including a peculiar cytokine production profile (IL-10++TGF-[beta]1+IL-2low/[+]IL-4low/[+]low), an intrinsic low proliferative capacity, and a contact-independent suppression of antigen-driven proliferation. Tr cells generated ex vivo after exposure to G-CSF might be clinically relevant for transplantation medicine and for the treatment of human immune-mediated diseases.


http://www.bloodjournal.org/cgi/content/abstract/100/8/2926

Because suppressor of cytokine signaling (SOCS) proteins are negative regulators of cytokine-induced signaling, it has been hypothesized that aberrant SOCS expression confers resistance against cytokine therapy. This study reports on the constitutive expression of SOCS3 in most chronic myelogenous leukemia (CML) cell lines, which are resistant to treatment with interferon [alpha] (IFN-[alpha]). In contrast, the KT-1/A3 cell line, in which constitutive expression of SOCS3 is barely detectable, is sensitive to IFN-[alpha] treatment. Forced expression of SOCS3 in the KT-1/A3 cell line confers resistance to IFN-[alpha] treatment. Furthermore, most of the blast cells from patients in CML blast crisis, which are usually resistant to IFN-[alpha] therapy, showed constitutive expression of SOCS3. These findings indicate that constitutive SOCS3 expression affects the IFN-[alpha] sensitivity of CML cell lines and blast cells from patients with CML blast crisis.

http://www.bloodjournal.org/cgi/content/abstract/103/4/1311

The endothelial cell protein C (PC) receptor (EPCR) facilitates PC activation by the thrombin-thrombomodulin complex. A soluble form of this receptor (sEPCR) found in plasma inhibits both activated PC (aPC) activity and PC activation by competing for PC with membrane-associated EPCR. Elevated sEPCR levels are found in approximately 20% of healthy subjects, but the mechanisms underlying this interindividual variability are unknown. We measured sEPCR levels in 100 healthy male volunteers, and observed 2 phenotypic groups of subjects. The temporal stability of sEPCR levels suggested genetic control. Extensive analysis of the EPCR gene in these subjects revealed 13 polymorphisms in complete linkage disequilibrium; these defined 3 haplotypes, 1 of which (A3) was strongly associated with high sEPCR levels. The high constitutive sEPCR levels observed in A3 haplotype carriers might reduce the efficiency of the PC system and predispose these subjects to venous thrombosis. By studying 338 patients with venous thrombosis and 338 age- and sex-matched healthy subjects, we found that the A3 haplotype was overrepresented in the patients. In multivariate analysis, subjects carrying the A3 haplotype had an increased risk of thrombosis (odds ratio [OR] = 1.8; P =.004). Thus, the A3 haplotype, which is associated with elevated plasma sEPCR levels, is a candidate risk factor for venous thrombosis.


http://www.bloodjournal.org/cgi/content/abstract/102/2/613

Dendritic cells (DCs) are specialized antigen-presenting cells residing in tissues, from which they take up antigen. Activated DCs migrate through chemokine gradients from sites of inflammation to lymph nodes to stimulate T cells. At sites of inflammation, nucleotides, such as adenosine triphosphate (ATP), are released by activated or dying cells and can function as signaling molecules through P2 receptors (P2Rs). We investigated P2R expression in different DC populations and the effect of nucleotides on chemokine-directed migration. Exposure of monocyte-derived DCs (MoDCs) and CD1a+ dermal DCs to gradients of ATP inhibited their migratory capacity in a dose-dependent manner. Studies using P2R agonists and antagonists implicated signaling through the P2Y11R. On maturation, MoDCs down-regulated P2Y11R expression and were less sensitive to ATP-mediated inhibition of migration. In contrast, ATP did not inhibit the migration of CD1c+ peripheral blood (PB) DCs or interleukin-3 receptor-positive (IL-3R+) plasmacytoid DCs. Although all 4 DC populations expressed mRNA for P2Y11R, calcium-flux studies showed that blood DC types were unresponsive to P2Y11R agonists. In conclusion, DCs use distinct subtypes of P2R. The formation of ATP gradients at sites of inflammation may transiently inhibit the migration of local DCs, thus prolonging the time of antigen encounter. P2R inhibition may represent a new strategy to improve the migration of antigen-loaded DCs from the vaccination site to lymph nodes.

Polymorphisms in several DNA repair genes have been described. These polymorphisms may affect DNA repair capacity and modulate cancer susceptibility by means of gene-environment interactions. We investigated DNA repair capacity and its association with acute myeloblastic leukemia (AML). We studied polymorphisms in 3 DNA repair genes: XRCC1, XRCC3, and XPD. We also assessed the incidence of a functional polymorphism in the NQO1 gene, which is involved in protection of cells from oxidative damage. We genotyped the polymorphisms by using polymerase chain reaction-restriction fragment-length polymorphism analysis in 134 patients with de novo AML, 34 with therapy-related AML (t-AML), and 178 controls. The distributions of the XRCC3 Thr241Met and NQO1 Pro187Ser genotypes were not significantly different in patients and controls. However, the distribution of the XRCC1 Arg399Gln genotypes was significantly different when comparing the t-AML and control groups ([chi]2, P =.03). The presence of at least one XRCC1 399Gln allele indicated a protective effect for the allele in controls compared with patients with t-AML (odds ratio 0.44; 95% confidence interval, 0.20-0.93). We found no interactions between the XRCC1 or XRCC3 and NQO1 genotypes. We also found no differences in the distribution of the XPD Lys751Gln or XRCC1 Arg194Trp genotypes. Our data provide evidence of a protective effect against AML in individuals with at least one copy of the variant XRCC1 399Gln allele compared with those homozygous for the common allele.


The Janus kinase Jak1 has been implicated in tumor formation by the Abelson oncogene. In this study we show that loss of Jak1 does not affect in vitro transformation by v-abl as defined by the ability to induce cytokine-independent B-cell colony formation or establishment of B-cell lines. However, Jak1-deficient, v-abl-transformed cell lines were more tumorigenic than wild-type cells when transplanted subcutaneously into severe combined immunodeficient (SCID) mice or injected intravenously into nude mice. Jak1 deficiency was associated with a loss in the ability of interferon-(gamma) (IFN-(gamma)) to induce growth arrest and/or apoptosis of v-abl-transformed pre-B cells or tumor growth in SCID mice. Moreover, IFN-(gamma) mRNA could be detected in growing tumors, and tumor cells explanted from SCID mice had lost the ability to respond to IFN-(gamma) in 9 of 20 cases, whereas the response to interferon-(alpha) (IFN-(alpha)) remained intact. Importantly, a similar increase in tumorigenicity was observed when IFN-(gamma)-deficient cells were injected into SCID mice, identifying the tumor cell itself as the main source of IFN-(gamma). These findings demonstrate that Jak1, rather than promoting tumorigenesis as previously proposed, is critical in mediating an intrinsic IFN-(gamma)-dependent tumor surveillance.


Caspase 10 (Mch4/FLICE2) is a caspase homologous to caspase 8. A recent report described that inherited CASP10 gene mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome (ALPS). In this study, to explore the possibility that mutation of this gene might be involved in the development of non-Hodgkin lymphoma (NHL), we have analyzed the entire coding region and all splice sites of the CASP10 gene for the detection
of somatic mutations in 117 human NHLs. Overall, 17 NHLs (14.5%) were found to have CASP10 mutations, which were identified in the coding regions of the prodomain (n = 3), the p17 large protease subunit (n = 11), and the p12 small protease subunit (n = 3). We expressed the tumor-derived caspase 10 mutants in 293 cells and found that apoptosis was suppressed. These data suggest that the inactivating mutations of the CASP10 gene might lead to the loss of its apoptotic function and contribute to the pathogenesis of some human NHLs.


http://www.bloodjournal.org/cgi/content/abstract/104/2/356

Patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) generally have a poor prognosis and would benefit from the development of new therapeutic approaches. We previously demonstrated that an allosterically controllable ribozyme, maxizyme (Mz), can induce apoptosis in chronic myelogenous leukemia (CML) cells. Ph+ ALL cells harbor a bcrabl fusion gene (e1a2) encoding a 190-kDa fusion protein (p190) involved in disease pathogenesis. In this study, we have designed a Mz that specifically cleaves e1a2 mRNA and transduced this e1a2Mz into Ph+ ALL cells using a third-generation lentiviral vector system. In 3 of 5 Ph+ ALL cell lines, e1a2Mz transduction resulted in a significant decrease in viability and increased cell apoptosis. We observed a decrease in e1a2 mRNA in all Ph+ ALL cells transduced with e1a2Mz, and the e1a2 mRNA level was higher in e1a2Mz-resistant cells than in e1a2Mz-sensitive cells. All samples of primary Ph+ ALL cells tested showed e1a2Mz-induced growth inhibition and apoptosis. Importantly, e1a2Mz did not influence the colony formation of normal CD34+ cord blood cells. These results indicate that e1a2Mz kills Ph+ ALL cells specifically, suggesting that it may be used as a novel gene therapy strategy for Ph+ ALL. (Blood. 2004;104:356-363)


http://www.bloodjournal.org/cgi/content/abstract/102/5/1588

We used a sensitive real-time reverse transcription-polymerase chain reaction assay to quantify cyclin D1 mRNA levels in bone marrow samples collected at diagnosis from 74 newly diagnosed multiple myeloma (MM) patients who were randomized to undergo either single or double autologous peripheral blood stem cell transplantation as part of first-line therapy for their malignancy. In 46 cases, fluorescence in situ hybridization (FISH) analysis and/or conventional cytogenetics were performed to detect chromosome 11 abnormalities. Patients with the t(11;14) or trisomy 11 significantly overexpressed cyclin D1 (P <.0001) in comparison with patients without 11q abnormalities, who had cyclin D1 mRNA levels similar to healthy donors. Overall, 32 (43%) of 74 patients showed cyclin D1 overexpression. No difference was found between cyclin D1-positive (group A) and cyclin D1-negative (group B) patients with respect to presenting clinical and laboratory characteristics, including chromosome 13 abnormalities, as well as to response to therapy and overall survival, both of which were calculated on an intent-to-treat basis. Patients who overexpressed cyclin D1 had significantly longer duration of remission in comparison with patients who did not (41 vs 26 months, respectively; P =.02). As a result, median event-free survival (EFS) was longer in group A than in group B (33 vs 24 months, respectively; P =.055). We concluded that cyclin D1 overexpression is closely associated with 11q abnormalities and identifies a subset of MM patients who are more likely to have prolonged duration of remission.
and EFS following autologous transplantation.


Minor histocompatibility (H) antigens crucially affect the outcome of human leukocyte antigen (HLA)-identical allogeneic stem cell transplantation (SCT). To understand the basis of alloimmune responses against minor H antigens, identification of minor H peptides and their antigenicity-determining mechanisms is essential. Here we report the identification of HA-3 and its encoding gene. The HA-3 peptide, VTEPGTAQY (HA-3T), is encoded by the lymphoid blast crisis (Lbc) oncogene. We thus show for the first time that a leukemia-associated oncogene can give rise to immunogenic T-cell epitopes that may have participated in antihost and antileukemic alloimmune responses. Genotypic analysis of HA-3- individuals revealed the allelic counterpart VMEPGTAQY (HA-3M). Despite the lack of T-cell recognition of HA-3- cells, the Thr[-&gt;Met] substitution had only a modest effect on peptide binding to HLA-A1 and a minimal impact on recognition by T cells when added exogenously to target cells. This substitution did not influence transporter associated with antigen processing (TAP) transport, but, in contrast to the HA-3T peptide, HA-3M is destroyed by proteasome-mediated digestion. Thus, the immunogenicity of minor H antigens can result from proteasome-mediated destruction of the negative allelic peptide.


Low-grade marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type can transform into high-grade diffuse large B-cell lymphoma (DLBCL). Up to 60% of the MALT lymphomas contain the recently described t(11;18). However, this translocation has not been detected in any DLBCL so far. To elucidate the pathogenesis of these tumors, microsatellite screening of 24 gastric MALT lymphomas was performed and the results were compared with aberrations detected in a previous study on gastric DLBCL. The most frequent aberration, found in 21% of the MALT lymphomas that were exclusively t(11;18)-negative cases, was amplification of the 3q26.2-27 region (harboring the locus of the BCL6 gene). Allelic imbalances in regions 3q26.2-27, 6q23.3-25, 7q31, 11q23-24, and 18q21 were shared by both MALT lymphoma and DLBCL. Loss of heterozygosity in regions 5q21 (APC gene locus), 9p21 (INK4A/ARF), 13q14 (RB), and 17p13 (p53) and allelic imbalances in 2p16, 6p23, and 12p12-13 occurred exclusively in DLBCL. Only one of 10 t(11;18)-positive MALT lymphomas showed an additional clonal abnormality. These tumors thus display features of a clonal proliferation characterized by the presence of the t(11;18). However, they only rarely display secondary aberrations and do not seem to transform into DLBCL. In contrast, t(11;18)-negative MALT lymphomas show numerous allelic imbalances[---]some of them identical with aberrations seen in DLBCL[---]suggesting that this group is the source of tumors eventually transforming into high-grade DLBCL.

Acquired somatic mutations in ATRX, an X-linked gene encoding a chromatin-associated protein, were recently identified in 4 patients with the rare subtype of myelodysplastic syndrome (MDS) associated with (alpha) thalassemia (ATMDS). Here we describe a series of novel point mutations in ATRX detected in archival DNA samples from marrow and/or blood of patients with ATMDS by use of denaturing high-performance liquid chromatography (DHPLC), a technique sensitive to low-level mosaicism. Two of the new mutations result in changes in amino acids altered in previously described pedigrees with germ line ATRX mutations (ATR-X syndrome), but the hematologic abnormalities were much more severe in the patients with ATMDS than in the corresponding constitutional cases. In one ATMDS case where DNA samples from several time points were available, the proportion of ATRX-mutant subclones correlated with changes in the amount of hemoglobin H. This study strengthens the link between acquired, somatic ATRX mutations and ATMDS, illustrates how molecular defects associated with MDS and other hematologic malignancies masked by somatic mosaicism may be detected by DHPLC, and shows that additional factors increase the severity of the hematologic phenotype of ATRX mutations in ATMDS.

The frequently occurring T-cell receptor delta (TCRD) deletions in precursor-B-acute lymphoblastic leukemia (precursor-B-ALL) are assumed to be mainly caused by V(delta)2-J(alpha) rearrangements. We designed a multiplex polymerase chain reaction tified clonal V(delta)2-J(alpha) rearrangements in 141 of 339 (41%) childhood and 8 of 22 (36%) adult precursor-B-ALL. A significant proportion (44%) of V(delta)2-J(alpha) rearrangements in childhood precursor-B-ALL were oligoclonal. Sequence analysis showed preferential usage of the J(alpha)29 gene segment in 54% of rearrangements. The remaining V(delta)2-J(alpha) rearrangements used 26 other J(alpha) segments, which included 2 additional clusters, one involving the most upstream J(alpha) segments (ie, J(alpha)48 to J(alpha)61; 23%) and the second cluster located around the J(alpha)9 gene segment (7%). Real-time quantitative PCR studies of normal lymphoid cells showed that V(delta)2 rearrangements to upstream J(alpha) segments occurred at low levels in the thymus (10^{-2} to 10^{-3}) and were rare (generally below 10^{-3}) in B-cell precursors and mature T cells. V(delta)2-J(alpha)29 rearrangements were virtually absent in normal lymphoid cells. The monoclonal V(delta)2-J(alpha) rearrangements in precursor-B-ALL may serve as patient-specific targets for detection of minimal residual disease, because they show high sensitivity (10^{-4} or less in most cases) and good stability (88% of rearrangements preserved at relapse).

Immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements are excellent patient-specific polymerase chain reaction (PCR) targets for detection of minimal residual disease (MRD) in acute
lymphoblastic leukemia (ALL), but they might be unstable during the disease course. Therefore, we performed detailed molecular studies in 96 childhood precursor-B-ALL at diagnosis and at relapse \((n = 91)\) or at presumably secondary acute myeloid leukemia \((n = 5)\). Clonal Ig and TCR targets for MRD detection were identified in 94 patients, with 71% of these targets being preserved at relapse. The best stability was found for IGK-Kde rearrangements \((90\%)\), followed by TCRG \((75\%)\), IGH \((64\%)\), and incomplete TCRD rearrangements \((63\%)\). Combined Southern blot and PCR data for IGH, IGK-Kde, and TCRD genes showed significant differences in stability at relapse between monoclonal and oligoclonal rearrangements: 89% versus 40%, respectively. In 38% of patients all MRD-PCR targets were preserved at relapse, and in 40% most of the targets were. In 22% of patients most targets \((10\) cases) or all targets \((10\) cases) were lost at relapse. The latter 10 cases included 4 patients with secondary acute myeloid leukemia with germline Ig/TCR genes. In 5 other patients additional analyses proved the clonal relationship between both disease stages. Finally, in 1 patient all Ig/TCR gene rearrangements were completely different between diagnosis and relapse, which is suggestive of secondary ALL. Based on the presented data, we propose stepwise strategies for selection of stable PCR targets for MRD monitoring, which should enable successful detection of relapse in most \((95\%)\) of childhood precursor-B-ALL.


http://www.bloodjournal.org/cgi/content/abstract/100/4/1113

A new way to identify tumor-specific genes is to compare gene expression profiles between malignant cells and their autologous normal counterparts. In patients with multiple myeloma, a major plasma cell disorder, normal plasma cells are not easily attainable in vivo. We report here that in vitro differentiation of peripheral blood B lymphocytes, purified from healthy donors and from patients with multiple myeloma, makes it possible to obtain a homogeneous population of normal plasmablastic cells. These cells were identified by their morphology, phenotype, production of polyclonal immunoglobulins, and expression of major transcription factors involved in B-cell differentiation. Oligonucleotide microarray analysis shows that these polyclonal plasmablastic cells have a gene expression pattern close to that of normal bone marrow-derived plasma cells. Detailed analysis of genes statistically differentially expressed between normal and tumor plasma cells allows the identification of myeloma-specific genes, including oncogenes and genes coding for tumor antigens. These data should help to disclose the molecular mechanisms of myeloma pathogenesis and to define new therapeutic targets in this still fatal malignancy. In addition, the comparison of gene expression between plasmablastic cells and B cells provides a new and powerful tool to identify genes specifically involved in normal plasma cell differentiation.


http://www.bloodjournal.org/cgi/content/abstract/99/12/4326

Constitutive activation of the FLT3 receptor tyrosine kinase, either by internal tandem duplication \((ITD)\) of the juxtamembrane region or by point mutations in the second tyrosine kinase domain \((TKD)\), has been described in patients with acute myelogenous leukemia \((AML)\). We analyzed the prevalence and the potential prognostic impact of FLT3 mutations in 979 AML patients. Results were correlated with cytogenetic data and the clinical response. FLT3-ITD mutations were found in 20.4% and FLT3-TKD mutations in 7.7% of the patients. Each mutation was associated with similar clinical characteristics and was more prevalent in patients with normal karyotype.
Significantly more FLT3 aberrations were found in patients with FAB M5, and fewer were found in patients with FAB M2 and M6. Although less frequent in patients with cytogenetic aberrations, FLT3-ITDs were found in 13 of 42 patients with t(15;17) and in 9 of 10 patients with t(6;9). The prevalence of the ITD allele on the DNA level was heterogeneous, ranging from faint mutant bands in some patients to predominant mutant bands in others. Based on quantitative analysis, the mutant-wild-type (wt) ratio ranged from 0.03 to 32.56 (median, 0.78). Patients with a high mutant/wt ratio (ie, greater than 0.78) had significantly shorter overall and disease-free survival, whereas survival in patients with ratios below 0.78 did not differ from those without FLT3 aberrations. Multivariate analysis confirmed a high mutant/wt ratio to be a strong independent prognostic factor. Taken together, these data confirm that FLT mutations represent a common alteration in adult AML. Constitutive activation may be associated with monocytoid differentiation. A high mutant/wt ratio in ITD-positive patients appears to have a major impact on the prognostic relevance.


http://www.bloodjournal.org/cgi/content/abstract/101/4/1558

Acute promyelocytic leukemia (APL) is associated with a reciprocal and balanced translocation involving the retinoic acid receptor-[alpha] (RAR[alpha]). All-trans retinoic acid (ATRA) is used to treat APL and is a potent morphogen that regulates HOX gene expression in embryogenesis and organogenesis. HOX genes are also involved in hematopoiesis and leukemogenesis. Thirty-nine mammalian HOX genes have been identified and classified into 13 paralogous groups clustered on 4 chromosomes. They encode a complex network of transcription regulatory proteins whose precise targets remain poorly understood. The overall function of the network appears to be dictated by gene dosage. To investigate the mechanisms involved in HOX gene regulation in hematopoiesis and leukemogenesis by precise measurement of individual HOX genes, a small-array real-time HOX (SMART-HOX) quantitative polymerase chain reaction (PCR) platform was designed and validated. Application of SMART-HOX to 16 APL bone marrow samples revealed a global down-regulation of 26 HOX genes compared with normal controls. HOX gene expression was also altered during differentiation induced by ATRA in the PML-RAR[alpha]+ NB4 cell line. PML-RAR[alpha] fusion proteins have been reported to act as part of a repressor complex during myeloid cell differentiation, and a model linking HOX gene expression to this PML-RAR[alpha] repressor complex is now proposed.


http://www.bloodjournal.org/cgi/content/abstract/105/8/3011

Monocyte cytokines (ie, monokines) induce natural killer (NK) cells to produce interferon-{gamma} (IFN-{gamma}), which is critical for monocyte clearance of infectious pathogens and tumor surveillance. Human CD56bright NK cells produce far more IFN-{gamma} in response to monokines than do CD56dim NK cells. The kinases and phosphatases involved in regulating IFN-{gamma} production by monokine-activated NK cells are not clearly identified. SHIP1 is a 5' inositol phosphatase that dephosphorylates the phosphatidylinositol-3 kinase (PI-3K) product PI3,4,5P3. Here, we show that constitutive expression of SHIP1 is distinctly lower in CD56bright NK cells compared with CD56dim NK cells, suggesting it could be an important negative regulator of IFN-{gamma} production in monokine-activated NK cells. Indeed, overexpression of SHIP1 in
CD56bright NK cells followed by monokine activation substantially lowered IFN-γ production. This effect was not seen when NK cells were infected with a SHIP1 mutant containing an inactive catalytic domain. Finally, NK cells in SHIP1−/− mice produced more IFN-γ in response to monokines in vivo than did NK cells from wild-type mice. Collectively, these results demonstrate that SHIP1 negatively regulates monokine-induced NK cell IFN-γ production in vitro and in vivo and provide the first molecular explanation for an important functional distinction observed between CD56bright and CD56dim human NK subsets.


http://www.bloodjournal.org/cgi/content/abstract/101/10/4013

The formation of terminally differentiated plasma cells represents the critical final step in B-cell differentiation. In this study, utilizing oligonucleotide microarray analysis, we describe the highly specialized genetic profile exhibited by terminally differentiated plasma cells. A total of 1476 known genes were differentially expressed by plasma cells compared with B cells. Plasma cells displayed an up-regulation, induction, or a selective retention of a unique constellation of transcription factors, including members of the AP-1, nuclear factor-κB (NF-κB), nuclear factor of activated T cells (NFAT), and octamer binding factor families. Interestingly, plasma cells also displayed a down-regulation of several RNA polymerase I-related factors, consistent with terminal differentiation, and exhibited a down-regulation of the TATA box binding protein. Furthermore, plasma cells displayed alterations in multiple components of the Wnt and Notch signaling pathways and showed a unique pattern of apoptosis and proliferation-associated genes. Unexpectedly, plasma cells displayed an up-regulation of 2 factors normally associated with microenvironmental positioning of neuronal cells, reelin and neuropilin-1. These results supply insight into the developmental genetics of plasma cell differentiation and provide a foundation for further analysis of plasma cell biology.


http://www.bloodjournal.org/cgi/content/abstract/104/3/667

Truncated granulocyte colony-stimulating factor receptors (G-CSF-Rs) are implicated in severe congenital neutropenia (SCN) and the consecutive development of acute myeloid leukemia (AML). Mice expressing G-CSF-R truncation mutants (gcsfr-d715) show defective receptor internalization, an increased signal transducer and activator of transcription 5 (STAT5)/STAT3 activation ratio, and hyperproliferative responses to G-CSF treatment. We determined whether a lack of negative feedback by suppressor of cytokine signaling (SOCS) proteins contributes to the signaling abnormalities of G-CSF-R-d715. Expression of SOCS3 transcripts in bone marrow cells from G-CSF-treated gcsfr-d715 mice was approximately 60% lower than in wild-type (WT) littermates. SOCS3 efficiently suppressed STAT3 and STAT5 activation by WT G-CSF-R in luciferase reporter assays. In contrast, while SOCS3 still inhibited STAT3 activation by G-CSF-R-d715, STAT5 activation was no longer affected. This was due mainly to the loss of the SOCS3 recruitment site Tyr729, with an additional contribution of the internalization defects of G-CSF-R-d715. Because Tyr729 is also a docking site for the Src homology 2-containing protein tyrosine phosphatase-2 (SHP-2), which binds to and inactivates STAT5, we suggest a model in which reduced SOCS3 expression, combined with the loss of recruitment of both SOCS3 and SHP-2 to the activated receptor complex, determine the increased STAT5/STAT3 activation ratio and the resulting signaling abnormalities projected by truncated G-CSF-R mutants.

http://www.bloodjournal.org/cgi/content/abstract/102/7/2522

The safe application of new strategies for the treatment of graft-versus-host disease (GVHD) is hampered by the lack of a clinically relevant model for preclinical testing. Current models are based on intraperitoneal transfer of human peripheral blood mononuclear cells (huPBMCs) into NOD-SCID (nonobese diabetic-severe combined immunodeficient)/SCID mice. Intravenous transfer would be preferred but this has always been ineffective. We developed a new model for xenogeneic GVHD (X-GVHD) by intravenous transfer of huPBMCs into RAG2-/- (gamma)c-/- mice. Our results show a high human T-cell chimerism of more than 20% (up to 98%) in more than 90% of mice, associated with a consistent development of XGVHD within 14 to 28 days and a total mortality rate of 85% shorter than 2 months. After murine macrophage depletion, engraftment was earlier and equally high with lower doses of huPBMCs. Human macrophages were also absent in these mice. Purified huCD3+ cells showed a similar X-GVH effect with contribution of both CD4 and CD8 phenotypes. Human immunoglobulins and cytokines were produced in diseased mice. One of 30 mice developed chronic X-GVHD with skin histology similar to human GVHD. In conclusion, we present a new model for X-GVHD by intravenous transfer of huPBMCs in RAG2-/- (gamma)c-/- mice. Murine and human macrophages do not seem to be necessary for acute X-GVHD in this model. (Blood. 2003;102:2522-2531)


http://www.bloodjournal.org/cgi/content/abstract/101/4/1596

We established the molecular basis for pyruvate kinase (PK) deficiency in a white male patient with severe nonspherocytic hemolytic anemia. The paternal allele exhibited the common PKLR cDNA sequence (c.) 1529G>A mutation, known to be associated with PK deficiency. On the maternal allele, 3 in cis mutations were identified in the erythroid-specific promoter region of the gene: one deletion of thymine [-]248 and 2 single nucleotide substitutions, nucleotide (nt) [-]324T>A and nt [-]83G>C. Analysis of the patient's RNA demonstrated the presence of only the 1529A allele, indicating severely reduced transcription from the allele linked to the mutated promoter region. Transfection of promoter constructs into erythroleukemic K562 cells showed that the most upstream [-]324T>A and [-]248delT mutations were nonfunctional polymorphisms. In contrast, the [-]83G>C mutation strongly reduced promoter activity. Site-directed mutagenesis of the promoter region revealed the presence of a putative regulatory element (PKR-RE1) whose core binding motif, CTCTG, is located between nt [-]87 and nt [-]83. Electrophoretic mobility shift assay using K562 nuclear extracts indicated binding of an as-yet-unidentified trans-acting factor. This novel element mediates the effects of factors necessary for regulation of pyruvate kinase gene expression during red cell differentiation and maturation.


http://www.bloodjournal.org/cgi/content/abstract/100/4/1123
The phenotype induced by the GATA-1low (neo[delta]HS) mutation is here further characterized by analyzing the hemopoietic system during the aging (up to 20 months) of a GATA-1low colony (135 mutants and 40 normal littermates). Mutants expressed normal hematocrit values (Hct = 45.9 +/- 4.0) until 12 months but became anemic from 15 months on (Hct = 30.9 +/- 3.9; P < .05). Anemia was associated with several markers of myelofibrosis such as the presence of tear-drop poikilocytes and progenitor cells in the blood, collagen fibers in the marrow and in the spleen, and hemopoietic foci in the liver. Semiquantitative reverse transcription-polymerase chain reaction showed that growth factor genes implicated in the development of myelofibrosis (such as osteocalcin, transforming growth factor-[beta]1, platelet-derived growth factor, and vascular endothelial growth factor) were all expressed in the marrow from the mutants at higher levels than in corresponding normal tissues. The GATA-1low mutants experienced a slow progression of the disease because the final exitus was not observed until at least 15 months with a probability of survival more favorable than that of W/Wv mice concurrently kept in the animal facility (P < .001, by Kaplan-Meier analysis). In conclusion, impaired GATA-1 expression may contribute to the development of myelofibrosis, and the GATA-1low mutants may represent a suitable animal model for the human disease that may shed light on its pathogenesis.


http://www.bloodjournal.org/cgi/content/abstract/100/13/4303

Stroke is a major cause of morbidity and mortality in sickle cell (SS) disease. Genetic risk factors have been postulated to contribute to this clinical outcome. The human genome project has substantially increased the catalog of variations in genes, many of which could modify the risk for manifestations of disease outcome in a monogenic disease, namely SS. VCAM1 is a cell adhesion molecule postulated to play a critical role in the pathogenesis of SS disease. We identified a total of 33 single nucleotide polymorphisms (SNPs) by sequencing the entire coding region, 2134 bp upstream of the 5' end of the published cDNA, 217 bp downstream of the 3' end of the cDNA, and selected intronic regions of the VCAM1 locus. Allelic frequencies for selected SNPs were determined in a healthy population. We subsequently analyzed 4 nonsynonymous coding, 2 synonymous coding, and 4 common promoter SNPs in a genetic association study of clinically apparent stroke in SS disease conducted in a cohort derived from a single institution in Jamaica (51 symptomatic cases and 51 matched controls). Of the 10 candidate SNPs analyzed in this pilot study, the variant allele of the nonsynonymous SNP, VCAM1 G1238C, may be associated with protection from stroke (odds ratio [OR] 0.35, 95% confidence interval [CI] 0.15-0.83, P = .04). Further study is required to confirm the importance of this variant in VCAM1 as a clinically useful modifier of outcome in SS disease.


http://www.bloodjournal.org/cgi/content/abstract/101/2/752

The Scianna blood group encompasses the high-frequency antigens Sc1 and Sc3 and the low-frequency antigen Sc2. Another low-frequency antigen Rd (Radin) was suggested to belong to the Scianna blood group. The molecular basis of the Scianna blood group was unknown. The erythrocyte membrane-associated protein (ERMAP) shared the genomic location, protein product size, and localization to the red blood cell (RBC) membrane surface with Scianna. The ERMAP gene was sequenced in probands with known Scianna and Radin phenotypes. In a Sc:-1,-2 proband, only an ERMAP allele with a 2-bp deletion in exon 3 causing a frameshift could be detected. A Sc:-1,2 proband was homozygous for the ERMAP(Gly57Arg) allele. An Rd+ proband
was heterozygous for the ERMAP(Pro60Ala) allele. Polymerase chain reaction with sequence-specific priming (PCR-SSP) systems was developed to detect the Sc2 and Rd alleles of the ERMAP gene. The 2 alleles occurred with about 1% and less than 1% frequency in the population, which was compatible with the frequency of the Sc2 and Rd antigens known in whites. Two Sc2+ and one Rd+ samples that were found by genotyping were confirmed by serology. The antigens of the Scianna blood group include Rd and are expressed by the human ERMAP protein. Sc2 is caused by an ERMAP(Gly57Arg) allele and Rd by an ERMAP(Pro60Ala) allele. Scianna is the last of the previously characterized protein-based blood group systems whose molecular basis was discerned. Hence, the phenotype prediction by genotyping became possible for all human blood group systems encoded by proteins.


The human peripheral B-cell compartment displays a large population of immunoglobulin M-positive, immunoglobulin D-positive CD27+ (IgM+IgD+CD27+) "memory" B cells carrying a mutated immunoglobulin receptor. By means of phenotypic analysis, complementarity-determining region 3 (CDR3) spectratyping during a T-independent response, and gene-expression profiling of the different blood and splenic B-cell subsets, we show here that blood IgM+IgD+CD27+ cells correspond to circulating splenic marginal zone B cells. Furthermore, analysis of this peripheral subset in healthy children younger than 2 years shows that these B cells develop and mutate their immunoglobulin receptor during ontogeny, prior to their differentiation into T-independent antigen-responsive cells. It is therefore proposed that these IgM+IgD+CD27+ B cells provide the splenic marginal zone with a diversified and protective preimmune repertoire in charge of the responses against encapsulated bacteria.


The design and evaluation of therapies for the sickle cell and [beta]-thalassemia syndromes rely on our understanding of hemoglobin accumulation during human erythropoiesis. Here we report direct measurements of hemoglobin composition and messenger RNA (mRNA) levels in cultured CD34+ cells and correlate those measurements with studies of freshly obtained bone marrow samples. Hemoglobin levels in differentiating cells were also compared with morphologic, immunophenotypic, and cell cycle assessments. A population of large preproerythroblasts was first identified within 24 hours and became the dominant population by day 5. The transition from proerythroblast to basophilic normoblast occurred later, from days 7 to 9, and correlated with a peak of 74.1% (+/-) 3.9% of the cells in the S phase of cell cycle. Orthochromatic normoblasts were the dominant and final cell type by day 13. High-performance liquid chromatography-based quantitation of fetal (HbF) and adult (HbA) hemoglobin and real-time polymerase chain reaction globin mRNA quantitation demonstrated a coordinate rise in the accumulation of both proteins and mRNA among these developmentally staged populations. Quantitative analyses on freshly sorted bone marrow populations demonstrated a similar rising pattern with [beta]-globin and HbA as the dominant species at both early and late stages of differentiation. We found no evidence for HbF dominant populations or switching during differentiation in adult cells. Instead, rapid increases in both HbF (heterocellular) and HbA (pancellular) content were observed, which coincided with the apex in cell cycling and the proerythroblast-basophilic normoblast transition. Based on these measurements, we conclude that HbF and HbA content are regulated with the
Chemokine stromal cell-derived factor-1 (SDF-1) is expressed by bone marrow (BM) stromal cells and plays key roles in BM cell migration. Modulation of its expression could affect the migratory capacity of cells trafficking the BM, such as hematopoietic progenitor and leukemic cells. Transforming growth factor-β1 (TGF-β1) is present in the BM environment and constitutes a pivotal molecule controlling BM cell proliferation and differentiation. We used the BM stromal cell line MS-5 as a model to investigate whether SDF-1 expression constitutes a target for TGF-β1 regulation and its functional consequences. We show here that TGF-β1 down-regulates SDF-1 expression, both at the mRNA level, involving a decrease in transcriptional efficiency, and at the protein level, as detected in lysates and supernatants from MS-5 cells. Reduction of SDF-1 in supernatants from TGF-β1-treated MS-5 cells correlated with decreased, SDF-1-dependent, chemotactic, and transendothelial migratory responses of the BM model cell lines NCI-H929 and Mo7e compared with their responses to supernatants from untreated MS-5 cells. In addition, supernatants from TGF-β1-exposed MS-5 cells had substantially lower efficiency in promoting integrin (α4β1)-mediated adhesion of NCI-H929 and Mo7e cells to soluble vascular cell adhesion molecule-1 (sVCAM-1) and CS-1/fibronectin than their untreated counterparts. Moreover, human cord blood CD34+ hematopoietic progenitor cells displayed SDF-1-dependent reduced responses in chemotaxis, transendothelial migration, and up-regulation of adhesion to sVCAM-1 when supernatants from TGF-β1-treated MS-5 cells were used compared with supernatants from untreated cells. These data indicate that TGF-β1-controlled reduction in SDF-1 expression influences BM cell migration and adhesion, which could affect the motility of cells trafficking the bone marrow.


http://www.bloodjournal.org/cgi/content/abstract/99/1/88

To investigate the role of viral expression in individuals infected with human T-cell lymphotrophic virus type 1 (HTLV-1), a real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) of HTLV-1 tax messenger RNA (mRNA) using ABI Prism 7700 Sequence Detection System was developed. Using this system, the HTLV-1 tax mRNA load was compared with HTLV-1 proviral DNA load, HTLV-1 Tax protein expression, HTLV-1 Tax-specific CD8+ T-cell frequency, and disease severity of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). This approach was a sensitive and specific technique for the precise quantification of HTLV-1 tax mRNA. The total amount of HTLV-1 tax mRNA and mRNA expression level in HTLV-1-infected cells (mRNA/DNA ratio) were higher in HAM/TSP patients than in asymptomatic HTLV-1 carriers. The HTLV-1 tax mRNA load correlated with the HTLV-1 proviral DNA load ex vivo, the Tax protein expression in vitro, and the Tax-specific CD8+ T-cell frequency ex vivo. The HTLV-1 tax mRNA load also correlated with disease severity in HAM/TSP patients. These data suggest that increased HTLV-1 expression plays an important role in the pathogenesis of HAM/TSP, and the HTLV-1 tax mRNA level could be a useful predictor of disease progression in patients with HAM/TSP.

http://www.bloodjournal.org/cgi/content/abstract/103/1/325

An immune pathophysiology for acquired aplastic anemia (AA) has been inferred from the responsiveness of the patients to immunosuppressive therapies and experimental laboratory data. To address the transcriptome of hematopoietic cells in AA, we undertook GeneChip analysis of the extremely limited numbers of progenitor and stem cells in the marrow of patients with this disease. We pooled total RNA from highly enriched bone marrow CD34 cells of 36 patients with newly diagnosed AA and 12 healthy volunteers for analysis on oligonucleotide chips. A large number of genes implicated in apoptosis and cell death showed markedly increased expression in AA CD34 cells, and negative proliferation control genes also had increased activity. Conversely, cell cycle progress-enhancing genes showed low expression in AA. Cytokine/chemokine signal transducer genes, stress response genes, and defense/immune response genes were up-regulated, as anticipated from other evidence of the heightened immune activity in AA patients' marrow. In summary, detailed genetic analysis of small numbers of hematopoietic progenitor cells is feasible even in marrow failure states where such cells are present in very small numbers. The gene expression profile of primary human CD34 hematopoietic stem cells from AA was consistent with a stressed, dying, and immunologically activated target cell population. Many of the genes showing differential expression in AA deserve further detailed analysis, including comparison with other marrow failure states and autoimmune disease.


http://www.bloodjournal.org/cgi/content/abstract/104/12/3731

Although phospholipid scramblase 1 (PLSCR1) was originally identified based on its capacity to promote transbilayer movement of membrane phospholipids, subsequent studies also provided evidence for its role in cell proliferation, maturation, and apoptosis. In this report, we investigate the potential role of PLSCR1 in leukemic cell differentiation. We show that all-trans retinoic acid (ATRA), an effective differentiation-inducing agent of acute promyelocytic leukemic (APL) cells, can elevate PLSCR1 expression in ATRA-sensitive APL cells NB4 and HL60, but not in maturation-resistant NB4-LR1 cells. ATRA- and phorbol 12-myristate 13-acetate (PMA)-induced monocytic differentiation is accompanied by increased PLSCR1 expression, whereas only a slight or no elevation of PLSCR1 expression is observed in U937 cells differentiated with dimethyl sulfoxide (DMSO), sodium butyrate, or vitamin D3. Cell differentiation with ATRA and PMA, but not with vitamin D3 or DMSO, results in phosphorylation of protein kinase C(\(\delta\)) (PKC(\(\delta\))), and the PKC(\(\delta\))-specific inhibitor rottlerin nearly eliminates the ATRA- and PMA-induced expression of PLSCR1, while ectopic expression of a constitutively active form of PKC(\(\delta\)) directly increases PLSCR1 expression. Finally, decreasing PLSCR1 expression with small interfering RNA inhibits ATRA/PMA-induced differentiation. Taken together, these results suggest that as a protein induced upon PKC(\(\delta\)) activation, PLSCR1 is required for ATRA- and PMA-triggered leukemic cell differentiation.
BACKGROUND: The mechanism(s) of intravenous immunoglobulin (IVIG) towards inhibition of monocyte phagocytic activity involves the function and/or the expression of inhibitory Fc(\(\gamma\))RIIb in a murine model. To confirm these findings in human monocytes, we used a human monocyte phagocytic model in vitro to study the effects of IVIG on the phagocytic activity and the expression of Fc(\(\gamma\))R genes. METHODS Part A: Monolayer Monocyte Phagocytosis Assay. Normal volunteer's peripheral blood mononuclear cells (PBMC) were isolated from heparin anticoagulated blood by Ficoll-Hypaque (Pharmacia Biotech) density separation. The PBMCs were washed and the monocytes were purified using a magnetic bead-positive selection method with anti-CD14 antibody (Miltenyl Biotec). 105 monocytes were incubated in a microtiter plate at 37\(^\circ\)C for 1 hour before exposure to IVIG 0.5 g/L. Anti-D (WinRho) sensitized Rh positive (R2R2) red cells were added to the monocytes at 0.5 hour and 18 hour post-IVIG treatment. After 1 hour incubation with sensitized RBC, monocytes phagocytic activity is measured by chemiluminescence detection with a LumiCount (Packard). The readings were normalized with maximal chemiluminescence signal achieved by the monocytes without prior exposure to IVIG (positive control). Part B: RT-PCR of Fc(\(\gamma\))RIIa and Fc(\(\gamma\))RIIb. After 18 hours of exposure to two different concentrations of IVIG (0.5 and 5 gm/L), monocytes were collected and total RNA was isolated with TRIzol reagent (Invitrogen). 1 \(\mu\)g of RNA was used to generate first strand cDNA using Superscript II RT kit (Invitrogen). Fc(\(\gamma\))RIIa and IIb were amplified with AmpliTaq Gold DNA polymerase system (Applied Biosystems). The PCR products were evaluated by polyacrylamide gel electrophoresis. RESULTS Part A: Dose-response curves were generated by plotting normalized chemiluminescence against the concentration of anti-D used to sensitize the red cells. Anti-D sensitized red cells were phagocytosed by monocytes in a dose-dependent manner. There is a time-dependent inhibition of monocyte phagocytic activity when monocytes were incubated with IVIG at 0.5 gm/L. (Fig. 1) Part B: There is no significant difference in the gene expression of FcR(\(\gamma\))RIIb and Fc(\(\gamma\))RIIa in the adherent monocytes after incubating with either low dose (0.5 gm/L) or high dose (5 gm/L) of IVIG for 18 hours. (Fig 2) CONCLUSION: Delayed inhibition of phagocytic activity with 18-hour exposure to IVIG is not directly mediated via the modulation of Fc(\(\gamma\))RIIb gene expression in human monocytes. Other mechanisms, such as intracellular signalling or receptor coupling, might be involved in the delayed inhibitory effects of IVIG.

http://www.sciencedirect.com/science/article/B6WBV-4DCWF7N-3/2/701e168ff5212444a04306395f955139

HFE-hemochromatosis is the most common form of hereditary hemochromatosis. The disorder is associated with the homozygous C282Y mutation and has variable phenotype, being modulated by environmental and genetic factors. Candidate modifier genes are hemojuvelin and hepcidin, which are responsible for juvenile hemochromatosis. We used DHPLC to scan mutations in these genes in a cohort of unrelated patients with C282Y mutation. They consisted of 136 C282Y homozygous, 43 heterozygous, and 42 C282Y/H63D compound heterozygous, plus 62 controls subjects. Mutations and polymorphisms were found in 16 patients and 4 controls. Abnormally high indices of iron status were found in subjects C282Y/H63D heterozygous for the N196K hemojuvelin mutation and the -72C>T hepcidin substitution. The already described G71D mutation of hepcidin did not induce evident modification of the C282Y/H63D phenotype. The data show that heterozygous mutations of the hemojuvelin gene contribute like those of hepcidin to the phenotypic heterogeneity of hemochromatosis. However, they are rare and explain only a minor portion of the variable penetrance of the disorder.


http://www.sciencedirect.com/science/article/B6WBV-480CJVK-3/2/3c9ed5029850a13510a591052243c032

Iron overload was found to be the major cause of disability in Chinese HbH disease patients although they were not on regular blood transfusion. The transferrin receptor 2 (TFR2) and hereditary hemochromatosis (HFE) genes were examined to see if inheritance of these gene defects may be a possible cause of iron overload in 45 HbH patients. A novel intronic (IVS6 (+6) T->A) mutation of the TFR2 gene was identified in one patient, and six others were found to carry a known missense mutation (exon 5, I238M) that was also present in one normal control subject. One HbH patient and one normal control carried the H63D mutation of the HFE gene. Since only eight out of 45 iron-overloaded HbH patients carry a defect in the TFR2 or HFE gene in the heterozygote state and their iron loading status was comparable to the matched controls without such defects, it would appear that the accumulation of excess iron in HbH disease is more likely a result of increase dietary absorption secondary to ineffective erythropoiesis.


http://www.sciencedirect.com/science/article/B6WBV-481MTT1-1/2/462b4bfe5d7bc3f24dc9ee8456e163d3

Congenital dyserythropoietic anemias (CDA) are genetic disorders characterized by anemia and ineffective erythropoiesis. Three main types of CDA have been distinguished: CDA I, CDAII and CDA III, whose loci have been already mapped. After the identification of the locus for CDA II, also known as HEMPAS (hereditary erythroblast multinuclearity with positive acidified serum test), on the long arm of chromosome 20 (20q11.2) we have analyzed by a mutational search seven candidate genes in a large series of CDA II patients. In particular, the following genes have
been investigated: integrin beta 4 binding protein, ribophorin II, ubiquitin protein ligase ITCH, mannosil-oligosaccharide alpha-1,2-mannosidase like protein, erythrocyte protein band 4.1 like protein, zinc finger protein PLAGL2, and finally novel zinc finger protein. None of them resulted as the causative gene but several protein variants and DNA polymorphisms have been identified. These data exclude the role of the above mentioned genes in causing CDA II and add further information in the process of cloning the CDA II gene.


http://www.sciencedirect.com/science/article/B6WBV-487KHJG-8/2/3308680c19b2784fc37aae42e9aeeeef

Hereditary hemochromatosis (HH) is an autosomal recessive disease caused by mutations in the HFE gene that mainly affects populations of European descent. Recently a novel mutation (IVS5+1 G->A) has been described in a Vietnamese patient with HH that was not detected in a European control population. We have developed a novel method to screen for this mutation based on restriction enzyme digestion of a PCR product using a modified forward primer. We have screened 314 Vietnamese people from several ethnic groups and 154 people from Thailand for this mutation and have detected two heterozygotes in the Vietnamese subjects (allele frequency 0.003). Analysis of these heterozygotes indicates that the mutation is on the same haplotype as that found in the original proband. Screening for the widely distributed HFE mutation, H63D, gave an allele frequency of 0.049 in the Vietnamese subjects and 0.032 in the subjects from Thailand. This is the first report of H63D allele frequencies in these populations. We suggest that the presence of the IVS5+1 G->A and H63D mutations should be considered when investigating iron overload in Vietnamese patients and those of mixed origin as co-inheritance of both mutations is likely to be a risk factor for iron overload.


http://www.sciencedirect.com/science/article/B6WBV-4BKN0G0-2/2/8d3c058513ffdaef37e579aa7388f382

Acute intermittent porphyria (AIP) is a very rare autosomal dominant disorder with low penetrance. Mutations in the gene of the porphobilinogen deaminase (PBG-D), also called hydroxymethylbilane synthase (HMBS), cause a partial deficiency of this enzyme of the heme biosynthetic pathway. Overstimulation of heme biosynthesis causes clinical symptoms. Because of the variability of the symptoms, diagnosis is often delayed. Using two approaches for genetic analysis, first in a stepwise manner, then sequencing extensive parts of the gene, the screening of the DNA of 20 unrelated individuals revealed 20 different mutations, 11 of which had not been reported previously. The novel mutations affected intron 1 (33 + 2 T->C), exon 5 (181 G->C), intron 6 (267-61 del 8 bp), intron 7 (345-1 G->C), intron 9 (498 + 15 G->T and 499-13 [Delta]-14 bp indel TGA), intron 13 (825 + 1 G->C and 825 + 2 T->C), exon 15 (962 G-A, 1067 del A and 1067-1068 ins 5 bp). The other nine mutations detected affected intron 14, exons 6, 7, 8, 9, 10 (3x) and 12. In the majority of AIP patients, the genotype does not predict phenotypic expression. Since the sudden manifestation of the disease maybe prevented by early diagnosis, identification of AIP gene carriers is the best preventive measure. This was performed in five families, revealing 10 additional AIP gene carriers.
Objectives: To investigate the role of human papillomavirus (HPV) in the development of cervical neoplasia in women with no previous cervical cytological abnormalities; whether the presence of virus DNA predicts development of squamous intraepithelial lesion; and whether the risk of incident squamous intraepithelial lesions differs with repeated detection of the same HPV type versus repeated detection of different types. Design: Population based prospective cohort study. Setting: General population in Copenhagen, Denmark. Participants: 10 758 women aged 20-29 years followed up for development of cervical cytological abnormalities; 370 incident cases were detected (40 with atypical squamous cells of undetermined significance, 165 with low grade squamous intraepithelial lesions, 165 with high grade squamous intraepithelial lesions). Main outcome measures: Results of cervical smear tests and cervical swabs at enrolment and at the second examination about two years later. Results: Compared with women who were negative for human papillomavirus at enrolment, those with positive results had a significantly increased risk at follow up of having atypical cells (odds ratio 3.2, 95% confidence interval 1.3 to 7.9), low grade lesions (7.5, 4.8 to 11.7), or high grade lesions (25.8, 15.3 to 43.6). Similarly, women who were positive for HPV at the second examination had a strongly increased risk of low (34.3, 17.6 to 67.0) and high grade lesions (60.7, 25.5 to 144.0). For high grade lesions the risk was strongly increased if the same virus type was present at both examinations (813.0, 168.2 to 3229.2). Conclusions: Infection with human papillomavirus precedes the development of low and high grade squamous intraepithelial lesions. For high grade lesions the risk is greatest in women positive for the same type of HPV on repeated testing. What is already known on this topic Persistence of infection with human papillomavirus (HPV) is thought to have a role in the development of cervical neoplasia Previous studies have included only a few cases of high grade squamous intraepithelial lesions, and few have randomly sampled women from the general population What this study adds In women aged 20-29, HPV infection preceded the development of high grade lesions Persistent HPV infection with a specific HPV type was an indicator of incident high grade lesions among young women in the general population The association between persistence and high grade cervical lesions was more pronounced among women aged over 25
Parathyroid hormone (PTH) is a potent stimulator of osteoblastic cell function in vitro and bone resorption and formation in vivo; however, the details of the molecular mechanism(s) responsible for PTH action and the regulation of gene expression in response to PTH remain unknown. In this study, we employed an mRNA differential display (DRD) approach to examine the initial events in gene expression in human osteoblast-like Saos-2/B10 cells exposed to 10^-7 mol/L bPTH(1-34). This approach identified several differentially regulated mRNA species, including a novel paired-class homeobox protein, osteoblast-specific factor-2 (OSF-2), and a unique clone with no known sequence homology (clone G18). G18 is a previously unidentified human gene, expressed in a wide variety of human tissues, including heart, brain, placenta, skeletal muscle, and kidney, and is regulated by PTH in osteoblastic cells in vitro. This mRNA appears to be the product of a single gene, which is alternatively spliced to produce multiple transcript sizes observed in several tissues, except bone and bone-derived cells, in which a single predominant ~1.8 kb transcript is observed. Our study has identified several genes that have expression altered significantly by treatment with bPTH(1-34), and which may provide insight into the immediate effects of PTH on osteoblast-like cells and ultimately on the mechanism of action and bioactivity of PTH.


http://www.sciencedirect.com/science/article/B6T4Y-3RJPBBB-1F/2/d5bed5215a5044db78c84513fc842ba4

Mutations in the arg201 codon of the [alpha]s G protein subunit have been associated with a variety of disorders, but analysis of such mutations has been complicated by their mosaic presentation. To overcome the problems associated with the analysis of genomic mutations that may be present in low and variable yield throughout the body, a polymerase chain reaction (PCR)-based technique has been developed that allows the selective amplification of products from the mutant allele. This technique uses site-directed mutagenesis to generate a PCR product from the normal allele that is susceptible to restriction endonuclease digestion, whereas that from the mutant allele is resistant to digestion. Consecutive and repeated cycles of amplification and digestion allow selective enrichment of the product from the mutant allele. The technique has been applied to the analysis of patients with fibrous dysplasia of bone, where the consequence of G[alpha]s mutations may vary from monostotic to polyostotic lesions, and has been performed with DNA isolated from either bone biopsy specimens or peripheral blood leukocytes. In addition to the previously described arg -> his and arg -> cys substitutions, the analyses have detected a novel arg -> ser substitution in one of the patients. This patient presented with a panostotic disease and may represent a unique subgroup of fibrous dysplasia.


http://www.sciencedirect.com/science/article/B6T4Y-3VGKPV5-J/2/417a2149729490b21562ff6022f3c028

Bone loss is observed after exposure to weightlessness in both astronauts and inflight animals. Histological and biochemical studies on rats have shown a decrease in bone formation, probably as a result of altered osteoblast function. To investigate whether microgravity alters osteoblast differentiation in vitro, the human osteosarcoma cell line MG-63 was used as a model. MG-63
cells can be induced to differentiate by treating the cells with 1,25(OH)2D3 (10-7 mol/L) and transforming growth factor-beta 2 (TGF[beta]2) (10 ng/mL). The message level of differentiation-related genes was quantitated via competitive reverse transcription-polymerase chain reaction (RT-PCR), both in untreated and hormone-treated cells cultured under microgravity for 9 days aboard the unmanned Foton 10 spaceflight, and compared to ground and inflight unit-gravity cultures. At microgravity, gene expression for collagen I[alpha]1 following treatment was reduced to 51% of unit-gravity levels (p<0.02). In conclusion, microgravity reduces the differentiation of osteoblastic MG-63 cells in response to systemic hormones and growth factors.


http://www.sciencedirect.com/science/article/B6T4Y-4CYNT1G-1/2/0d141cb52d79031eb4acbf45ea82e80bd

Bisphosphonates (BPs) are widely used in the treatment of a variety of bone-related diseases, particularly where the bone turnover is skewed in favor of osteolysis. The mechanisms by which BPs reduce bone resorption directly acting on osteoclasts are now largely clarified even at molecular level. Researches concerning the BP's effects on osteoblast have instead shown variable results. Many in vitro studies have reported positive effects on osteoblasts proliferation and mineralization for several BPs; however, the observed effects differ, depending on the variety of different model system that has been used. Objectives. We have investigated if neridronate, an aminobisphosphonate suitable for pulsatory parenteral administration, could have an effect on human osteoblastic proliferation and differentiation in vitro. Methods. We have investigated whether prolonged addition of neridronate (from 10-3 to 10-11 M) to different human osteoblasts cultures, obtained from 14 different bone specimens, could affect the cells number, the endogenous cellular alkaline phosphatase (ALKP) activity, and the formation of mineralized nodules. Results. Our results show that neridronate does not negatively affect in vitro the viability, proliferation, and cellular activity of normal human osteoblasts even after a long period addition of the drug (20 days) at concentrations equal or lower than 10-5 mol/l (therapeutic dose). In addition, neridronate seems to enhance the differentiation of cultured osteoblasts in mature bone-forming cells. A maximum increase of alkaline phosphatase activity (+50% after 10 days; P<0.001). Conclusions. These results encourage the use of neridronate in long-term therapy of demineralizing metabolic bone disorders.


http://www.sciencedirect.com/science/article/B6T4Y-42YF54G-5/2/fd81755726a256e87db3b8c8379af770

Bone morphogenetic protein (BMP)-2, a member of the transforming growth factor-[beta] (TGF-[beta]) superfamily, is able to induce osteoblastic differentiation of C2C12 cells. Both Smad and mitogen-activated protein kinase (MAPK) pathways are essential components of the TGF-[beta] superfamily signaling machinery. Although Smads have been demonstrated to participate in the BMP-2-induced osteoblastic differentiation of C2C12 cells, the role of MAPK has not been addressed. This report shows that BMP-2 activates ERK and p38, but not JNK, in C2C12 cells. Pretreatment of cells with the p38 inhibitor, SB203580, dramatically reduced BMP-2-induced expression of the osteoblast markers alkaline phosphatase (ALP) and osteocalcin (OC). Nevertheless, overexpression of MKK3, a protein kinase that phosphorylates and activates p38, failed to induce ALP or OC expression in the absence of BMP-2, indicating that p38 activation is
necessary but not sufficient for the acquisition of the osteoblast phenotype by these cells. Although ALP induction was increased slightly in the presence of PD-98059, a selective inhibitor of the ERK cascade, this compound significantly inhibited both steady-state and BMP-2-induced OC RNA levels. Our results indicate that p38 and ERK cascades play a crucial role in the osteoblast differentiation of C2C12 cells mediated by BMP-2.


We have conducted a genome-wide scan on a pedigree containing 372 adult members, of whom 49 have PDB. In the present study, we report linkage of a large pedigree to the PDB3 region on chromosome 5q35-qter with a peak multipoint LOD score of 6.77. Sequestosome 1 (SQSTM/p62) has been identified as the causative PDB gene in this region. Six sequestosome 1 mutations have been described to date. Four mutations have been identified in exon 8, 1210delT and 1215delC both resulting in premature stop codon at amino acid 394, 1215C to T (P392L), 1224insT (E396X), one mutation in exon 7, 1200C to T (P387L) and a G to A splice junction mutation at IVS7+1. These mutations cluster in the C terminus of the protein and are predicted to disrupt the ubiquitin binding properties of sequestosome 1. Sequence analysis of the gene encoding sequestosome 1 revealed a single base pair deletion (1215delC) segregating with the majority of affected members in the pedigree. This deletion introduces a stop codon at position 394, resulting in premature termination of the protein (L394X) and loss of the ubiquitin-associated binding domain. Screening of affected members from 10 further PDB families identified the previously reported P392L mutation in one family. No SQSTM1/p62 coding mutations were found in the remaining 9 families or in 113 age-matched controls.


Osteoarthritis (OA) is a common age-related joint disease resulting in progressive degenerative damage to articular cartilage. The etiology of primary OA has not yet been determined. However, there is evidence supporting the hypothesis that primary OA is a disease affecting bone remodeling in addition to articular cartilage. In this study, we have used cDNA microarray analysis to compare gene expression in bone between normal (CTL) and OA individuals. Trabecular bone was sampled from the intertrochanteric region of the proximal femur, a site distal to the diseased hip joint. Total RNA was extracted from three pairs of age- and sex-matched CTL and OA bone samples, reverse-transcribed and radioactively labeled to generate cDNA probes, before hybridization with the Research Genetics GF211 human gene microarray filter. The CTL and OA samples were found to have similar levels of gene expression for more than 4000 known human genes. However, forty-one genes were identified that were differentially expressed, twofold or more, between all three CTL-OA sample pairs. Using semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis, three genes, fms-like tyrosine kinase 1 (FLT1), plexin B1 (PLXNB1), and small inducible cytokine A2 (SCYA2), were confirmed to be consistently expressed at lower levels in OA, in a majority of twenty age- and sex-matched CTL-OA bone sample pairs tested. FLT1, PLXNB1, and SCYA2 have known or potential roles in angiogenesis and bone remodeling. Down-regulation of these genes is consistent with a role for bone in the pathogenesis of OA.
We determined the effect of basic fibroblast growth factor (bFGF) on osteoclast-like cell (OCL) formation in bone marrow cultures using C57BL/6 mice. Cells were cultured for 7 days with or without bFGF at various concentrations or 10-8 mol/L 1,25(OH)2 vitamin D3 [1,25(OH)2D3]. bFGF dose-dependently increased OCL formation per well (10-10 mol/L = 40 +/- 2; 10-9 mol/L = 146 +/- 13; 10-8 mol/L = 156 +/- 12) compared with control (-9 and 10-8 mol/L were similar to that of 10-8 mol/L 1,25(OH)2D3 (154 +/- 11 per well). OCLs formed by bFGF were multinuclear, tartrate-resistant acid phosphatase (TRAP)-positive, expressed calcitonin receptors, and formed characteristic resorption pits. We also determined whether bFGF enhanced OCL formation during the early proliferative or late differentiating phases of the cultures. When bFGF (10-8 mol/L) was added only on days 1-2 or days 3-4 of 6 day cultures, there was a significant increase in OCL formation. In contrast, when bFGF was added only on days 5-6 few OCLs formed. Addition of bFGF at days 1-6 or days 1-2 and days 5-6 caused similar increases in OCL formation, which were greater than OCL formation induced by treatment for days 1-2 or days 1-4. We examined the production of prostaglandin E2 (PGE2) in the cultures because bFGF is a potent stimulator of PGE2 synthesis in bone, and PGE2 stimulates OCL formation. bFGF treatment significantly increased PGE2 levels in 7 day cultures (controls = 1.4 +/- 0.1 nmol/L, 10-8 mol/L bFGF = 132.5 +/- 0.7 nmol/L). In addition, treatment of marrow cultures with the prostaglandin synthesis inhibitors, indomethacin or NS-398 (both at 10-6 mol/L), completely blocked bFGF-induced OCL formation. We conclude that bFGF stimulates OCL formation in C57BL/6 bone marrow cultures by mechanisms that require prostaglandin synthesis. This pathway is likely to be one mechanism by which bFGF stimulates resorption.


Prostaglandin (PG) E2 displays physiological and pharmacological action in various tissues including bone. It increases intracellular Ca, and stimulates or inhibits CAMP production through the PGE receptor subtypes EP1, EP2, and EP3 respectively. These receptor subtypes have been recently cloned. In the present study, we investigate the expression of these receptor subtypes in bone tissue. RT-PCR revealed that EP1, EP2, and EP3 were expressed in rat calvariae and that osteoblastic cells (MC3T3-E1) expressed EP1 and EP2. In situ hybridization analysis using cryosection of neonatal calvariae revealed that EP2 was expressed by osteoblasts and cells not in contact with bone, probably including preosteoblasts. EP2 expression was observed at an early stage in calvarial development, at 14 days prenatal. EP2 expression was also observed at day 3 in rat bone marrow cell culture in which bone-like mineralized nodules are formed at day 8. It has been established that PGE2 response accompanying cAMP production is one of the characteristics of osteoblasts. The present results indicate that this phenotype appears at an early stage of osteoblastic differentiation and bone development.

Kitahama, S., M. A. Gibson, et al. (2000). "Expression of fibrillins and other microfibril-associated proteins
Fibrillin-containing microfibrils are structural components of extracellular matrices of a diverse range of tissues, including bone. Their importance in bone biology is illustrated by the skeletal abnormalities manifest in the congenital disorder, Marfan syndrome, which results from mutations in the fibrillin-1 gene. We investigated the expression of fibrillins and other microfibril-associated proteins in human bone and bone-derived osteoblasts. Analysis of RNA extracted from cancellous bone showed expression of mRNAs encoding fibrillin-1 and -2, MAGP-1 and -2, LTBP-2, and MP78/70 (Big-h3). In demineralized normal mature bone, fibrillin-1 was immunolocalized to fibrils within the bone matrix and pericellularly to cells lining the endosteal surfaces of trabecular bone, some osteocytes, and cells associated with blood vessels. LTBP-2 was also identified at the endosteal surface and within the bone matrix in a lamellar fashion. In addition, primary osteoblast-like cells cultured from human trabecular bone (obtained from patients at joint replacement surgery) were found to express abundant mRNA for fibrillins and associated glycoproteins. Moreover, using western blot analysis, fibrillin-1 protein was shown to be secreted into the medium and to be deposited into the cell layer. Immunofluorescence staining of the cell layer visualized fibrillin-1 in the matrix as a three-dimensional network of fine filaments. Expression of fibrillin-1 by osteoblast-like cells was constitutive, and a number of skeletally active agents had little effect on mRNA or protein levels. These results show that human osteoblasts from mature bone express fibrillins and other microfibril-associated proteins, and suggest a role for these molecules in adult human bone.


Transforming growth factor (TGF)-[beta]1 is the most abundant growth factor in human bone. It is produced by osteoblasts and inhibits osteoclast proliferation and activity and stimulates proliferation and differentiation of preosteoblasts. Several polymorphisms have been described in the TGF-[beta]1 gene. Previously, we and others have found associations between some of these polymorphisms and bone mass. We therefore wanted to examine if these polymorphisms are also predictors of osteoporotic fractures. The polymorphisms G-1639-A, C-1348-T, C-765insC, T29-C, G74-C, 713-8delC, C788-T, and T816-20-C were examined using RFLP and sequencing in 296 osteoporotic patients with vertebral fractures and 330 normal individuals. Bone mineral density (BMD) was examined at the lumbar spine and at the femoral neck by DXA. Genotype distributions were in H-W equilibrium. Linkage disequilibrium was found between the polymorphisms. The T816-20-C genotypes were distributed differently among osteoporotic patients and normal controls. The TT genotype was less common in individuals with osteoporotic fractures ([chi]2 = 6.02, P 816-20-C) at the lumbar spine, 0.960 +/- 0.173 g/cm2 compared with individuals with the TC or CC genotypes: 0.849 +/- 0.181 g/cm2 and 0.876 +/- 0.179 g/cm2, respectively (P -1348-T) had higher bone mass at the femoral neck: 0.743 +/- 0.134 g/cm2 compared with 0.703 +/- 0.119 g/cm2 in individuals with TC or CC genotypes (P 2 vs. 0.818 +/- 0.149 g/cm2, respectively (P 816-20-C polymorphism is less common in patients with osteoporotic fractures and is associated with higher bone mass both at the lumbar spine and at the hip. The C-1348-T and T29-C polymorphisms were distributed similarly in osteoporotic patients and normal controls, however, the rare genotypes were associated with higher bone mass at the hip.
Langdahl, B. L., J. Y. Knudsen, et al. (1997). "A sequence variation: 713-8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women." Bone 20(3): 289.

Bone mass is partly genetically determined. The genes involved are, however, still largely unknown. Transforming growth factor-beta 1 (TGF-[beta]1) is considered a putative regulator of osteoclastic-osteoblastic interaction (coupling). The aim of the present study was therefore to examine whether possible variants of the TGF-[beta]1 gene are related to bone mass and osteoporosis. We examined 161 osteoporotic women (at least one low energy spinal fracture) and 131 normal women. We investigated sequence variations in the TGF-[beta]1 gene using the single-stranded conformation polymorphism (SSCP) technique combined with DNA sequencing. Seven patients were heterozygous for a cytosine to thymidine base substitution at position 76 in exon 5 (C788-T) (corresponding to position 788 in the TGF-[beta]1 cDNA), resulting in a threonine to isoleucine amino acid shift at position 263 in the TGF-[beta]1 propeptide (Thr263-Ile). Ten other patients had a one base deletion in the intron sequence 8 bases prior to exon 5 (713-8delC), which could influence splicing. Five normal women exhibited the C788-T sequence variant, and two the 713-8delC. The prevalence of 713-8delC was significantly higher in the osteoporotic group ([chi]2 = 4.02, p < 0.05). The sequence variation, 713-8delC, in the TGF-[beta]1 gene is more frequent in patients with osteoporosis compared to normal controls. The 713-8delC variant seems to be associated with very low bone mass in osteoporotic women with low bone mass and increased bone turnover in both osteoporotic and normal women.


We studied differences in ectopic osteoinduction in eight mouse inbred strains and an outbred strain. Antigen-extracted autolyzed rat bone gelatin was implanted under hind limb muscle fascia of 12-week-old males, and new bone formation was morphologically assessed on serial sections. Four weeks after implantation, less than half of the implants from CBA/J, A/J, BALB/cJ, and C3Hf/Bu mice showed induction of only cartilage. New cartilage was observed in all, and bone and bone marrow in 80% of the implants from AKR/J, C57BL/6J, DBA/2J, and RFM/Rij mice. Volume of the newly formed tissue ranged from 1.3% of the old matrix in A/J strain to 74.6% in DBA/2J strain. Outbred CD1 mice showed only weak cartilage induction. The "good" responders differed among themselves in the volume and type of newly induced tissue: DBA/2J, RFM/Rij, and AKR/J mice had a similar ratio of new bone and cartilage and abundant bone marrow, whereas the predominant newly induced tissue in C57BL/6J mice was cartilage. The pattern of the expression of BMP-2, -4, and -7, alkaline phosphatase, osteocalcin, interferon-[gamma], and granulocyte-macrophage colony-stimulating factor, measured by reverse transcriptase polymerase chain reaction, did not correlate with the type and the quantity of the newly induced tissue. Our results show that adult mice of inbred strains differ not only in the peak bone mass and morphology, but also ability to form new bone after an osteoinductive stimulus. Ectopic osteoinduction may be a useful in vivo model to investigate genetic determinants of endochondral osteogenesis, especially its immunological component.
Nelson, A. E., R. S. Mason, et al. (1998). "Tumor expression studies indicate that HEM-1 is unlikely to be the active factor in oncogenic osteomalacia." Bone 23(6): 549.

http://www.sciencedirect.com/science/article/B6T4Y-3V5TMGJ-9/2/4a74de1eebd1f2a4623cfa0c2c2793

HEM-1 was isolated as a putative factor responsible for oncogenic osteomalacia by Kumar et al. (Proc Assoc Am Phys 107:296-305; 1995). The cDNA was identified on the basis of PTH-like immunoreactivity; however, no studies have been reported of the expression of HEM-1 mRNA in oncogenic osteomalacia tumors. In this study, expression of HEM-1 mRNA was investigated in two oncogenic osteomalacia tumors and in a series of normal tissues. An HEM-1 PCR product was amplified from a cDNA library from one of the tumors, with six base changes identified, as compared with the published sequence. No expression was detected, however, in the oncogenic osteomalacia tumors either by Northern blot analysis or by reverse transcriptase PCR. This indicates that, although a region of HEM-1 sequence is present in the tumor cell cDNA library, any HEM-1 expression must be at very low levels. It is unlikely, therefore, that the HEM-1 product is the active factor responsible for oncogenic osteomalacia. In the normal tissues examined, human placenta, fibroblasts, parathyroid gland, liver, fetal bone, and rat kidney cortex, HEM-1 mRNA was not detected, suggesting that it does not have a physiological role in these tissues.


http://www.sciencedirect.com/science/article/B6T4Y-4FM5D26-9/2/e1bb8c311437401c68354c3e89313f11

The pathogenesis of osteoporosis involves both genetic and environmental factors. On the basis of linkage data suggesting gene effects on bone density at chromosome 14q and data locating the BMP4 gene to 14q, we performed a positional candidate study to examine a possible association of BMP4 gene polymorphisms, hip bone density (n = 1012) and fracture rates (n = 1232) in postmenopausal women (mean age 75). On genotype analysis of the three selected single nucleotide polymorphisms (SNP), the 6007C > T polymorphism was associated with total and intertrochanteric hip BMD and BMD was lower in the 32% of subjects homozygous for the C allele. This polymorphism codes for a nonsynonymous amino acid change with the T allele coding for valine, while the C allele codes for alanine. The difference in BMD was 3.1% (TT vs. CC) and 2.3% (CT versus CC) for the total hip (P = 0.023), and 3.7% (TT vs. CC) and 2.8% (CT versus CC) for the intertrochanter site (P = 0.012). Haplotype analysis demonstrated 6 haplotypes of frequency greater than 2%. A major haplotype defined by G-C-T alleles in SNPs -5826G > A, 3564C > T and 6007C > T respectively, showed association with high bone mass. No SNP showed association with fracture rates. We conclude that a polymorphism found in the BMP4 gene, affecting amino acid sequence, is associated with hip bone density in postmenopausal women, presumably via regulation of anabolic effects on the skeleton.


http://www.sciencedirect.com/science/article/B6T4Y-3Y6HHKJ-3G/2/2c3df3f0d8b4be61fe2eef344f7c52aac9
Tartrate-resistant acid phosphatase (TRAP) is expressed at high levels in osteoclasts and may play an important role in the bone resorptive process. However, factors regulating human TRAP gene expression have not been clearly defined. Therefore, we isolated a genomic clone (CL-9) for TRAP containing a 14-kb insert. A restriction map was generated for this insert, and a 2.6-kb Apal fragment containing the 5'-flanking region was subcloned. Sequence analysis of this fragment revealed the presence of candidate transcription factor-binding sequences for H-APF-1, SP1, GATA2, and the c-Myc proto-oncogene. PCR analysis of RNA isolated from human osteoclastomas and pagetic bone revealed a 276-bp intron at -1 by to -276 bp relative to the ATG and a transcript originating from this intron. Rapid amplification of the 5' end of the human TRAP mRNA by PCR indicated the presence of a 93-bp untranslated region 5' from the intron. Promoter activity was detected in the DNA fragment from +1 bp to -1903 by relative to the ATG initiation codon, which drove the transient expression of a luciferase reporter gene when transfected into HRE H9 rabbit endometrial cells. Comparison of the human TRAP 5'-flanking region with mouse TRAP and uteroferrin revealed 41% and 47% homology, respectively. This suggests that regulation of human TRAP gene expression may differ from that for the murine TRAP gene.


Silicon deficiency in animals leads to bone defects. This element may therefore play an important role in bone metabolism. Silicon is absorbed from the diet as orthosilicic acid and concentrations in plasma are 5-20 \([\mu]\)M. The in vitro effects of orthosilicic acid (0-50 \([\mu]\)M) on collagen type 1 synthesis was investigated using the human osteosarcoma cell line (MG-63), primary osteoblast-like cells derived from human bone marrow stromal cells, and an immortalized human early osteoblastic cell line (HCC1). Collagen type 1 mRNA expression and prolyl hydroxylase activity were also determined in the MG-63 cells. Alkaline phosphatase and osteocalcin (osteoblastic differentiation) were assessed both at the protein and the mRNA level in MG-63 cells treated with orthosilicic acid. Collagen type 1 synthesis increased in all treated cells at orthosilicic acid concentrations of 10 and 20 \([\mu]\)M, although the effects were more marked in the clonal cell lines (MG-63, HCC 1.75- and 1.8-fold, respectively, \(P < 0.004\)). The effect of orthosilicic acid was abolished in the presence of prolyl hydroxylase inhibitors. No change in collagen type 1 mRNA level was seen in treated MG-63 cells. Alkaline phosphatase activity and osteocalcin were significantly increased (1.5, 1.2-fold at concentrations of 10 and 20 \([\mu]\)M, respectively, \(P < 0.05\)). Gene expression of alkaline phosphatase and osteocalcin also increased significantly following treatment. In conclusion, orthosilicic acid at physiological concentrations stimulates collagen type 1 synthesis in human osteoblast-like cells and enhances osteoblastic differentiation.


Skeletal unloading results in an inhibition of bone formation associated with a decrease in osteoblast number, impaired mineralization of bone, and altered proliferation and differentiation of osteoprogenitor cells. Although such changes are likely to be mediated by multiple factors, resistance to the growth-promoting action of insulin-like growth factor I (IGF-I) has been hypothesized to play an important role. To determine whether skeletal unloading induces resistance to IGF-I on bone formation, we examined the response of unloaded (hindlimb
elevation) and normally loaded tibia and femur to IGF-I administration. To eliminate the variable of endogenous growth hormone production and secretion during exogenous IGF-I administration, we used growth hormone-deficient dwarf rats (dw-4). The rats were given IGF-I (2.5 mg/kg/day) or vehicle during 7 and 14 days of unloading or normal loading. This significantly increased the serum level of IGF-I in both the normally loaded and unloaded rats. Unloading did not affect the serum level of IGF-I in the vehicle-treated rats. IGF-I markedly increased periosteal bone formation at the tibiofibular junction of normally loaded rats. Unloading decreased bone formation in the vehicle-treated rats, and blocked the ability of IGF-I to increase bone formation. On the other hand, IGF-I increased periosteal bone formation at the midpoint of the humerus (normally loaded in this model) in both hindlimb-elevated and normally loaded rats. IGF-I significantly increased osteogenic colony number, total ALP activity, and total mineralization in bone marrow osteoprogenitor (BMOp) cells of normally loaded rats. Unloading reduced these parameters in the vehicle-treated rats, and blocked the stimulation by IGF-I. Furthermore, IGF-I administration (10 ng/ml) in vitro significantly increased cell proliferation of the BMOp cells isolated from normally loaded bone, but not that of cells from unloaded bone. These results indicate that skeletal unloading induces resistance to IGF-I on bone formation.


Interleukin-6 (IL-6) has been attributed to induction of osteoclastogenic-precursor cell proliferation and maturation. Estrogens suppress IL-6 production in stromal/osteoblastic cells in vitro. Conversely, estrogen withdrawal is associated with increased IL-6 production. IL-6 is therefore thought to be an important mediator of the increased bone resorption after menopause. However, evidence supporting a rise in the expression of IL-6 or the IL-6 receptor in human bone tissue with menopause is still lacking. To address this question, we established a 5'-nuclease assay to quantitate the expression of human IL-6 and the gp80 subunit of the IL-6 receptor in human bone samples. The number of mRNA copies was normalized to the number of copies of beta actin mRNA. Osteocalcin expression served as an independent control. The study population consisted of 169 women (mean age 52.4 +/- 11.6 years) who underwent surgery for early breast cancer. Serum IL-6 was measured by enzyme-linked immunosorbsent assay, serum crosslaps as a marker of bone resorption were measured by electrochemiluminescent assay, and serum osteocalcin was measured by chemoluminescence assays. RNA expression of osteocalcin in bone tissue from early postmenopausal women was higher compared with premenopausal women. Local expression was positively associated with circulating osteocalcin and crosslaps concentrations. Postmenopausal women also had higher circulating IL-6 concentrations. In contrast, bone samples from postmenopausal women lacked an increased expression of either IL-6 or gp80 compared with bone samples from premenopausal women. In conclusion, we failed to detect local increases in IL-6 or IL-6 receptor expression in human bone tissue with menopause. If direct changes in the IL-6 system in bone tissue are involved in postmenopausal bone loss, these changes appear to be below the detection limit of our assay system.


Ferritin, a metal-binding protein responsible for maintaining the bioavailability of iron, has been
demonstrated in cells of the osteoblastic lineage. Messenger RNAs encoding the light and heavy chain subunits of ferritin were detected in ROS 17/2.8, ROS 25/1, and UMR106 rat osteosarcoma cell lines, in fetal rat calvaria, and in primary cultures of rat calvarial osteoblast-like cells. In vivo, the expression of ferritin light-chain mRNA was observed in both active osteoblasts and in osteocytes. A 450-kD iron-binding protein was immunoprecipitated from ROS 17/2.8 cells by an antiferritin antiserum. This protein comigrated with native ferritin, and could be dissociated into subunits comigrating with ferritin light and heavy chains. Addition of extracellular Fe59-transferrin to cultures of ROS 17/2.8 cells resulted in the sequestration of the iron in intracellular ferritin. These observations demonstrate that cells of the osteoblastic lineage possess a functional ferritin-based iron uptake and storage system capable of regulating metal homeostasis in bone.


http://www.sciencedirect.com/science/article/B6T4Y-4447H0C-2/2/6a0d95cc6a20a02acb7bbdf6e63e3fd4

Several members of the transforming growth factor-[beta] (TGF-[beta]) superfamily have been demonstrated to play regulatory roles in osteoblast differentiation and maturation, but the mechanisms by which these cells at different developmental stages remain largely unknown. We studied the effects of TGF-[beta]1 and bone morphogenetic protein-2 (BMP-2) on the differentiation/maturation of osteoblasts using the murine cell lines MC3T3-E1 and C3H10T1/2. BMP-2 induced or enhanced the expression of the osteoblast differentiation markers alkaline phosphatase (ALP) and osteocalcin (OC) in both cells. In contrast, TGF-[beta]1 was not only unable to induce these markers, but it dramatically inhibited BMP-2-mediated OC gene expression and ALP activity. In addition, TGF-[beta]1 inhibited the ability of BMP-2 to induce MC3T3-E1 mineralization. TGF-[beta]1 did not sensibly modify the increase of Osf2/Cbfa1 gene expression mediated by BMP-2, thus demonstrating that the inhibitory effect of TGF-[beta]1 on osteoblast differentiation/maturation mediated by BMP-2 was independent of Osf2/Cbfa1 gene expression. Finally, it is shown that TGF-[beta]1 does not affect BMP-2-induced Smad1 transcriptional activity in the mesenchymal pluripotent cells studied herein. Our data indicate that in vitro BMP-2 and TGF-[beta]1 exert opposite effects on osteoblast differentiation and maturation.


http://www.sciencedirect.com/science/article/B6T4Y-4BX2KR5-26/2/d89bc1bf0f18f265200669f2b0517aaa

Osteogenesis imperfecta (OI) is a group of inherited disorders characterized by a predisposition to bone fracturing, and usually resulting from mutations in the genes encoding type I collagen. This report describes the molecular defects in a patient with type II OI and another with type III OI. These patients were demonstrated to possess point mutations resulting in glycine -> arginine substitutions within the triple helical domain of the [alpha]1(I) or [alpha]2(I) collagen polypeptide chain. The defect in the type II OI patient affected residue 211 of the [alpha]1(I) triple helical domain, and constitutes the most amino-terminal lethal glycine -> arginine substitution described to date. The substitution in the type III OI patient affected residue 427 of the [alpha]2(I) triple helical domain. Both defects were informative in that they identified the regions of the [alpha]1(I) and [alpha]2(I) collagen chains in which the phenotypes associated with glycine -> arginine substitutions undergo a transition between lethal and nonlethal forms, thereby allowing a more reliable prognosis of disease severity. The histological examination of bone from these patients...
revealed striking abnormalities in the quantity and organization of mineralized bone structures, compared with age-matched controls. Although the patients were differently classified, no major differences in the magnitude of bone architectural changes could be perceived, consistent with the presence of their defects near a common phenotypic transition. The results are compatible with there being a gradient in severity between OI types II and III, and that parameters external to the gene mutations might account for the survival differences in the 2 cases presented in this study.


Several lines of evidence suggest that vitamin K has nutritional and pharmacological effects against bone loss. To clarify effects of vitamin K on bone marrow cells, which contains progenitors of both osteoblasts and osteoclasts, we examined mouse bone marrow cell cultures in the presence of vitamin K1 (K1) and menatetrenone (MK4), a vitamin K2 with four isoprene units. Treatment with MK4 but not K1 inhibited the formation of adipocytes and stimulated alkaline phosphatase activity, an early differentiation marker of osteoblast. Although nuclear receptor PPAR[gamma]2 plays a pivotal role in adipogenesis, MK4 had no effects on the expression of PPAR[gamma]2 mRNA and PPAR[gamma]2-dependent transcriptional activity. MK4 inhibited the expression of osteoclast differentiation factor (ODF)/RANK ligand and the formation of osteoclast-like cells induced by 1,25-dihydroxyvitamin D3. These results suggest that MK4 specifically influences differentiation and functions of bone marrow cells to inhibit adipogenesis and osteoclastogenesis. At the expense of adipogenesis, MK4 might stimulate osteoblastogenesis in bone marrow cells. Therefore, MK4 may favor bone metabolism to spare bone mass as a compound that modulates cellular differentiation and functions in bone marrow in addition to as a nutrient factor.

Br. J. Ophthalmol. (4)


http://bjo.bmjournals.com/cgi/content/abstract/86/3/328

Background: Besides the three known genes (RHO, RDS/Peripherin, NRL) involved in autosomal dominant retinitis pigmentosa (adRP), a fourth gene, RP1, has been recently identified. Initial reports suggest that mutations in the RP1 gene are the second most frequent cause of adRP. The clinical findings were described in a family with adRP and a novel mutation in the RP1 gene. Method: Index patients from 15 independent families with adRP in which RHO mutations had been excluded in previous examinations were screened for mutations in the RP1 gene by means of direct DNA sequencing. Evaluation of the RP1 phenotype in patients included funduscopby, kinetic perimetry, dark adapted final threshold test, standard electroretinography and, in one case, multifocal electroretinography. Results: One novel nonsense mutation (Lys778ter) in one of these 15 patients was detected. Cosegregation of the mutation with the disease phenotype could
be established in the index patient's family. The phenotype comprises variable expression of clinical disease probably including one case of incomplete penetrance, a onset of symptoms beginning in adulthood, and evidence of regionally varying retinal function loss. Conclusion: The Lys778ter mutation localises inside the critical region harbouring all mutations described so far. The ophthalmic findings support previous observations that variation of disease expression appears as a typical feature of the RP1 phenotype.


http://bjo.bmjournals.com/cgi/content/abstract/87/7/893

Aim: To characterise the phenotype and identify the underlying genetic defect in a family with deafness segregating with a North Carolina-like macular dystrophy (NCMD). Methods: Details of the family were obtained from the Moorfields Eye Hospital genetic clinic database and comprised eight affected, four unaffected members, and two spouses. Pedigree data were collated and leucocyte DNA extracted from venous blood. Positional candidate gene and genetic linkage strategies utilising polymerase chain reaction (PCR) based microsatellite marker genotyping were performed to identify the disease locus. Results: The non-progressive ocular phenotype shared similarities with North Carolina macular dystrophy. Electro-oculography and full field electroretinography were normal. Progressive sensorineural deafness was also present in all affected individuals over the age of 20 years. Hearing was normal in all unaffected relatives. Haplotype analysis indicated that this family is unrelated to previously reported families with NCMD. Genotyping excluded linkage to the MCDR1 locus and suggested a potential novel disease locus on chromosome 14q (Z=2.92 at (theta)=0 for marker D14S261). Conclusion: The combination of anomalies segregating in this family represents a novel phenotype. This molecular analysis indicates the disease is genetically distinct from NCMD.


http://bjo.bmjournals.com/cgi/content/abstract/86/7/767

Background: Glaucomatous neuropathy is a type of cell death by apoptosis. The p53 gene is one of the regulatory genes of apoptosis. Recently, p53 codon 72 polymorphism has been extensively studied to determine the risk factors responsible for many diseases. In the p53 gene, a single base change from G to C causes the alternation of amino acid residue 72 from arginine to proline. In this study the association between p53 codon 72 polymorphism and primary open angle glaucoma (POAG) patients was evaluated. Methods: 58 POAG patients and 59 healthy volunteers were enrolled in this study. Polymerase chain reaction based analysis was used to resolve the p53 codon 72 polymorphism. Results: There were significant differences in the distribution of the polymorphism between the control subjects and the POAG patients (p = 0.00782) The proline form of p53 gene codon 72 appears to be a significant risk factor in the development of POAG (odds ratio 2.389, 95% confidence interval: 1.14 to 5.01). Conclusions: Retinal ganglion cells die during POAG by apoptosis. The tumour suppressor protein, p53, is one of the primary regulators steps of apoptosis, and the results of our study are compatible with this concept.

http://bjo.bmjournals.com/cgi/content/abstract/88/6/752

Aim: To determine the disease causing gene defects in two patients with Meesmann's corneal dystrophy. Methods: Mutational analysis of domains 1A and 2B of the keratin 3 (K3) and keratin 12 (K12) genes from two patients with Meesmann's corneal dystrophy was performed by polymerase chain reaction amplification and direct sequencing. Results: Novel mutations of the K12 gene were identified in both patients. In one patient a heterozygous point mutation (429A[-&gt;]C = Arg135Ser) was found in the 1A domain of the K12 gene. This mutation was confirmed by restriction digestion. In the second patient a heterozygous 27 bp duplication was found inserted in the 2B domain at nucleotide position 1222 (1222ins27) of the K12 gene. This mutation was confirmed by gel electrophoresis. The mutations were not present in unaffected controls. Conclusion: Novel K12 mutations were linked to Meesmann's corneal dystrophy in two different patients. A missense mutation replacing a highly conserved arginine residue in the beginning of the helix initiation motif was found in one patient, and an insertion mutation, consisting of a duplication of 27 nucleotides, was found before the helix termination motif in the other.

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http://brain.oupjournals.org/cgi/content/abstract/126/6/1293

The autosomal dominant cerebellar ataxias (ADCA) are a clinically, pathologically and genetically heterogeneous group of disorders. Ten responsible genes have been identified for spinocerebellar ataxia types SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12 and SCA17, and dentatorubral pallidoluysian atrophy (DRPLA). The mutation is caused by an expansion of a CAG, CTG or ATTCT repeat sequence of these genes. Six additional loci, SCA4, SCA5, SCA11, SCA13, SCA14 and SCA16 have also been mapped. The growing heterogeneity of the autosomal dominant forms of these diseases shows that the genetic aetiologies of at least 20% of ADCA have yet to be elucidated. We ascertained and clinically characterized a four-generation Chinese pedigree segregating an autosomal dominant phenotype for cerebellar ataxia. Direct mutation analysis, linkage analysis for all known SCA loci and a genome-wide linkage study were performed. Direct mutation analysis excluded SCA1, 2, 3, 6, 7, 8, 10, 12, 17 and DRPLA, and genetic linkage analysis excluded SCA4, 5, 11, 13, 14 and 16. The genome-wide linkage study suggested linkage to a locus on chromosome 1p21-q23, with the highest two-point LOD score at D1S1167 (Zmax = 3.46 at (theta) = 0.00). Multipoint analysis and haplotype reconstruction traced this novel SCA locus (SCA22) to a 43.7-cM interval flanked by D1S206 and D1S2878 (Zmax = 3.78 under four liability classes, and 2.67 using affected-only method). The age at onset ranged from 10 to 46 years. All affected members had gait ataxia with variable features of dysarthria and hyporeflexia. Head MRI showed homogeneous atrophy of the cerebellum without involvement of the brainstem. In six parent-child pairs, median onset occurred 10 years earlier in offspring than in their parents, suggesting anticipation. This family is distinct from other families with SCA and is characterized by a slowly progressive, pure cerebellar ataxia.

http://brain.oupjournals.org/cgi/content/abstract/127/12/2693

The pathophysiological mechanisms underlying the development of spasticity are not clear, but the excitability of the disynaptic reciprocal inhibitory pathway is affected in many patients with spasticity of different origin. Patients with genetically identified autosomal dominant pure spastic paraparesis (ADPSP) develop spasticity and paresis in the legs, but usually have no symptoms in the arms. Comparison of the spinal and supraspinal control of the legs and arms in these patients may therefore provide valuable information about the pathophysiology of spasticity. In the present study, we tested the hypothesis that one of the pathophysiological mechanisms of spasticity in these patients is abnormal corticospinal transmission and that this may lead to decreased reciprocal inhibition. Ten patients and 15 healthy age-matched control subjects were investigated. The patients were all spastic in the legs (with hyperactive tendon reflexes, increased muscle tone and Babinski sign), but had no neurological symptoms in the arms (except for one patient). Disynaptic reciprocal Ia inhibition of flexor carpi radialis (FCR) and soleus (SOL) motoneurons was measured (as the depression of the background FCR and SOL EMG activity and as the short latency inhibition of the FCR and SOL H-reflex evoked by radial and peroneal nerve stimulation). In addition, the latency of motor evoked potentials (MEPs) in the FCR muscle and the tibialis anterior (TA) muscle was measured. In the patients, the mean reciprocal inhibition was normal in the arms, while it was significantly decreased in the leg compared with the healthy subjects. In the patients, the average latency of MEPs in the FCR muscle was normal, while the latency to the MEP in TA muscle was significantly longer than that found in healthy subjects. Four patients, however, differed from the other patients by having significant reciprocal inhibition in the leg and a significantly shorter latency of TA MEPs than found in the other patients. The six patients without reciprocal inhibition in the leg instead had significant short latency facilitation of the SOL H-reflex and a longer TA MEP latency than seen in the healthy subjects and in the four patients with retained reciprocal inhibition. These findings support the hypothesis that disynaptic reciprocal inhibition and short latency facilitation are involved in the development of spasticity and, furthermore, they suggest a positive correlation between impairment of corticospinal transmission and decrease of reciprocal inhibition/appearance of reciprocal facilitation.


http://brain.oupjournals.org/cgi/content/abstract/128/4/711

Glutaric acidemia type 1 (GA-1) is an autosomal recessive disorder characterized by a deficiency of glutaryl-CoA dehydrogenase (GCDH) activity. GA-1 is often associated with an acute encephalopathy between 6 and 18 months of age that causes striatal damage resulting in a severe dystonic movement disorder. Ten autopsy cases have been previously described. Our goal is to understand the disorder better so that treatments can be designed. Therefore, we present the neuropathological features of six additional cases (8 months-40 years), all North American aboriginals with the identical homozygous mutation. This cohort displays similar pathological characteristics to those previously described. Four had macroencephaly. All had striatal atrophy with severe loss of medium-sized neurons. We present several novel findings. This natural time course study allows us to conclude that neuron loss occurs shortly after the encephalopathical crisis and does not progress. In addition, we demonstrate mild loss of large striatal neurons, spongiform changes restricted to brainstem white matter and a mild lymphocytic infiltrate in the early stages. Reverse transcriptase-PCR to detect the GCDH mRNA revealed
normal and truncated transcripts similar to those in fibroblasts. All brain regions demonstrated markedly elevated concentrations of GA (3770-21 200 nmol/g protein) and 3-OH-GA (280-740 nmol/g protein), with no evidence of striatal specificity or age dependency. The role of organic acids as toxic agents and as osmolytes is discussed. The pathogenesis of selective neuronal loss cannot be explained on the basis of regional genetic and/or metabolic differences. A suitable animal model for GA-1 is needed.


http://brain.oupjournals.org/cgi/content/abstract/126/1/134

Three genes commonly causing Charcot-Marie-Tooth disease (CMT) encode myelin-related proteins: peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ) and connexin 32 (Cx32). Demyelinating versus axonal phenotypes are major issues in CMT associated with mutations of these genes. We electrophysiologically, pathologically and genetically evaluated demyelinating and axonal features of 205 Japanese patients with PMP22 duplication, MPZ mutations or Cx32 mutations. PMP22 duplication caused mainly demyelinating phenotypes with slowed motor nerve conduction velocity (MCV) and demyelinating histopathology, while axonal features were variably present. Two distinctive phenotypic subgroups were present in patients with MPZ mutations: one showed preserved MCV and exclusively axonal pathological features, while the other was exclusively demyelinating. These axonal and demyelinating phenotypes were well concordant among siblings in individual families, and MPZ mutations did not overlap among these two subgroups, suggesting that the nature and position of the MPZ mutations mainly determine the axonal and demyelinating phenotypes. Patients with Cx32 mutations showed intermediate slowing of MCV, predominantly axonal features and relatively mild demyelinating pathology. These axonal and demyelinating features were present concomitantly in individual patients to a variable extent. The relative severity of axonal and demyelinating features was not associated with particular Cx32 mutations. Median nerve MCV and overall histopathological phenotype changed little with disease advancement. Axonal features of diminished amplitudes of compound muscle action potentials (CMAPs), axonal loss, axonal sprouting and neuropathic muscle wasting all changed as disease advanced, especially in PMP22 duplication and Cx32 mutations. Median nerve MCVs were well maintained independently of age, disease duration and the severity of clinical and pathological abnormalities, confirming that median nerve MCV is an excellent marker for the genetically determined neuropathic phenotypes. Amplitude of CMAPs was correlated significantly with distal muscle strength in PMP22 duplication, MPZ mutations and Cx32 mutations, while MCV slowing was not, indicating that clinical weakness results from reduced numbers of functional large axons, not from demyelination. Thus, the three major myelin-related protein mutations induced varied degrees of axonal and demyelinating phenotypic features according to the specific gene mutation as well as the stage of disease advancement, while clinically evident muscle wasting was attributable to loss of functioning large axons.


http://brain.oupjournals.org/cgi/content/abstract/127/3/505

Symptoms of Huntington's disease may be caused by a toxic insult triggered by the mutant human huntingtin (Htt) protein itself, by a maladaptive protective mechanism initiated in response to an insult, or by a combination of these. We observed a protection from N-methyl-D-aspartate (NMDA) receptor-induced excitotoxicity in striata of symptomatic N171-82Q mice, a new
A transgenic model of Huntington's disease. The goal of this study was to determine if NMDA receptor-mediated signalling pathways are altered in these mice. Multiple proteins of NMDA receptor and dopamine D1 receptor pathways are being regulated in ways predictive of the protection we observe. Although examining NMDA receptor subunit proteins showed no change in NR1, NR2A, or NR2B in the striata of the symptomatic mice, we observed a decrease in phosphorylation of NR1 at Ser897, previously reported to decrease NMDA receptor current. The dopamine D1 receptor, responsible for protein kinase A activation and subsequent phosphorylation of Ser897 of NR1, also showed an age-related decrease. Other proteins regulated in this disease were associated with PSD-95-like scaffolding proteins of the NMDA receptor. Specifically, we observed a decrease in membrane-associated neuronal nitric oxide synthase (nNOS), a decrease in PSD-95-like proteins, which link nNOS to the NMDA receptor complex, and a decrease in citron, a protein associated with dendritic spine formation. From these data, we conclude that the N171-82Q mice seem to be regulating, in a protective direction, many of the known effector pathways of NMDA receptor-induced excitotoxicity. These regulations, although seemingly effective in decreasing neuronal death, may in fact be causing some of the symptoms associated with the disease.


http://brain.oupjournals.org/cgi/content/abstract/126/1/32

To date, two point mutations, G209A and G88C, have been reported in the coding region of the {alpha}-synuclein gene in autosomal dominant familial Parkinson's disease. When translated, these lead to the missense mutations Ala53Thr and Ala30Pro, respectively. Reduced mRNA expression of the G209A allele was reported recently in a Greek-American family. Here, we show that {alpha}-synuclein mRNA is normally expressed in blood cells and report the results of an analysis of {alpha}-synuclein mRNA and protein expression in lymphoblastoid cell lines established from kindreds with the G209A and G88C mutations. mRNA expression was characterized using a TaqMan real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay. We assessed five affected and three unaffected members of a German family with the G88C mutation and two affected members in different, unrelated Greek families with the G209A mutation. The ratio of wild-type to mutant {alpha}-synuclein allele expression ranged from 2.2 to 9.2 in the affected individuals with a severe clinical phenotype. The ratios of the expression levels of the wild-type to mutant alleles were only slightly decreased in mild cases and were less than 1.0 in two asymptomatic heterozygotes. Sequence analysis of the RT-PCR products showed only the presence of G in position 88 and G in position 209 in severely affected heterozygotes of the German and Greek families, respectively. High performance liquid chromatography/mass spectrometry demonstrated that, relative to wild-type {alpha}-synuclein, there is a reduction of Ala30Pro (alpha)-synuclein in lymphoblastoid cell lines originating from severely affected, but not mildly affected G88C/+ heterozygotes. Taken together, these data indicate that there is haploinsufficiency at the {alpha}-synuclein gene and that the ratio of expression of the wild-type to mutant alleles correlates with the severity of the clinical phenotype. Furthermore, these findings suggest that haploinsufficiency of {alpha}-synuclein mutations may contribute to disease progression in these forms of familial Parkinson's disease.


http://brain.oupjournals.org/cgi/content/abstract/127/11/2540

Autosomal recessive demyelinating Charcot-Marie-Tooth disease (CMT4), Dejerine-Sottas
disease and congenital hypomyelinating neuropathy are variants of hereditary demyelinating neuropathy of infancy, a genetically heterogeneous group of disorders. To explore the spectrum of early-onset demyelinating neuropathies further, we studied the clinicopathological and genetic aspects of 20 patients born to unaffected parents. In 19 families out of 20, consanguinity between the parents or presence of an affected sib suggested autosomal recessive transmission. Screening of various genes known to be involved in CMT4 revealed six mutations of which five are novel. Four of these novel mutations occurred in the homozygous state and include: one in GDAP1, one in MTMR2, one in PRX and one in KIAA1985. One patient was heterozygous for a novel MTMR2 mutation and still another was homozygous for the founder mutation, R148X, in NDRG1. All patients tested negative for mutations in EGR2. Histopathological examination of nerve biopsy specimens showed a severe, chronic demyelinating neuropathy, with onion bulb formation, extensive demyelination of isolated fibres and axon loss. We did not discern a specific pattern of histopathology that could be correlated to mutations in a particular gene.


http://brain.oupjournals.org/cgi/content/abstract/126/8/1873

Interleukin (IL)-6 is a multifunctional cytokine with diverse actions and has been implicated in the pathophysiology of many neurological and inflammatory disorders. In this study, we investigated the role of IL-6 in pneumococcal meningitis. Cerebral infection in wild-type (WT) mice caused an increase in vascular permeability and intracranial pressure (ICP), which were significantly reduced in IL-6-/- mice. In contrast, meningitis in IL-6-/- mice was associated with a significant increase in CSF white blood cell count compared with infected WT mice, indicating an enhanced inflammatory response. Analysis of mRNA expression in the brain showed an increase in tumour necrosis factor (TNF)-{alpha}, IL-1{beta}, and macrophage inflammatory protein 2 (MIP-2) levels, but decreased expression of granulocyte-macrophage colony-stimulating factor in infected IL-6-/- mice compared with infected WT controls. Similar results were obtained when rats challenged with pneumococci were systemically treated with neutralizing anti-IL-6 antibodies, resulting in an increased pleocytosis but at the same time a reduction of vascular permeability, brain oedema formation, and ICP, which was not accompanied by a downregulation of matrix metalloproteinases. Our data indicate that IL-6 plays an important anti-inflammatory role in bacterial meningitis by reducing leukocyte infiltration but contributes to the rise in intracranial pressure by increasing blood-brain barrier (BBB) permeability. These findings suggest that the migration of leukocytes across the BBB and the increase in vascular permeability are two independent processes during bacterial meningitis.


http://brain.oupjournals.org/cgi/content/abstract/126/4/814

The majority of cases with frontotemporal dementia (FTD) have no tau deposition in the brain, yet mutations in the tau gene lead to a similar clinical phenotype with insoluble tau depositing in neuropathological lesions. We report two tau gene mutations at positions +19 and +29, in the intronic sequences immediately following the stem loop structure in exon 10, which segregate with FTD. Exon-trapping experiments showed that these gene mutations alter the splicing out of exon 10 and produce an increase in tau isoforms with three microtubule binding domains (three repeat tau). Mutagenesis experiments demonstrated that the +19 mutation was responsible for the increase in three repeat tau, possibly by altering an intron silencer modulator sequence element found at this region of the gene. Microtubule binding experiments revealed a significant
decrease in microtubule assembly with increasing amounts of three and decreasing amounts of four repeat tau. Brain autopsy was available in one case. Analysis of the type of soluble tau isoforms revealed an increase in three repeat tau and an absence of tau isoforms with exon 3 inserts. No insoluble tau was isolated in the tissue fractions, consistent with the absence of tau-positive histopathology. There was also an increase in tau degradation products suggestive of increased proteolysis. This increase in tau breakdown products was associated with TUNEL- and activated caspase-3-positive neurons identified histologically. These studies show that increases in soluble three repeat tau can be responsible for FTD in cases with tau gene mutations in the intronic region immediately adjacent to the stem loop in exon 10. These cases of FTD have tau isoforms (without exon 3 inserts) that do not form abnormal aggregates and appear more prone to proteolysis. The increase in tau proteolysis was associated with increased evidence of apoptosis. This mechanism of neurodegeneration may be more applicable to the majority of FTD cases, which do not accumulate insoluble tau deposits.

Brain and Development (3)


http://www.sciencedirect.com/science/article/B6T50-3SV3PJ8-4/2/48f3df10b9ae4b8914455b77a42d77f9

On dystrophin gene analysis by multiplex polymerase chain reaction (PCR), 76 of 130 (58.5%) Japanese patients with Duchenne muscular dystrophy had a deletion or duplication in genomic DNA. Of the remaining 54 patients who had no identifiable gene mutations, muscle biopsy tissue was available in 16 for RNA extraction. The full length of the coding regions of dystrophin cDNA was amplified in 10 fragments by reverse transcription nested PCR (RT-PCR). Five of 16 patients (31%) had dystrophin cDNA of abnormal size. One patient had a deletion, and two duplications that were not covered by multiplex PCR; one an exon-skipping of exon 51 caused by a 5' consensus splice site mutation of intron 51, and one 172 bp or 202 bp insertion in the cDNA between exon 25 and 26. Nested RT-PCR from the total RNA extracted from muscle biopsy was useful for screening patients who had no identifiable gene abnormality by multiplex PCR.


http://www.sciencedirect.com/science/article/B6T50-3W0NC53-C/2/3dbdda46e49bccc43fede87765b4a66cc

We report a rare case of intractable frontal lobe epilepsy with mental deterioration, in which the measles virus gene was detected from the cerebrospinal fluid (CSF) and peripheral mononuclear cells (PBMC) obtained 9 years after the first epileptic episode using reverse transcriptase-polymerase chain reaction (RT-PCR). The patient had been immunized with an attenuated measles vaccine and had no history of clinically apparent acute measles infection. However the analysis of the sequence of the PCR product from CSF showed the circulating wild strain
genotype at the time when the patient complained of his first epileptic episode.


http://www.sciencedirect.com/science/article/B6T50-44KD8HY-1T/2/e2e4a66639957372da9bdf9ba8f0f76f

Rett syndrome (RTT) is an X-linked dominant neurological disorder, which appears to be the most common genetic cause of profound combined intellectual and physical disability in Caucasian females. This syndrome has been associated with mutations of the MECP2 gene, a transcriptional repressor of unknown target genes. We report a detailed mutational analysis of a large cohort of RTT patients from the UK and Italy. This study has permitted us to produce a hot spot map of the mutations identified. Bioinformatic analysis of the mutations, taking advantage of structural and evolutionary data, leads us to postulate the existence of a new functional domain in the MeCP2 protein, conserved among brain-specific regulatory factors.

Brain Research (48)


http://www.sciencedirect.com/science/article/B6SYR-484DDGJ-17V/2/e25ec85d08e03c10c220c4caaad81c5a

Previous electrophysiological studies suggested that GABAA receptors in rat hippocampal neurons might be less sensitive to ethanol than mouse neurons. Therefore, we examined the effects of ethanol (0.5-850 mM) in cultured mouse (C57BL/6) and rat (Sprague-Dawley) neurons. In 35% of the mouse neurons, the Cl- current was potentiated by ethanol starting at 0.5 mM. In all of the rat neurons examined, on the other hand, the current was potentiated by concentrations starting at 200 mM. We also studied the effects of GABA and other GABAergic ligands. GABAA receptors in rat and mouse neurons displayed EC50s for GABA of 9 +/- 0.3 and 17 +/- 0.8 [mu]M, respectively and ethanol did not significantly change these values. The EC50 for diazepam was 92 +/- 3 and 120 +/- 8 nM in rat and mouse, respectively. Pentobarbital enhanced the current with EC50s of 84 +/- 3 and 106 +/- 6 [mu]M in rat and mouse, respectively. The sensitivity for Cl- was similar in all the neurons. RO 15-4513, an inverse partial agonist to the benzodiazepine receptor, was not effective in reversing the potentiation of the Cl- current in rat neurons and only slightly reduced the potentiation in mouse neurons. The receptors in rat neurons were more sensitive to external Zn2+; the current was inhibited by 50% with a concentration of 93 +/- 3 and 244 +/- 9 [mu]M in rat and mouse, respectively. Analysis of mRNA encoding for the [gamma]2L receptor subunit showed similar levels in rat and mouse neurons. The data suggest that most pharmacological properties of hippocampal GABAA mouse and rat receptors are comparable, with the exception of the sensitivity to ethanol and Zn2+. These differences can not be explained by differential expression of [gamma]2L subunits. We provide good experimental evidence indicating a difference in the sensitivity of the GABAA receptor to GABAergic ligands in two distinct animal.
The neurotrophin brain-derived neurotrophic factor (BDNF) is considered to be a key factor for neuronal survival, differentiation and plasticity. According to a proposed hypothetical model BDNF expression might play a central role in the pathogenesis of depression. The BDNF gene is rather complex in its structure and it can express four different mRNA isoforms by alternative splicing, each producing the same protein. This might reflect fine tuning of gene regulation by different signalling networks. Since the BDNF gene has been reported to be upregulated by antidepressants, the expression of the four BDNF mRNA isoforms was measured by real-time quantitative RT-PCR in rat hippocampi after chronic and acute treatment with the antidepressant drug fluoxetine and GR205171, a selective NK-1 receptor antagonist with anxiolytic-like properties. The aim of this study was to test the hypothesis of differential regulation of the mRNA isoforms by those compounds. Our results indicate that the expression of BDNF mRNA isoforms is not affected by chronic or acute treatment with fluoxetine or GR205171.


Real-time Taqman(TM) RT-PCR was used to make quantitative comparisons of the levels of PrRP mRNA expression in micropunch brain samples from rats at different stages of the oestrous cycle and in lactation. The nucleus of the solitary tract and ventrolateral reticular nuclei of the medulla oblongata contained significantly (PP<0.05) lower levels of expression, including the rostral and caudal dorsomedial hypothalamus. Very low levels of PrRP expression were observed in the arcuate nucleus, paraventricular nucleus, medial preoptic nucleus and ventrolateral aspect of the ventromedial hypothalamus. No significant changes in PrRP expression were noted in any sampled region between proestrus, oestrus or dioestrus. Similarly, PrRP expression in hypothalamic regions did not differ between lactating and non-lactating (dioestrous) animals. During validation of RT-PCR techniques we cloned and sequenced a novel splice variant of PrRP from the hypothalamus. This variant arises from alternative splicing of the donor site within exon 2, resulting in an insert of 64 base pairs and shift in the codon reading frame with the introduction of an early stop codon. In the hypothalamus and brainstem, mRNA expression of the variant was restricted to regions that expressed PrRP. These results suggest that PrRP expression in the hypothalamus may be more widespread than previously reported. However, the relatively low level of PrRP in the hypothalamus and the lack of significant changes in expression during the oestrous cycle and lactation provides further evidence that PrRP is unlikely to be involved in the regulation of prolactin secretion.

the expression of metallothionein-III (GIF) mRNA in immortalized mouse brain glial cells (VR-2g)." Brain Research 792(2): 335.

http://www.sciencedirect.com/science/article/B6SYR-3SVRYNV-P/2/bfe3c2f44f9789c4bb0fde71b2c8cc99

Metallothionein (MT)-III, originally discovered as a growth inhibitory factor (GIF), is a brain specific isomer of MTs and is markedly reduced in the brain of Alzheimer's disease patients (AD) and in several other neurodegenerative diseases. We analyzed the level and regulation of mRNA expression of MT-III in immortalized fetal mouse brain glial cells (VR-2g) by reverse transcriptase-polymerase chain reaction (RT-PCR). The basal expression level of MT-III mRNA is very low in VR-2g cells. 4-Methylcatechol, dopamine (DA) and levodopa (-3,4-dihydroxyphenylalanine), which stimulate the synthesis of nerve growth factor (NGF), further increased the expression of MT-III mRNA in VR-2g cells.


http://www.sciencedirect.com/science/article/B6SYR-4007GTG-5H/2/c56123f3a00ded65e0eb12a9e29c2294138

Previously, we established a stable transfectant, Nf-1, from normal rat kidney (NRK) fibroblasts transfected with a human metallothionein II A (hMT-IIA) promoter/human genomic c-fos fusion gene to produce c-Fos protein. Since the hMT-IIA promoter can be activated by heavy metals, the level of human c-fos gene expression can be increased by addition of heavy metals to the culture medium of Nf-1 cells and the anchorage-independent growth of Nf-1 in soft agar is markedly enhanced in the presence of transforming growth factor-[beta] (TGF-[beta]) and epidermal growth factor (EGF). In this study, we found that the hMT-IIA promoter can be activated by zinc, resulting in the elevation of fused c-fos gene expression in Nf-1 cells. We transplanted NRK and Nf-1 cells into the striatum of the rat brain and investigated whether expression of the human c-fos gene could be modified in the brain by exogenous zinc. After 8 weeks, we found that the Nf-1 cells could survive in the rat brain without any immunosuppression and grafts of Nf-1 induced angiogenesis when zinc was administered. Such implants enhanced the expression of c-fos mRNA by zinc. These results indicated that the transplanted cells continued expressing the c-fos transgene when the rats were given drinking water containing zinc, resulting in the promotion of cell growth and of neovascularization. This study will present a useful animal model of gene therapy by control of transgene expression in the brain.


http://www.sciencedirect.com/science/article/B6SYR-3RBYH5J-25/2/375abfc6c754dc651ed5bfaa7bc89f8c

mRNA transcripts for insulin-like growth factor I (IGF-I) and its receptor are expressed in the lumbar region of the spinal cord. Accordingly, we examined the involvement of IGF-I in nociceptive transmission. An intrathecal injection of IGF-I (200-1000 ng) produced a dose-dependent elevation in nociceptive threshold as indicated by tail flick/withdrawal latency. In contrast, comparable doses of insulin had no significant effect. The time-response curve (15-75 min) revealed that the peak for IGF-I's antinociceptive effect is attained at 30 min. Our data provide evidence that the IGF-I system within the spinal cord may serve as a target for novel
analgesics.


http://www.sciencedirect.com/science/article/B6SYR-4BJMD9C-4/2/0f4e01ccae13f6daa1d097094e57a394

In situ hybridization (ISH) is an essential technique for mapping gene expression in the brain. Although many ISH protocols provide for quantitative analysis of individual mRNAs in different brain regions or across experimental conditions, this technique has lacked the necessary standardization for quantitative comparisons between different mRNA transcripts. We have developed a standardized quantitative ISH (SQuISH(TM)) protocol that utilizes multiple radioactive oligonucleotide probes, providing for increased sensitivity, decreased background and accurate comparison of relative mRNA levels. We evaluated the SQuISH(TM) protocol against a riboprobe-based ISH procedure by comparing the mRNA expression levels in the brain for two transcripts, insulin receptor substrate p53 (IRSp53) and Calsenilin. The results of these two methods were then validated by real-time quantitative PCR. Both protocols exhibited identical mRNA expression patterns for IRSp53 and Calsenilin. In three brain regions analyzed, the levels of IRSp53 mRNA expression were ~1.5-fold higher with the riboprobe-based ISH than with the SQuISH(TM) procedure, although the relative abundance in regional expression levels was similar between the two methods. In contrast, the levels of Calsenilin mRNA expression were 10-17-fold higher with the riboprobe-based ISH than with the SQuISH(TM) procedure and the relative abundance in regional expression levels was different. When compared to the real-time PCR results, the SQuISH(TM) method showed almost identical relative levels of IRSp53 to Calsenilin mRNA in all three brain regions analyzed, while the riboprobe-based procedure showed a completely opposite trend. These results support the accuracy of the SQuISH(TM) protocol for determining relative mRNA levels in the brain.


http://www.sciencedirect.com/science/article/B6SYR-414NVSM-7/2/4e34ff40da06b9a7588cb21433061c15

[alpha]2-Adrenoceptor agonists, such as clonidine, attenuate hypoxia-induced damage to brain and retinal neurones by a mechanism of action which likely involves stimulation of [alpha]2-adrenoceptors. In addition, the neuroprotective effect of [alpha]2-adrenoceptor agonists in the retina may involve stimulation of bFGF production. The purpose of this study was to examine more thoroughly the neuroprotective properties of clonidine. In particular, studies were designed to ascertain whether clonidine acts as a free radical scavenger. It is thought that betaxolol, a [beta]1-adrenoceptor antagonist, acts as a neuroprotective agent by interacting with sodium and L-type calcium channels to reduce the influx of these ions into stressed neurones. Studies were therefore undertaken to determine whether clonidine has similar properties. In addition, studies were undertaken to determine whether i.p. injections of clonidine or betaxolol affect retinal bFGF mRNA levels. In vitro data were generally in agreement that clonidine and bFGF counteracted the effect of NMDA as would occur in hypoxia. No evidence could be found that clonidine interacts with sodium or L-type calcium channels, reduces calcium influx into neurones or acts as a free radical scavenger at concentrations below 100 [mu]M. Moreover, i.p. injection of clonidine, but not betaxolol, elevated bFGF mRNA levels in the retina. The conclusion from this study is that the neuroprotective properties of [alpha]2-adrenoceptor agonists, like clonidine, are very different
from betaxolol. The fact that both betaxolol and clonidine blunt hypoxia-induced death to retinal ganglion cells suggests that combining the two drugs may be a way forward to producing more effective neuroprotection.


http://www.sciencedirect.com/science/article/B6SYR-3RSGJD4-1J/2/408c881cd296fe096b8ee787f957382b

Infection of neonatal mice with ts1, the neuropathogenic mutant of the Moloney murine leukemia virus, results in motor neuronal death in the brainstem and the spinal cord, with gliosis and demyelination, but no inflammatory cell infiltration into the CNS. To evaluate the possible mechanism(s) of ts1-induced neuropathogenesis, we measured CNS expression of cytokines and cell death-related genes in ts1-infected mice with neurological signs and compared with control uninfected mice. In the brainstem, the expression of Fas and tumor necrosis factor [alpha] (TNF-[alpha]) was increased in the ts1-infected mice. Both TNF-[alpha] and Fas were detected in astrocytes, and Fas was also detected in neurons in the brainstem. Some TNF-[alpha]-immunolabeled cells also appeared to be microglial cells. Most Fas-positive cells, including astrocytes and neurons, showed cytoplasmic vacuolization and other degenerative changes. In addition, Fas ligand-immunolabeled cells were also detected in sites where spongiform degeneration occurred. This study suggests that neural cell death in ts1-induced neurodegeneration is likely due to Fas- and TNF-[alpha]-mediated cell death mechanisms.


http://www.sciencedirect.com/science/article/B6SYR-41SSWPP-N/2/230f09a824e09250ebdb29df49c6e35d

Sleep and waking differ significantly in terms of behavior, metabolism, and neuronal activity. Recent evidence indicates that sleep and waking also differ with respect to the expression of certain genes. To systematically investigate such changes, we used mRNA differential display and cDNA microarrays to screen ~10000 transcripts expressed in the cerebral cortex of rats after 8 h of sleep, spontaneous waking, or sleep deprivation. We found that 44 genes had higher mRNA levels after waking and/or sleep deprivation relative to sleep, while 10 were upregulated after sleep. Known genes that were upregulated in waking and sleep deprivation can be grouped into the following categories: immediate early genes/transcription factors (Arc, CHOP, IER5, NGFI-A, NGFI-B, N-Ras, Stat3), genes related to energy metabolism (glucose type I transporter Glu1, Vgf), growth factors/adhesion molecules (BDNF, TrkB, F3 adhesion molecule), chaperones/heat shock proteins (BiP, ERP72, GRP75, HSP60, HSP70), vesicle- and synapse-related genes (chromogranin C, synaptotagmin IV), neurotransmitter/hormone receptors (adrenergic receptor [alpha]1A and [beta]2, GABAA receptor [beta]3, glutamate NMDA receptor 2A, glutamate AMPA receptor GluR2 and GluR3, nicotinic acetylcholine receptor [beta]2, thyroid hormone receptor TR[beta]), neurotransmitter transporters (glutamate/aspartate transporter GLAST, Na+/Cl- transporter NTT4/Rxt1), enzymes (aryl sulfotransferase, c-jun N-terminal kinase 1, serum/glucocorticoid-induced serine/threonine kinase), and a miscellaneous group (calmodulin, cyclin D2, LMO-4, metallothionein 3). Several other genes that were upregulated in waking and all the genes upregulated in sleep, with the exception of the one coding for membrane protein E25, did not match any known sequence. Thus, significant changes in gene expression occur across behavioral states, which are likely to affect basic cellular functions such as RNA and protein synthesis, neural plasticity, neurotransmission, and metabolism.

http://www.sciencedirect.com/science/article/B6SYR-3RSFFTM-F/2/8b425bc53d49c116673bda26f19dd9fa

Melatonin binding sites were identified over the leptomeninges surrounding the human fetal brain using quantitative in vitro autoradiography and the melatonin agonist, 2-[125I]iodomelatonin. Binding was found to be saturable and of high affinity (dissociation constant (Kd)=54 pM and maximal theoretical binding (Bmax)=13 fmol/mg protein), and inhibited by guanosine-5'-o-(3-thiotriphosphate) (GTP[gamma]S) suggesting that these binding sites represent G protein-coupled melatonin receptors. RT-PCR performed on mRNA isolated from the human fetal leptomeninges detected expression of the G protein-coupled melatonin receptor Mel1a, but not Mel1b. In situ hybridisation confirmed the localisation of Mel1a mRNA transcripts over the leptomeninges of the fetal brain. The identification of 2-[125I]iodomelatonin and Mel1a melatonin receptor expression in the fetal leptomeninges implies that melatonin may play a role in the early growth and development of the human brain.


http://www.sciencedirect.com/science/article/B6SYR-4007GRG-57/2/b87f806a39781314383fc84dfa35cd5e

Treatment of castrated quail with testosterone (T) reliably activates male copulatory behavior and, at the same time, increases the aromatase activity (AA), the number of aromatase-immunoreactive (ARO-ir) cells and the concentration of aromatase mRNA as measured by RT-PCR in the brain. All these effects can be mimicked by estrogens. The behavioral effects of T can be blocked by a variety of aromatase inhibitors and, in parallel, the AA is strongly inhibited in the preoptic area (POA). We showed recently that the steroidal inhibitor, 4-OH-androstenedione (OHA) markedly decreases the immunostaining density of brain ARO-ir cells while the non-steroidal inhibitor, R76713 (racemic Vorozone; VOR) unexpectedly increased the density of this staining, despite the fact that the enzyme activity was completely inhibited. To generalize these findings and try to identify the underlying mechanism, we compared here the effects of two steroidal (OHA and androstatrienedione [ATD]) and two non-steroidal (VOR and Fadrozole [FAD]) aromatase inhibitors on the aromatase immunostaining and aromatase mRNA concentration in the brain of castrated quail concurrently treated with T. The 4 inhibitors significantly blocked the activation by T of male copulation. The two steroidal inhibitors decreased the immunostaining of brain ARO-ir cells but both VOR and FAD markedly enhanced the density of this staining. In parallel, OHA and ATD completely blocked the T-induced increase in aromatase mRNA concentration, while VOR and FAD had no effect on these RNA concentrations in the POA-anterior hypothalamus and they decreased them only slightly in the posterior hypothalamus. Taken together these results suggest that the inhibition of AA by ATD or OHA and the subsequent removal of locally produced estrogens blocks the synthesis of aromatase presumably at the transcriptional level. By contrast, the two non-steroidal inhibitors tested here block AA but in parallel increase the aromatase immunostaining. This effect does not result from an enhanced transcription and it is therefore speculated that these compounds increase either the translation of the aromatase mRNA or the half-life of the protein itself.

We investigated the effectiveness of lipopolysaccharide (LPS) and muramyl dipeptide (MDP) administered into the brain to induce anorexia in acutely fasted Wistar rats allowed to refeed. We also assayed for changes in mRNA levels of IL-1 system components, TNF-[alpha], TGF-[beta]1, glycoprotein 130 (gp 130), leptin receptor (OB-R), pro-opiomelanocortin (POMC), neuropeptide Y (NPY), glucocorticoid receptor (GR), and CRF receptor (CRF-R) in selected brain regions. The data show that LPS and MDP induced anorexia differentially during refeeding. LPS-induced anorexia was of a stronger magnitude and duration than that of MDP. RNase protection assays showed that LPS and MDP significantly increased the expression of IL-1[beta], IL-1 receptor type I, and TNF-[alpha] mRNAs in the cerebellum, hippocampus, and hypothalamus; LPS was more potent in all cases. MDP treatment, on the other hand, induced a stronger increase in hypothalamic levels of IL-1 receptor antagonist (IL-1Ra) and TGF-[beta]1 mRNAs relative to LPS. In addition, competitive RT-PCR analysis showed that LPS induced an eleven-fold increase in IL-1[a] mRNA in the hypothalamus relative to vehicle. These findings suggest that LPS and MDP mediate anorexia through different cytokine mechanisms. A stronger up-regulation of anti-inflammatory cytokines (IL-1Ra and TGF-[beta]1) mRNA expression by MDP may be involved in the weaker MDP-induced anorexia relative to LPS. No significant changes were observed in the peptide components examined except for an up-regulation in cerebellar gp 130 mRNA and down-regulation of hypothalamic GR mRNA expression in response to LPS or MDP. This study shows that LPS and MDP induce anorexia in fasted rats allowed to refeed, and suggests an important role for endogenous cytokine-cytokine interactions.


The subventricular zone (SVZ) generates the largest number of migratory cells in the adult brain. SVZ neuroblasts migrate to the olfactory bulbs (OB) in the adult, whereas during development, SVZ cells migrate into many adjacent nuclei. Previously, we showed that cerebral cortex injury in the adult causes molecular and cellular changes which may recapitulate the developmental migratory directions. Consistent with this, growth factors, as well as models of illness or injury can cause adult SVZ cells to migrate into non-olfactory bulb nuclei. Here, we tested the hypothesis that cerebral cortex injury in the adult mouse induces changes in migration, by labeling adult SVZ cells with a retroviral vector and examining the distribution of cells 4 days and 3 weeks later. Four days after cortical lesions, disproportionately fewer retrovirally-labeled cells had migrated to the olfactory bulb in lesioned mice than in controls. Conversely, the number of cells found in non-olfactory bulb regions (primarily the area of the lesion and the corpus callosum) was increased in lesioned mice. The morphology of these emigrated cells suggested that they were differentiating into glial cells. Three weeks after cortical injury, the majority of retrovirally-labeled cells in both groups of mice had migrated into the granule and periglomerular layers of the olfactory bulb. At 3 weeks, we still observed retrovirally-labeled glial cells in the corpus callosum and in the area of the injury in lesioned mice. These results suggest that cortical lesions cause a transient change in migration patterns of SVZ progeny, which is characterized by decreases in migration to the olfactory bulb but increased migration towards the injury. Our studies also suggest that cortical lesions induce the production of new glial cells which survive for at least 3 weeks after injury. The data support the concept that in the adult, SVZ cells can generate progeny that migrate towards
injured areas and thus potentially be harnessed for neural repair.

Grattan, D. R., M. S. Rocca, et al. (1996). "GABAergic neuronal activity and mRNA levels for both forms of glutamic acid decarboxylase (GAD65 and GAD67) are reduced in the diagonal band of Broca during the afternoon of proestrus." Brain Research 733(1): 46.

http://www.sciencedirect.com/science/article/B6SYR-49HGFSK-55/2/6dcc00ef2f08b9c623cd6757b59ef5b1

There is considerable evidence that GABAergic neurons play an important role in the regulation of gonadotropin-releasing hormone (GnRH) secretion, and that these neurons may mediate the feedback actions of gonadal steroids on GnRH neurons. The aim of the present study was to investigate whether endogenous changes in ovarian steroid secretion during the estrous cycle influenced GABAergic neuronal activity in the preoptic region of the hypothalamus, and in other steroid-sensitive brain regions. Intact, adult female rats were sacrificed at various times during the days of metestrus or proestrus. GABAergic neuronal activity was estimated by measuring the rate of accumulation of GABA in microdissected brain regions after pharmacological inhibition of GABA degradation. Concentrations of mRNA for both forms of glutamic acid decarboxylase (GAD65 and GAD67) were quantified in microdissected brain regions by a microlysate ribonuclease protection assay. In the diagonal band of Broca at the level of the organum vasculosum of the lamina terminalis (DBB(ovlt)), GABAergic neuronal activity was significantly reduced during the afternoon of proestrus compared with the morning of either proestrus or metestrus. In the lateral septal nucleus, GABAergic neuronal activity was significantly increased in the afternoon of proestrus compared with the morning. There were no significant effects of time of day or day of estrous cycle in the medial preoptic nucleus, median eminence, ventromedial nucleus, suprachiasmatic nucleus, medial septal nucleus, hippocampus (CAI region), or cingulate cortex. In the DBB(ovlt), mRNA levels for both GAD65 and GAD67 were significantly reduced in the afternoon of proestrus compared with the afternoon of metestrus. By contrast, there was no change in GAD65 and GAD67 mRNA levels in the cingulate cortex at any of the times examined. These results demonstrate that GABAergic neuronal activity, and mRNA levels for both GAD65 and GAD67 are reduced in the DBB(ovlt) during the afternoon of proestrus. These results support the hypothesis that decreased GABAergic neuronal activity in this region plays a major permissive role in the generation and maintenance of the estrogen-induced LH surge.


http://www.sciencedirect.com/science/article/B6SYR-3VTRJTX-5/2/223a6b6329e8cf78278e238bc2f2a016

There is increasing evidence that the inflammatory response plays an important role in CNS ischemia. The murine model of focal ischemia, however, remains incompletely characterized. In this study we examined expression of several cytokines and the vascular adhesion molecule E-selectin, in order to characterize the molecular events following stroke in the C57BL/6J mouse. Using a multi-probe RNAse protection assay (RPA), mRNA for 19 cytokines was analyzed following permanent and transient occlusion of the middle cerebral artery in mice. In addition, samples from the same mice were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) to evaluate E-selectin mRNA expression levels. Several cytokine mRNAs showed a similar expression pattern in both permanent and transient CNS ischemia while others showed a temporal expression pattern that was dependent on the type of stroke. For both models, mRNA levels of TNF[alpha] rose early (4 h) followed by IL-6 (10-18 h) and a comparatively late increase (96 h) in TGF[beta1]. IL-1[alpha], IL-1[beta] and IL-1ra levels showed a model dependent shift in
temporal expression. Reperfusion appeared to delay the induction of these cytokines. Temporal changes in cytokine mRNA expression in the mouse CNS occur following ischemic damage. Our findings demonstrate the utility and power of multi-probe RPA for evaluation of changes in cytokine mRNA levels. Moreover, this study is, to our knowledge the first to show temporal changes in cytokine mRNA in mouse cerebral ischemia, forming a basis for further exploration of the roles of these cytokines in modulating ischemic neuronal damage in this model.


http://www.sciencedirect.com/science/article/B6SYR-46SP11B-F/2/e91064c291811d59b4ce2a4f33d1724f

Microglial cyclo-oxygenase (COX) expression is considered to be important in the pathogenesis of Alzheimer's disease (AD) and, therefore, constitutes a key target for therapeutic intervention. We investigated the influence of AD plaque associated factors on COX-1 and COX-2 expression and activity in adult human microglial cells in vitro. COX-2 immunoreactivity and mRNA were induced by lipopolysaccharide (LPS), not by AD plaque associated cytokines interleukin (IL)-1[alpha], IL-1[beta], IL-6, tumor necrosis factor (TNF)-[alpha], or amyloid (A)[beta]1-42. To assess functional COX activity, the release of PGE2 into the culture medium was determined. LPS and also arachidonic acid (AA) dose-dependently stimulated PGE2 release. The effects of AA are independent from induction of COX mRNA expression, or of de novo protein synthesis. No effects of either plaque-associated cytokines or A[beta]1-42 on PGE2 secretion were seen, even when cells were co-stimulated with AA, to provide enough substrate. COX isotype selective inhibitors were used to discern relative contributions of COX-1 and COX-2 activities to microglial PGE2 secretion. COX-2 and in part COX-1-selective inhibitors inhibited LPS-induced PGE2 secretion, whereas the AA-induced PGE2 secretion was reduced by COX-1-selective inhibitors only. Apparently, adult human microglia in vitro (1) constitutively express COX-1, and (2) do not express COX-2 upon exposure to either A[beta] or plaque associated cytokines. In the light of microglial COX activity as a potential therapeutical target in AD, the data presented in this study suggest that classical NSAIDs, rather than selective COX-2 inhibitors, are more potent in reducing microglial prostaglandin secretion.


http://www.sciencedirect.com/science/article/B6SYR-47PPG5V-2/2/e09b2e25fd0dad5fa1a3aa227561b622

Nociceptin is a peptide transmitter belonging to the opioid family. Nociceptin has recently attracted considerable interest since it appears to exhibit a number of differences to the other opioids. In the present study, we used a nociceptin antibody to map the distribution of nociceptin in the human trigeminal ganglion. In addition, we studied the nociceptin receptor at mRNA levels by RT-PCR and the vasomotor response to nociceptin in human cerebral vessels using a sensitive in vitro method. About 70% of all neuronal cells in trigeminal ganglia were nociceptin immunopositive. Nociceptin was predominantly (78%) expressed in medium-sized cells (30-60 [mu]m). Nociceptin also distributed in small-sized cells (14% of positive cell bodies; 60 [mu]m). Double immunostaining showed that in the human trigeminal ganglion nociceptin colocalized with calcitonin gene-related peptide (CGRP), substance P (SP), nitric oxide synthase (NOS) or pituitary adenylate cyclase activating peptide (PACAP). About 61% of nociceptin positive cells contained CGRP, 54% contained SP, 50% contained NOS and 68% contained PACAP.
Immunoreactivity to nociceptin was not detected in human cerebral blood vessels. Reverse transcriptase-polymerase chain reaction detected the expression of nociceptin receptor mRNA in trigeminal ganglia but not in basilar arteries. To further examine whether there are functional nociceptin receptors in human cerebral arteries, a pharmacological study was done, where cerebral arteries revealed strong contractions to 60 mM K+ and U466166 and strong relaxation to CGRP. Nociceptin failed to elicit contraction or relaxation. In conclusion, nociceptin is expressed in human trigeminal ganglia but not in cerebral blood vessels. Nociceptin is colocalized with CGRP, SP, NOS and PACAP. Nociceptin receptor mRNA is expressed in human trigeminal ganglia but not in basilar arteries. The functional role of nociceptin may be at the presynaptic level.


Basic fibroblast growth factor (FGF2) stimulates proliferation of the globose basal cells, the neuronal precursor in the olfactory epithelium. The present study investigates the expression of basic fibroblast growth factor and fibroblast growth factor receptors in the adult olfactory epithelium. FGF2 immunoreactivity was expressed widely in the olfactory epithelium, with the highest density of immunoreactivity in the supporting cells. In contrast, most cells in the epithelium expressed FGF2 mRNA. Fibroblast growth factor receptor-1 (FGFr1) immunoreactivity was densest in the basal cell and neuronal layers of the olfactory epithelium and on the apical surface of supporting cells. In the lamina propria FGF2 immunoreactivity and mRNA were densest in cells close to the olfactory nerve bundles. FGFr1 immunoreactivity was heaviest on the olfactory ensheathing cells. Using reverse transcriptase-polymerase chain reaction analysis, the olfactory epithelium was shown to express only three receptor splice variants, including one (FGFr1c) with which basic fibroblast growth factor has high affinity. Other receptor splice variants were present in the lamina propria. Taken together, these observations indicate endogenous sources of FGF2 within the olfactory epithelium and lamina propria and suggest autocrine and paracrine pathways via which FGF2 might regulate olfactory neurogenesis. The observation of only three receptor splice variants in the olfactory epithelium limits the members of the fibroblast growth factor family which could act in the olfactory epithelium. The widespread distribution of receptors suggests that fibroblast growth factors may have roles other than proliferation of globose basal cells.


Analyzing variation of bovine norepinephrine transporter (NET) at the 3'-region by RT-PCR in the adrenal glands and the brain revealed four isoforms of NET produced by alternative splicing of four cassettes (C0, C1, C2 and C3) encoded by exons 12-15, designated bNET1a (C0-C1-C2, formerly designated bNET1), bNET1b (C0-C2), bNET2a (C0-C1-C3) and bNET2b (C0-C3, formerly designated bNET2), respectively. Expression of these isoforms in COS-7 cells revealed that the isoforms that contain the C1 cassette encoded by exon 13 (bNET1a and bNET2a) showed a significant increase in [3H]norepinephrine uptake and [3H]nisoxetine binding, whereas the isoforms which lack the C1 cassette (bNET1b and bNET2b) failed to display those activities.
despite the selection of either exon 14 or exon 15. These results suggest that the region encoded by exon 13 is indispensable for NET functional expression.


http://www.sciencedirect.com/science/article/B6SYR-40N79W5-1/2/501a9f6ed9574261a86f2c2079db910

This study evaluated complement mRNA expression in human brain microvessel endothelial cells (HBMEC), human umbilical vein endothelial cells (HUVEC), and cells of the human derived ECV304 line. Cerebral endothelial cells and HUVEC expressed detectable levels of complement gene mRNAs for the C1q B-chain, C1r, C1s, C2, C3, C4, C7, C8 gamma-subunit and C9. In addition to C6 mRNA, C1q and C9 were not detected in ECV304 cells. These results indicate that endothelial cells may be a source of complement proteins in brain and other organs of the body.


http://www.sciencedirect.com/science/article/B6SYR-43KBBX1-W/2/03d44b8a4dc8e737b2088c2f36772d0e

Even though nicotine has been shown to modulate mRNA expression of a variety of genes, a comprehensive high-throughput study of the effects of nicotine on the tissue-specific gene expression profiles has been lacking in the literature. In this study, cDNA microarrays containing 1117 genes and ESTs were used to assess the transcriptional response to chronic nicotine treatment in rat, based on four brain regions, i.e. prefrontal cortex (PFC), nucleus accumbens (NAs), ventral tegmental area (VTA), and amygdala (AMYG). On the basis of a non-parametric resampling method, an index (called jackknifed reliability index, JRI) was proposed, and employed to determine the inherent measurement error across multiple arrays used in this study. Upon removal of the outliers, the mean correlation coefficient between duplicate measurements increased to 0.978+/-0.0035 from 0.941+/-0.045. Results from principal component analysis and pairwise correlations suggested that brain regions studied were highly similar in terms of their absolute expression levels, but exhibited divergent transcriptional responses to chronic nicotine administration. For example, PFC and NAs were significantly more similar to each other (r=0.7; P-14) than to either VTA or AMYG. Furthermore, we confirmed our microarray results for two representative genes, i.e. the weak inward rectifier K+ channel (TWIK-1), and phosphate and tensin homolog (PTEN) by using real-time quantitative RT-PCR technique. Finally, a number of genes, involved in MAPK, phosphatidylinositol, and EGFR signaling pathways, were identified and proposed as possible targets in response to nicotine administration.


http://www.sciencedirect.com/science/article/B6SYR-4CMJG28-5/2/8452f388c8116a1ef96c8eba25a6fbe7

Copy numbers of mRNAs for GFR[alpha]-1 and GFR[alpha]-2, the preferred receptors for glial
cell line-derived neurotrophic factor (GDNF) and neurturin (NTN) were determined by real-time quantitative RT-PCR (QRT-PCR). Receptor expression was assessed in striatum (ST) and substantia nigra (SN) of normal rats and rats acutely or progressively lesioned by 6-OHDA injected into the medial forebrain bundle or ST, respectively. GFRα-1 mRNA was clearly detected in normal ST. In normal SN, significantly higher expression of both receptors was observed. At 4 weeks after acute lesion, GFRα-2 mRNA was markedly decreased in SN bilaterally, whereas GFRα-1 mRNA in SN and ST was not affected. A progressive lesion resulted in a progressive decrease of GFRα-1 mRNA in ST bilaterally. In SN, levels of GFRα-1 mRNA were not significantly affected by a progressive lesion, whereas GFRα-2 mRNA was markedly decreased bilaterally. Quantitative western blotting standardized against tyrosine hydroxylase (TH) protein from PC12 cells revealed the expected decrease in TH protein in lesioned SN, but also significant increases in TH protein in contralateral, unlesioned SNs at 4 weeks after both acute and progressive lesions. These data suggest that previously unrecognized compensatory changes in the nigrostriatal system occur in response to unilateral dopamine depletion. Since the changes observed in receptor expression did not always parallel loss of dopamine neurons, cells in addition to the nigral dopamine neurons appear to be affected by a 6-OHDA insult and are potential targets for the neurotrophic factors, GDNF and NTN.


http://www.sciencedirect.com/science/article/B6SYR-49JPSWT-6/2/60d76ff46a989501d886e0ed99860a15

We have previously reported that chronic nicotine administration (4.0 mg/kg/day by i.p. injection over 14 days) up-regulates orexin/hypocretin and neuropeptide Y (NPY) mRNA expression and peptide levels within the hypothalamus. Since there exists a coregulation between these neuropeptides and the protein leptin, the present study was undertaken to determine whether nicotine has a regulatory effect on leptin signaling. Under the same experimental regimen used previously, we found that nicotine down-regulates plasma leptin concentration by 48.8% (PP<0.05). On the other hand, we found that chronic nicotine administration increased the expression levels of OB-Rb mRNA by 12% and OB-R mRNA by 25% in the medial basal hypothalamus compared to control rats. Subsequent radioligand binding assays indicated that nicotine also significantly increased leptin binding in ventromedial hypothalamic area (VMA), medial basal hypothalamic area (MBA), arcuate nucleus/median eminence, paraventricular nuclei and piriform cortex. Taken together, our results revealed that nicotine is involved in the regulation of leptin signaling, suggesting that leptin and its receptor play a role in the anorectic effects of nicotine on food intake and body weight in rats.


http://www.sciencedirect.com/science/article/B6SYR-40CS12SM/2/7dc038da626a8a23b1af93a35a3ed024

Epidemiological studies have shown an inverse relationship between cigarette smoking and body weight. In rodents, a negative correlation between nicotine and body weight has been reported, but this observation was largely derived from studies where relatively high doses of nicotine (~12 mg/kg/day) were used. In the current study, we showed that a negative relationship also holds for low doses of nicotine that are comparable to that consumed by average human smokers (P<0.01) in the free-feeding nicotine-treated group compared to saline controls. No significant differences in body weight were detected between the nicotine-treated and pair-fed groups. To determine
whether the effects of nicotine on food intake and body weight were related to neuropeptide Y (NPY) expression, semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and radioimmunoassay were utilized to measure NPY mRNA and peptide levels in various regions of the hypothalamus. Significantly higher levels of NPY mRNA (ca. 20-50%) and peptide (ca. 24-69%) were only detected in the nicotine-treated groups. In addition, significantly higher NPY contents were also obtained in two hypothalamic areas of pair-fed control animals. In summary, our data suggest that the pharmacological effects of nicotine on food intake and body weight may be mediated by changes in hypothalamic NPY levels, a neuropeptide that is pivotal to the hypothalamic regulation of food intake.


http://www.sciencedirect.com/science/article/B6SYR-4002KTT-C3/2/74e35b976970c84a6a31fd59cab2a3cf

Opioid compounds have potent analgesic and euphoric properties. They act with specific cell-membrane receptors which have been pharmacologically defined into three major classes, [mu], [kappa] and [delta]. These receptors are highly regulated with respect to their gene expression, resulting in a temporally and spatially specific pattern of distribution for each receptor. To characterize the promoter sequence of the [mu] opioid receptor (MOR) gene, a mouse genomic DNA library was screened under high stringency with a rat MOR (MOR-1) cDNA probe and genomic sequences for the mouse MOR gene were isolated. From one genomic clone, a 2.3-kb EcoRi fragment, which hybridized to the 5’-end of the rat MOR-1 cDNA probe, was subcloned and sequenced. This fragment contains 1.3 kb of sequence upstream of the initiation codon, extends downstream through exon 1 and includes a portion of intron 1. Primer extension analysis using mouse brain poly (A)+ RNA identified a transcription initiation site 793 bp upstream from the translation start site. Chimeric constructs of mouse MOR deletion fragments fused to a luciferase reporter gene were transfected into a human neuroblastoma cell line, SK-N-SH, which constitutively expresses endogenous MOR. These transient expression studies indicated that the 0.2-kb region upstream from the transcription site possesses a functional promoter, which directs the expression of the reporter gene in vitro and may possess promoter activity for the mouse MOR gene in vivo.


http://www.sciencedirect.com/science/article/B6SYR-475BF7F-16/2/777d7cd5a9f86fafa1bc6f1e035998bb0

The output of GABAergic medium-sized spiny neurons in the dorsal striatum is controlled in part by glutamatergic input from the neocortex and the thalamus, and dopaminergic input from ventral midbrain. We acutely isolated these neurons from juvenile (P14-24) rats to study the consequences of the interaction between glutamate and dopamine for neuronal excitability. Single-cell RT-PCR analysis was used to identify the expression patterns of dopamine receptors. D1 and D2 dopamine receptor mRNA was detected in 11/22 and 3/22 of isolated neurons, respectively. Receptor mRNA co-expression was detected in 1/22 cells tested. Whole-cell voltage clamp recording (Vh=-70 mV) was combined with local or bath application of dopaminergic and glutamatergic agonists to explore dopamine receptor modulation of glutamatergic excitation. Glutamate-evoked inward currents (5 [mu]M, Mg2+-free, 1 [mu]M glycine) were attenuated by dopamine (5 [mu]M) to 83.2+/-3.6% (n=31). NMDA-evoked (20 [mu]M), APV-sensitive currents were attenuated by dopamine to 80.9+/-4.5% (n=24). NMDA-induced responses were also
attenuated by the D1 receptor agonist SKF 38393 (1 \([\mu]\text{M}; n=28\)), while the D2/3 receptor agonist quinpirole (10 \([\mu]\text{M}\) had no effect. The currents evoked by application of AMPA (5 \([\mu]\text{M}\)) displayed a steady rundown. Application of dopamine abolished or significantly reduced the rundown in the cells tested \((n=17)\). A similar effect was observed after the application of SKF 38393 (1 \([\mu]\text{M}\)), while quinpirole (10 \([\mu]\text{M}\)) had no significant effect. Our results provide direct evidence for modulation by dopamine of glutamatergic responses of striatal medium spiny neurons, and demonstrate that the effects of this neuromodulator are receptor subtype specific. Disruption of this modulatory effect is likely to contribute to movement disorders associated with Parkinson's disease.


http://www.sciencedirect.com/science/article/B6SYR-3S0VH2Y-1Y/2/e700bfcbb72200d56ab691e4a89955

Schwann cell is a cell type that forms myelin sheath and provides trophic supports for neuronal cells by producing neurotrophic factors such as neurotrophins and neurokines in both normal and traumatic situations. It was recently reported that after lesion of sciatic nerve, mRNA for cholinergic differentiation factor (CDF)/leukemia inhibitory factor (LIF) is induced in nonneuronal cells in the nerve. However, the source of LIF-mRNA and the mechanism of LIF-mRNA regulation have remained largely unknown. In the present study, we searched for factors regulating the LIF-mRNA expression in cultured Schwann cells isolated from newborn rat sciatic nerve. Among various growth factors and cytokines tested, TGF-[beta]-1 exerted the most prominent effect on the induction of LIF-mRNA in the cultured Schwann cells. The effect of TGF-[beta]1 on the increase of LIF-mRNA levels was suppressed by either staurosporine or H-7 suggesting the role of PKC or PKC-like protein kinase activity in the induction of LIF-mRNA. The induction of LIF mRNA by TGF-[beta]1 was suppressed in the co-culture of the Schwann cells with embryonic rat DRG neurons. The addition of ascorbic acid, which is known to promote myelination in this co-culture system, further suppressed the TGF-[beta]1 induction of LIF-mRNA. These results suggest that Schwann cells respond to TGF-[beta]1 in a lesion situation to produce LIF, which supports neuronal survival and regeneration. The re-establishment of neuron-Schwann cell interaction would in turn suppress the LIF production to terminate its action during the lesion situation.


http://www.sciencedirect.com/science/article/B6SYR-4835X9K-NT/2/359d5f8cb25b85aede3e04e1599e1644

The membrane inhibitor of reactive lysis (MIRL) protects host cells from complement-mediated lysis. It was detected immunohistochemically in tangled neurons and dystrophic neurites of Alzheimer disease (AD) tissue in a pattern highly similar to that observed for the membrane attack complex of complement, C5b-9. MIRL was also detected in cultured IMR-32 neuroblastoma cells. The mRNA for MIRL was detected in RNA extracts of both AD and normal brain. These data provide the first evidence of brain neuronal expression of MIRL and its upregulation in neurons exposed to complement attack. They are consistent with the previously advanced hypothesis that complement-mediated neuronal injury may play a role in AD.

http://www.sciencedirect.com/science/article/B6SYR-4835Y66-139/2/cb10fd5359649bfd827b0ae43126f7cd

While interleukin-2 (IL-2) has been shown to produce a variety of effects in the CNS and has recently been implicated as an endogenous brain neurokine, little is known about the molecular biology of IL-2 receptors in normal brain. The present investigation provides the first evidence that mRNA for IL-2 receptor-\([\beta]\) (IL-2R[\beta]), an essential subunit for signal transduction by peripheral immune cells, is expressed in normal murine forebrain. Using polymerase chain reaction (PCR) cloning, a partial cDNA (349 bp) corresponding to the extracellular domain was cloned and found to have the identical sequence as the lymphocyte IL-2R[\beta]. IL-2R[\beta] mRNA expression was confirmed by a ribonuclease protection assay, and using in situ hybridization histochemistry, IL-2R[\beta] mRNA was localized in the hippocampus where an intense signal was present over the neuron-rich granule cells of the dentate gyrus and Ammon's horn. Moreover, cDNA clones obtained from two murine neuroblastoma cell lines exhibited the same sequence as IL-2R[\beta] CDNA from normal brain. IL-2R[\beta] gene expression was also detected in the frontal cortex and striatum using PCR. Further in situ hybridization studies will be important to extent this initial observation to determine the brain regional localization and cell-specific anatomy of IL-2R[\beta] mRNA in the CNS.


http://www.sciencedirect.com/science/article/B6SYR-42D2C5C-15/2/456e297f31b4bd7f6d76b8872a3405eb

The mitochondrial cytochrome c oxidase (CO) gene sequence was determined on a patient with Alzheimer's disease (AD). Compared to the standard Cambridge sequence to identify base changes, two missense mutations were found in the patient with AD. The mutations were a G to T transition at np 8206 and an A to T transition at np 8224. The np 8206 mutation changed a Met to an Ile and np 8224 mutation changed a Leu to a Phe. The normal bases and the mutations of mitochondrial DNA (mtDNA) coexist in the patient. Further studies will be required to demonstrate the role of the point mutations of mitochondrial DNA in the pathogenesis of Alzheimer's disease.


http://www.sciencedirect.com/science/article/B6SYR-3YBB92C-1B/2/bbd62d9658c37c40759b0361c4b78ff6

Calpain, a Ca2+-dependent cysteine protease, has previously been implicated in apoptosis or programmed cell death (PCD) in immune cells. Although oxidative stress and intracellular free Ca2+ are involved in neurodegenerative diseases, the mechanism of neuronal cell death in the central nervous system (CNS) due to these agents has not yet been defined. To explore a possible role for calpain in neuronal PCD under oxidative stress and Ca2+ influx, we examined the effects of H2O2 and A23187 on PC12 cells. Treatments caused PCD (light microscopy and TUNEL assay) with altered mRNA expression (RT-PCR) of bax (pro-apoptotic) and bcl-2 (anti-apoptotic) genes, resulting in a high bax/bcl-2 ratio. Control cells expressed 1.3-fold more
Calpain, a Ca2+-dependent cysteine protease, has been implicated in cytoskeletal protein degradation and neurodegeneration in the lesion and adjacent areas following spinal cord injury (SCI). To attenuate apoptosis or programmed cell death (PCD) in SCI, we treated injured rats with E-64-d, a cell permeable and selective inhibitor of calpain. SCI was induced on T12 by the weight-drop (40 g-cm force) method. Within 15 min, E-64-d (1 mg/kg) in 1.5% DMSO was administered i.v. to the SCI rats. Following 24 h treatment, a 5-cm long spinal cord section with the lesion in the center was collected. The spinal cord section was divided equally into five 1-cm segments (S1: distant rostral, S2: near rostral, S3: lesion or injury, S4: near caudal and S5: distant caudal) for analysis. Determination of mRNA levels by reverse transcriptase-polymerase chain reaction (RT-PCR) indicated that ratios of bax/bcl-2 and calpain/calpastatin were increased in spinal cord segments from injured rats compared to controls. Degradation of the 68-kD neurofilament protein and internucleosomal DNA fragmentation were also increased. All of these changes were maximally increased in the lesion and gradually decreased in the adjacent areas of SCI rats, while largely undetectable in E-64-d treated rats and absent in sham controls. The results indicate that apoptosis in rat SCI appears to be associated with calpain activity which can be attenuated by the calpain inhibitor E-64-d.


http://www.sciencedirect.com/science/article/B6SYR-43SVK93-4/2/6c51f2e0db9dc9e3abd0567238f90b6c

Upregulation of calpain, a Ca2+-activated cysteine protease, has been implicated in apoptosis and tissue degeneration in spinal cord injury (SCI) that over time spreads from the site of injury to the surrounding regions. We examined calpain content and activity, regulation of immediate early genes (IEGs) such as c-jun and c-fos, reactive astrogliosis as the expression of glial fibrillary acidic protein (GFAP), and apoptosis-related features such as caspase-3 mRNA expression and internucleosomal DNA fragmentation in 1-cm long spinal cord segments (S1, distant rostral; S2, adjacent rostral; S3, lesion or injury; S4, adjacent caudal; and S5, distant caudal) following SCI in rats. Calpain content and production of 150 kD calpain-cleaved [alpha]-fodrin fragment, expression of IEGs, reactive astrogliosis, and apoptotic features were highly increased in the lesion (S3), moderately in adjacent areas (S2 and S4), and slightly in distant areas (S1 and S5) in SCI rats when compared to sham animals. Administration of the calpain-specific inhibitor E-64-d (1 mg/kg) to SCI rats continuously for 24 h inhibited calpain activity and other factors contributing to apoptosis in the lesion and surrounding areas, indicating that calpain played a key role in the
The pathophysiology of SCI. The results obtained from this animal model of SCI suggest that calpain inhibitor can provide neuroprotection in patients with SCI.


http://www.sciencedirect.com/science/article/B6SYR-3VHX44K-F/2/91f899971557ce8688703717785d45e0

Calpain, a calcium-activated neutral proteinase, has been implicated in myelin and cytoskeletal protein degradation following spinal cord injury. In the present study, we examined the activity and transcriptional expression of calpain in spinal cord injury lesions via Western blotting analysis and RT-PCR, respectively. No increases in transcriptional expression of calpain or calpastatin, the endogenous inhibitor, were observed in the lesion at 1, 4, 24, and 72 h following injury. However, calpain activity (as measured by calpain-specific degradation of the endogenous substrate fodrin) was marginally increased at 4 h and significantly increased by 129.8% at 48 h compared to sham controls after injury. Calpain translational expression was localized in injured spinal cords using double immunofluorescent labeling which revealed increased calpain expression in astrocytes compared to sham controls. These results suggest that calpain produced by astrocytes located in or near spinal cord injury lesions may participate in myelin/axon degeneration following injury.


http://www.sciencedirect.com/science/article/B6SYR-3WJFC07-3/2/e889a6f3e563eb6ee8308cb7afa1e05b

Calpain, a Ca2+-activated cysteine protease, has been implicated in apoptosis of immune cells. Since central nervous system (CNS) is abundant in calpain, the possible involvement of calpain in apoptosis of CNS cells needs to be investigated. We studied calpain expression in rat C6 glioma cells exposed to reactive hydroxyl radical (OH) [formed via the Fenton reaction (Fe2++H2O2+H++ >Fe3+++H2O+·OH)], interferon-gamma (IFN-[gamma]), and calcium ionophore (A23187). Cell death, cell cycle, calpain expression, and calpain activity were examined. Diverse stimuli induced apoptosis in C6 cells morphologically (chromatin condensation as detected by light microscopy) and biochemically [DNA fragmentation as detected by dT-mediated dTTP nick-end labeling (TUNEL) assay]. Oxidative stress arrested a population of C6 cells at the G2/M phase of cell cycle. The levels of mRNA expression of six genes were analyzed by the reverse transcriptase-polymerase chain reaction (RT-PCR). Diverse stimuli did not alter [beta]-actin (internal control) expression, but increased calpain expression, and the upregulated bax (pro-apoptotic)/bcl-2 (anti-apoptotic) ratio. There was no significant increase in expression of calpastatin (endogenous calpain inhibitor). Western blot analysis showed an increase in calpain content and degradation of myelin-associated glycoprotein (MAG), a calpain substrate. Pretreatment of C6 cells with calpeptin (a cell-permeable calpain inhibitor) blocked calpain overexpression, MAG degradation, and DNA fragmentation. We conclude that calpain overexpression due to OH stress, IFN-[gamma] stimulation, or Ca2+ influx is involved in C6 cell death, which is attenuated by a calpain-specific inhibitor.

Tryptophan hydroxylase catalyses the rate-limiting step in the biosynthesis of serotonin, a neurotransmitter which has been implicated in the etiologies of clinically important psychiatric illnesses. Tryptophan hydroxylase is expressed in a tissue-specific manner, but little is known about its transcriptional regulation. By analysing transcriptional activities of a set of 5′-deletion constructs of promoter-reporter plasmids in P815-HTR mastocytoma cells, we found that transcription was activated by sequences between nucleotides -343 and -21. DNase I footprint analysis, using nuclear protein extracts from P815-HTR cells, revealed a protein-DNA interaction between nucleotides -77 and -46. A double stranded oligonucleotide, representing this binding site, specifically bound nuclear protein in a gel shift assay. Methylation interference analysis of this complex revealed that nuclear protein interacted with an inverted GGCCAAT element, which is a high-affinity binding motif for the transcription factor NF-Y (also known as CP1 or CBF). An NF-Y specific antibody abolished protein binding in a gel shift assay. Mutagenesis of specific base pairs abolished protein binding in vitro, and mutagenesis of the same base pairs in a reporter gene construct resulted in a 65% decrease in transcriptional activity. Our results suggest that the transcription factor NF-Y binds to a GGCCAAT motif in the tph proximal promoter and activates transcription.


Understanding mechanisms of estrogen effects on cognition is critical for designing therapies for post-menopausal women and others with dementia. Hippocampus, an area important to cognitive function, responds robustly to estrogen. ER[beta] and ER[alpha] transcripts were detected in the hippocampus and hypothalamus of an ovariectomized female monkey at a relatively high ER[beta]/ER[alpha] ratio. These results suggest that ER[beta] may play a role in mediating estrogen effects in the primate hippocampus and hypothalamus.


Immortalized SCN2.2 cells retain most biochemical and biophysical characteristics of the native rat SCN including the expression of clock genes and circadian regulatory proteins, and its distinctive pacemaker function. This study assessed the expression and signaling of MT1 and MT2 melatonin receptors in SCN2.2 cells. SCN2.2 cells express MT1 and MT2 receptors mRNA as detected by RT-PCR. In situ hybridization with digoxigenin-labeled probes demonstrated that mRNA for MT1 and MT2 melatonin receptors is expressed mostly in cells with neuronal-like morphology, representing 10.8+/−2.2% and 9.8+/−0.2%, respectively, of the SCN2.2 cell population. MT1 and MT2 melatonin receptor proteins are expressed in both rat SCN2.2 cells and rat SCN tissue as demonstrated by Western blot analysis with specific receptor antiserum. Melatonin (0.1-100 nM) inhibited forskolin (20 [μM])-stimulated cAMP formation in a dose-
dependent manner and this effect was blocked by the competitive melatonin receptor antagonist luzindole (100-1000 nM). Furthermore, melatonin (1 nM) stimulated protein kinase C (PKC) activity by ~2-fold. The selective MT2 receptor antagonist 4P-PDOT (100 nM) blocked this effect, indicating that the melatonin-mediated increase in PKC activity occurs through activation of MT2 melatonin receptors. We conclude that SCN2.2 cells express functional melatonin receptors, providing an in vitro model to unveil the melatonin signaling pathway(s) involved in the regulation of circadian rhythms.


http://www.sciencedirect.com/science/article/B6SYR-3YBB8YF-H/2/e26c8e73ce5cff2bb752f40866c9fce

Metallothionein (MT)-III, originally discovered as a growth inhibitory factor (GIF), is a brain specific isomer of MTs and is markedly reduced in the brain of patients with Alzheimer's disease (AD) or other neurodegenerative diseases. We analyzed the level and regulation of mRNA expression of MT-III in immortalized fetal mouse brain glial cells (VR-2g) by reverse transcriptase-polymerase chain reaction (RT-PCR). We have recently reported that dopamine (DA) increases the expression of MT-III mRNA in vitro. In this study, we investigated the mechanism of such increase by examining the effects of DA agonists (SKF38393 or bromocriptine) and DA antagonists (SCH23390 or sulpiride) on the expression of MT-III mRNA. MT-III mRNA did not change by either agonist and DA-increased MT-III mRNA was not inhibited by either antagonist. These results suggested that the induction of MT-III mRNA by DA was not mediated by stimulation of DA receptors. On the other hand, DA-induced MT-III mRNA expression was strongly inhibited by the addition of antioxidants (glutathione, vitamin E or ascorbic acid), indicating that DA-enhanced MT-III mRNA was mediated by reactive oxygen species. Our results suggest that oxidative stress may be one of the principle factors that modulate MT-III mRNA expression.


http://www.sciencedirect.com/science/article/B6SYR-3TWYPXD-D/2/041384cac73b64d6e80bd90d42e08da9

The present study analyzes the transcription of the human prodynorphin gene. Transfection experiments indicate that promoter activity for the 2.8 kb 'brain-type' human prodynorphin mRNA resides in the DNA region located 140-180 b upstream of the exon 1/intron A boundary and not 1.2 kb further upstream, as proposed by others [S. Horikawa, T. Takai, M. Toyosato, H. Takahashi, M. Noda, H. Kakidani, T. Kubo, T. Hirose, S. Tanayama, H. Hayashida, T. Miyata, S. Numa, Isolation and structural organization of the human preproenkephalin B gene, Nature 306, 1983, pp. 611-614]. The new data locates the human prodynorphin gene promoter for the brain-type mRNA in a position corresponding to the position of the rat prodynorphin gene promoter [J. Douglass, C.T. McMurray, J.E. Garett, J.P. Adelman, L. Calavetta, Characterization of the rat prodynorphin gene, Mol. Endocrinol. 3, 1989, pp. 2070-2078]. Three previously not described types of human prodynorphin mRNA of the same size, 2.8 kb, one expressed in fetal brain and two others in testis, were characterized in this study. These mRNAs are generated by alternative splicing of novel 5'-uppermost exons and transcription is probably initiated from other promoters. Human neuroblastoma SH-SY5Y and SK-N-MC cell lines previously used in studies of gene transcription have the 2.8 kb prodynorphin mRNA of adult brain alongside a more abundant, shorter 2.3 kb transcript. The latter transcript was also found in testis and in fetal brain. It lacked
the 5'-part of the 2.8 kb mRNA including the signal peptide encoding sequence. The complex pattern of prodynorphin gene expression and its functional consequences are issues for further studies.


http://www.sciencedirect.com/science/article/B6SYR-3WB8DYS-4/2/927b2e4e7680115e49e6f0ecce3cad86

A majority of the parasympathetic nerve fibers to cranial structures derive from the sphenopalatine and otic ganglia. In particular, blood vessels are invested with a rich supply of dilator fibers of parasympathetic origin. In the present study, we have examined the occurrence of noncholinergic neuromessengers and neuropeptide receptors in the human sphenopalatine and otic ganglia. Vasoactive intestinal peptide (VIP)-immunoreactive (ir) nerve cell bodies occurred in high numbers in the sphenopalatine and otic ganglia. Likewise, high numbers of NOS- and PACAP-containing nerve cell bodies were seen in both ganglia. Autofluorescent lipofuscin, characteristic of adult human nervous tissue, was present within many nerve cell bodies in both ganglia. Receptor mRNA was studied with reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA from the sphenopalatine and otic ganglia was successfully extracted. By using appropriate sense and antisense primers, oligonucleotides were designed from the human sequences derived from GenBank, corresponding to human NPY Y1, CGRP1 and VIP1 receptors. In the sphenopalatine ganglion, we revealed the presence of mRNA for the human NPY Y1 and VIP1 receptors but not the CGRP1 receptor. The otic ganglion was found to react positively only for primers to mRNA for VIP1 but not for CGRP1 or NPY Y1 receptors.


http://www.sciencedirect.com/science/article/B6SYR-46Y5GHP-B/2/815b88efc5c45d35936295a266e8d387

Areas of the limbic system of adult male Wistar rats were screened for kainic-acid-induced gene expression. Polymerase-chain-reaction-based differential display identified a 147-bp cDNA fragment, which represented an mRNA that was upregulated in the entorhinal cortex and hippocampus in the kainic-acid-treated animals. The sequence was 97.8% homologous to rat 14-3-3 [zeta] isoform mRNA. Detailed Northern analysis revealed increased mRNA levels in the entorhinal cortex 1 h after kainic acid exposure and continued elevation 24 h post-injection in both the entorhinal cortex and hippocampus. Western blot analyses confirmed that the protein product of this gene was also present in increased amounts over the same time period. Immunohistochemistry and terminal transferase-mediated dUTP nick end labelling (TUNEL) detected expression of 14-3-3 [zeta] protein exclusively in the entorhinal cortex and hippocampus, and only in TUNEL-positive neuronal cells. Expression of the tumor suppressor protein, p53 was also induced by kainate injection, and was co-localized with 14-3-3 [zeta] protein in selected cells only in the affected brain regions. The increase gene expression of 14-3-3 [zeta] represents a transcription-mediated response associated with region selective neuronal damage induced by kainic acid.

We have recently shown that CART peptides exhibit a diurnal rhythm in blood that is affected by food intake and glucocorticoids. In the present study, we extend our observations by demonstrating that CART peptides also exhibit a diurnal rhythm in several brain regions, notably the nucleus accumbens, hypothalamus and amygdala, but not in the midbrain. To examine whether the CART peptide rhythm was dependent on food intake, animals were food-deprived for 24 h. In regular-fed animals, CART peptide levels were lower in the morning compared to evening hours. However, this diurnal variation of CART peptide was not apparent in fasted animals, and CART peptide levels were reduced. The diurnal variation of CART mRNA in the nucleus accumbens paralleled the variation of CART peptide in this region. Similar to the peptide, the mRNA did not change in midbrain. These results show that CART peptide levels and gene expression undergo a diurnal variation in some brain regions, and the variation is altered by fasting. These findings suggest a variety of regulatory mechanisms for CART and additional considerations for CART's role in brain.


Individual neurons dissociated from rat suprachiasmatic nucleus can express independently phased circadian firing rhythms in culture. The phases of these rhythms are unperturbed by reversible blockade of neuronal firing lasting 2.5 days, indicating that multiple circadian clocks continue to operate in the absence of conventional synaptic transmission. The possibility remains, however, that these circadian rhythms might depend on some other form of intercellular communication. In the present study, a potential role for gap junctional coupling in SCN cultures was evaluated by introduction of the tracer molecule Neurobiotin into both neurons (n = 98) and astrocytes (n = 10), as well as by immunolabeling for specific connexins, the molecular components of gap junctions. Astrocytes were extensively coupled to each other by connexin43-positive gap junctions, but no evidence was found for coupling of neurons to each other or to astrocytes. These data support the hypothesis that neurons expressing independently phased circadian rhythms in SCN cultures ('clock cells') are autonomous, single cell circadian oscillators, but do not exclude a role for glia in synchronizing neuronal clock cells in vivo.


The presence of mRNAs for dopamine receptor subtypes and dopamine transporter in rat peripheral sensory and sympathetic ganglia was investigated using polymerase chain reaction (PCR) and DNA sequencing. Dopamine D1, D2, D3, D5 receptor subtype mRNAs and dopamine transporter mRNA were detected in both superior cervical sympathetic ganglia (SCG) and dorsal root ganglia (DRG) in the rat; the expression of D4 mRNA was only detected in DRG. While two alternatively spliced isoforms of D2 were detected in both ganglia, the alternative splicing
transcripts for D3 and D4 were only found in the DRG. These results are useful in further studying the roles of dopamine and the effects of dopaminergic agents in the peripheral nervous system.


The casein kinase-1 (Ck1) family are serine/threonine specific protein kinases. They are highly associated with Alzheimer disease (AD) brain-derived tau filaments and granulovacuolar bodies. Recently we have demonstrated that one family member, Cki[delta], colocalizes with tau containing neurofibrillary tangles (NFTs) and other tau deposits in a number of neurodegenerative diseases. Here we show that the association in AD is accompanied by a sharp upregulation of Cki[delta] mRNA in brain but not in peripheral organs. The degree of upregulation in AD brain is correlated with the degree of regional pathology. There was a 24.4-fold increase of Cki[delta] mRNA in AD hippocampus compared with control, 8.04-fold in the amygdala, 7.45 in the entorhinal cortex and 7.30-fold in the midtemporal gyrus. These are areas with a high burden of NFTs, neuropil threads and dystrophic neurites. In areas almost devoid of this tau pathology, such as the caudate nucleus, occipital cortex and cerebellum, the increases in AD compared to control brain were only 2.21-, 1.89- and 1.87-fold, respectively. Western blot analysis showed that the upregulation of Cki[delta] mRNA was paralleled by an upregulation of Cki[delta] protein. These data establish that the association of Cki[delta] with the tau pathology of AD is reflective of an increase in gene transcription. Since Alzheimer-like phosphoepitopes of tau can be generated by Ck1, the Cki[delta] isoform may play an important role in this fundamental aspect of AD pathology.


http://www.sciencedirect.com/science/article/B6SYR-4BJX728-5/2/d71e3c4ce26ce3d4de2bd632b4a4607

The adenosine A2A receptor (A2AR) is abundantly expressed in brain and emerging as an important therapeutic target for Parkinson's disease and potentially other neuropsychiatric disorders. To understand the molecular mechanisms of A2AR gene expression, we have characterized the genomic organization of the mouse and human A2AR genes by molecular and bioinformatic analyses. Three new exons (m1A, m1B and m1C) encoding the 5' untranslated regions (5'-UTRs) of mouse A2AR mRNA were identified by rapid amplification of 5' cDNA end (5' RACE), RT-PCR analysis and genome sequence analyses. Similar bioinformatics analysis also suggested six variants of the non-coding "exon 1" (h1A, h1B, h1C, h1D, h1E and h1F) in the human A2AR gene, which were confirmed by RT-PCR analysis, while three of the human exon 1 variants (h1D, h1E and h1F) were likewise verified by 5' oligonucleotide capping analysis suggesting multiple transcription start sites. Importantly, RT-PCR and quantitative PCR analysis demonstrated that the A2AR transcripts with different exon 1 variants displayed tissue-specific expression patterns. For instance, the mouse exon m1A mRNA was detected only in brain (specifically striatum) and the human exon h1D mRNA in lymphoreticular system. Furthermore, the determination of the three new transcription start sites of human A2AR gene by 5' oligonucleotide capping and bioinformatics analyses led to the identification of three corresponding promoter regions which contain several important cis elements, providing additional target for further molecular dissection of A2AR gene expression. Finally, our analysis indicates that A2AR mRNA and a novel transcript partially overlapping with the 3' exon h3, but in
opposite orientation to the A2AR gene, could conceivably form duplexes to mutually regulate transcript expression. Thus, combined molecular and bioinformatics analyses revealed a new A2AR genomic structure, with conserved coding exons 2 and 3 and divergent, tissue-specific exon 1 variants encoding for 5'-UTR. This raises the possibility of generating multiple tissue-specific A2AR mRNA species by alternative promoters with varying regulatory susceptibility.

Brain Research Bulletin  (4)


http://www.sciencedirect.com/science/article/B6SYT-4FD76R4-1/2/92d81e96df91a7e22d0dadc8b4b6f88

There is growing evidence that Vitamin D3 (1,25-dihydroxyvitamin D3) is involved in brain development. We have recently shown that the brains of newborn rats from Vitamin D3 deficient dams were larger than controls, had increased cell proliferation, larger lateral ventricles, and reduced cortical thickness. Brains from these animals also had reduced expression of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor. The aim of the current study was to examine if there were any permanent outcomes into adulthood when the offspring of Vitamin D3 deficient dams were restored to a normal diet. The brains of adult rats were examined at 10 weeks of age after Vitamin D3 deficiency until birth or weaning. Compared to controls animals that were exposed to transient early Vitamin D3 deficiency had larger lateral ventricles, reduced NGF protein content, and reduced expression of a number genes involved in neuronal structure, i.e. neurofilament or MAP-2 or neurotransmission, i.e. GABA-A[alpha]4. We conclude that transient early life hypovitaminosis D3 not only disrupts brain development but leads to persistent changes in the adult brain. In light of the high incidence of hypovitaminosis D3 in women of child-bearing age, the public health implications of these findings warrant attention.


http://www.sciencedirect.com/science/article/B6SYT-4F6SR7S-1/2/db8d070dcb7fa99da047e7fda3eb8549

The expression of neuronal nitric oxide synthase (nNOS) and the cGMP-dependent protein kinases cGKI and cGKII in rat cerebellum was evaluated at different developmental stages by quantitative RT-PCR and Western blotting. mRNAs coding for these proteins were detected in the cerebella of rats aged 7, 14 and 21 days. Expression levels, nevertheless, varied significantly at each of these developmental stages. While nNOS and cGKI mRNA levels steadily increased during development, cGKII mRNA showed a different behaviour pattern, with similar levels observed on postnatal days 7 and 14 and increased levels noted on postnatal day 21. Moreover, protein expression profiles for nNOS and cGKI showed similar patterns to the mRNAs encoding these proteins. Our results reveal the developmental regulation of the expression of these proteins in the cerebellum, giving rise to higher levels as the cerebellum matures.
To understand graft rejection in cell based therapies for brain repair we have quantified IL-1[beta], IL-2, IL-4, IL-10, IL-12p40, IFN-[gamma] and TNF-[alpha] mRNA levels using real-time PCR, at days 4, 14, and 42 post-transplantation, in rats engrafted with syngeneic, allogeneic, concordant and discordant xenogeneic neural tissues. In addition, in the discordant xenografts immunohistochemistry and in situ hybridization were applied to detect local expression of IFN-[gamma], TNF-[alpha], IL-10 and TGF-[beta]. Allografts remained non-rejected but expressed IL-1[beta], TNF-[alpha] and IL-4 transcripts but not IL-12p40 and IFN-[gamma]. Xenografts demonstrated distinct cytokine profiles that differed from syngeneic and allogeneic grafts. Non-rejected discordant xenografts contained higher levels of TNF-[alpha] transcripts and lower levels of IL-2 transcripts than the rejected ones at day 42. Discordant xenografts displayed a stronger and earlier expression of IL-1[beta] and TNF-[alpha], followed by T-helper 1 and T-helper 2 associated cytokine expression. The number of cells expressing mRNA encoding TNF-[alpha] and TGF-[beta] was significantly increased over time in the discordant group. In conclusion, the immunological disparity of the implanted tissue explains survival rates and is associated with different cytokine profiles. In allografts, a chronic inflammatory reaction was detected and in xenogeneic grafts a delayed hypersensitivity like reaction may be involved in rejection.

We reported previously that many neurodegenerative changes characteristic of apoptosis could be induced by a short fragment of [beta]-amyloid protein, A[beta]31-35, in cultured newborn mice cortical neurons, and that these changes were accompanied with alterations in expression of some genes. This study was designed to examine whether the apoptotic processes and related gene modulations in this model could be affected by coadministration of carbachol by electrophoretic analysis for DNA ladder formation and by RT-PCR assays for genomic modulation. The results showed that (1) simultaneous incubation with carbachol dose- and time-dependently blocked the specific DNA ladder formation induced by exposure to A[beta]31-35 and (2) the A[beta]31-35-induced downregulation of bcl-2 and upregulations of bax, p53, and c-fos genes were reversed or ameliorated by the coadministration of carbachol. It is proposed that A[beta]31-35-induced apoptosis can be prevented by carbachol through mechanisms that modulate the expression of related genes.
Detecting and quantifying generalized mitochondrial heteroplasmy is essential if the field of mitochondrial genetics is to advance in the arena of complex genetic disorders. The majority of techniques used to detect and quantify mitochondrial heteroplasmy focus on a known mutation or polymorphism. The necessity of knowing the mitochondrial DNA (mtDNA) change beforehand means that non-specific heteroplasmy in general cannot be assessed. In this study, we assessed the extent that denaturing high-performance liquid chromatography (dHPLC) could detect and quantify mitochondrial heteroplasmy from cerebrospinal fluid (CSF). Although we used a known polymorphism to assess reliability and sensitivity of this technique, a distinct advantage to using dHPLC for heteroplasmy detection is that the entire fragment is screened for variability and any unique fragments will be detected regardless of the placement or type of change. Our results demonstrate that dHPLC can consistently and reliably detect mitochondrial heteroplasmy in a CSF sample down to 0.01%. In addition, the level of heteroplasmy was consistent with peak height for each homoduplex, giving a reliable method to quantify level of heteroplasmy.


Comparing female and male brain structures reveals a variety of sex differences in many vertebrates. Some of these differences are thought to be induced during the fetal period by the effect of steroid hormones produced in the gonads. Not much is known about molecular mechanisms involved in gender-specific development of the brain. We have taken a broad approach to isolate sex-specific genes from 18.5 days post coitum brain (A. Eriksson, C. Wahlestedt and K. Nordqvist. 1999. Isolation of sex-specific cDNAs from fetal mouse brain using mRNA differential display and representational difference analysis. Mol. Brain Res., 74, 91-97). Female and male mouse brains were screened with the signal peptide differential display, developed in our laboratory, and with a modified representational difference analysis of cDNA. The resulting sex-specific fragments were verified by semi-quantitative RT-PCR. Here we describe these methods in detail.


Rapid cloning of 5'- and 3'-cDNA ends polymerase chain reaction (5'-/3'-RACE-PCR) is useful to determine unknown 5'- and 3'-cDNA termini. Even if the method can yield complete cDNA sequences within a couple of days, the RACE procedure bears some characteristic traps and often results in amplification of unspecific PCR-products. Here we used improved 5'- and 3'-RACE-PCR protocols to obtain the complete cDNA sequence of the G-protein-coupled receptor
kinase 6 (GRK6) from a rat brain cDNA library. The use of an anchored oligo-(dT)16-V-primer in the cDNA synthesis, the addition of single-sided PCR steps prior to the RACE-PCRs and the optimization of the dA-tailing reaction conditions in 5'-RACE enhanced RACE-PCR efficiency. Taken together, the method is a tool to determine unknown 5’ and 3’-cDNA ends and enables the detection of different transcription initiation sites and mRNA splice variants even from small tissue samples like distinct brain regions. The extensive troubleshooting section discusses typical problems of each substep and contains additional references for support protocols. Themes: Cell biology, neurotransmitters, modulators, transporters, and receptors. Topics: Gene structure and function: general, second messengers and phosphorylation.


http://www.sciencedirect.com/science/article/B6T3N-3Y8N7FJ-G/2/431cc8f4a6e35c8708c6ceec3cd46ce0

Gamma-aminobutyric acid is the major inhibitory neurotransmitter in the brain. GABA transporters (GATs) remove GABA from the synaptic cleft. Till now, five distinct GABA transporters have been cloned and termed consecutively GAT1 to GAT4 and vGAT. To study the mechanisms by which tolerance and dependence associated with drugs enhancing GABAergic transmission is brought upon we analysed the mRNA expression levels of GATs in various brain regions under different conditions. In this paper, we describe our protocol for measurement of GAT3 mRNA expression, and its validation through control experiments for the various steps. We performed competitive reverse transcription and polymerase chain reaction (RT-PCR) with a competitor cRNA as internal standard. Different amounts of competitor cRNA were added to total RNA prepared from different tissue samples, reverse-transcribed and PCR amplified. The PCR amplification gave two products: the GAT wild type fragment and the competitor fragment. PCR products were separated by gel electrophoresis and band intensities were determined from which the relative and absolute abundance of GAT3 mRNA was calculated by regression analysis. Validation experiments in our laboratory showed a 6% intra-assay and a 15% inter-assay variability of this method. Themes: Neurotransmitters, modulators, transporters, and receptors. Topics: GABA, uptake and transporters.


http://www.sciencedirect.com/science/article/B6T3N-454B8WG-7/2/a0e4c754c509b796b0773638e0316c20

Reverse transcription-polymerase chain reaction (RT-PCR) is a powerful tool to detect specific gene expression from a small amount of tissue, which is superior to the traditional RNA assays such as Northern blotting and in situ hybridization (ISH) in terms of sensitivity. However, conventional RT-PCR is not suitable for quantification due to its exponential nature. Recently, a real-time quantitative PCR method has been developed to overcome the weak points of RT-PCR, e.g. quantification. Here we describe the use of real-time quantitative PCR using a fluorescent TaqMan probe, to study the regional differences in expression of glutamate receptor subunit/subtype genes (NR1, NR2A, GluR2, KA2, mGluR1, mGluR7) in the central vestibular system including the vestibular nucleus complex, inferior olive and cerebellar flocculus. We found that real-time quantitative PCR yielded similar results to other techniques such as ISH but offered several advantages in terms of relative speed and ability to detect low levels of gene expression. We suggest that real-time quantitative PCR is a useful method to study gene expression for other
neurotransmitter receptors in the vestibular brainstem and cerebellum, and is also expected to be more accurate to assess the changes in gene expression following any treatment.


http://www.sciencedirect.com/science/article/B6T3N-48B59FN-7/2/18527882fa846e62dbf8ecbc0c888b9

The N-methyl-D-aspartate (NMDA)-selective subtype of ionotropic glutamate receptor is of importance in neuronal differentiation and synapse consolidation, activity-dependent forms of synaptic plasticity, and excitatory amino acid-mediated neuronal toxicity [Neurosci. Res. Program Bull. 19 (1981) 1; Lab. Invest. 68 (1993) 372]. NMDA receptors exist in vivo as tetrameric or pentameric complexes comprising proteins from two families of homologous subunits, designated NR1 and NR2(A-D) [Biochem. Biophys. Res. Commun. 185 (1992) 826]. The gene coding for the human NR1 subunit (hNR1) is composed of 21 exons, three of which (4, 20 and 21) can be differentially spliced to generate a total of eight distinct subunit variants. We detail here a competitive RT-PCR (cRT-PCR) protocol to quantify endogenous levels of hNR1 splice variants in autopsyed human brain. Quantitation of each hNR1 splice variant is performed using standard curve methodology in which a known amount of synthetic ribonucleic acid competitor (internal standard) is co-amplified against total RNA. This method can be used for the quantitation of hNR1 mRNA levels in response to acute or chronic disease states, in particular in the glutamatergic-associated neuronal loss observed in Alzheimer's disease [J. Neurochem. 78 (2001) 175]. Furthermore, alterations in hNR1 mRNA expression may be reflected at the translational level, resulting in functional changes in the NMDA receptor.


http://www.sciencedirect.com/science/article/B6T3N-48B59FN-8/2/06c35607188a9853f7be5c6f2db3a73f

The NMDA-selective ionotropic receptor constitutes one of the three principal classes of -glutamate receptors within the mammalian brain. It plays key roles in neuronal differentiation and synapse consolidation, activity-dependent forms of synaptic plasticity, and excitatory amino acid-mediated neuronal toxicity [Lab. Invest., 68 (1993) 372-387]. NMDA receptors exist as multimeric complexes comprising proteins from two families, NR1 and NR2(A-D) [J. Biol. Chem., 271 (1996) 15669-15674]. Studies on recombinant receptors have revealed that while homomeric NR2 receptors are non-functional, co-expression of an NR1 with an NR2 subunit modulates the efficacy of the resulting channel [Nature, 357 (1992) 70-74]. The RT-PCR assay we describe here was developed to allow quantitation of all hNR2 transcripts in a single-tube PCR assay. Each hNR2 isoform is quantified on the basis of standard curves in which a known amount of synthetic ribonucleic acid competitor is co-amplified against total RNA. The protocol has been applied to the quantitation of hNR2 mRNA levels in autopsy brain. Used in conjunction with a method for the quantitation of hNR1 transcripts [Brain Res. Protoc., in press], a complete analysis of NMDA receptor mRNA expression can be obtained.

Our laboratory has developed a one-step quantitative reverse transcription polymerase chain reaction (RT-PCR) procedure in which the reverse transcriptase enzyme and Taq DNA polymerase are combined in the one tube and a single, non-interrupted, thermal cycling program is performed. In the past, RT-PCR has been carried out with two separate steps: (1) reverse transcription of RNA to generate a cDNA pool and (2) polymerase chain reaction amplification of the cDNA. The two-step method can affect the accuracy of the procedure as the total number of manipulations is greater, thereby allowing a greater chance for pipetting errors. Quantitation by our method is achieved in a single reaction by the use of a competitive internal standard that is identical in sequence to the target RNA except for a deletion of 107 base pairs and uses identical primers and cycling conditions. Using this method, we have been able to quantify the amount of message of a G protein (Gz[alpha]), in small amounts of tissue, such as dorsal root ganglia, from embryonic as well as postnatal mice.


Differential gene expression plays an important role in normal development and pathophysiological conditions. The accurate quantitation of mRNA expression is critical to assess the differential gene expression. While a number of techniques, such as Northern analysis, (semi-)quantitative reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization, are available to measure the levels of mRNA expression, certain limitations exist, including the insensitive and inaccurate quantitation of mRNA expressed at low abundance. In the present study, we describe the application of a recently developed TaqMan real-time quantitative RT-PCR for the detection of interleukin-1[beta] (IL-1[beta]) mRNA expression in rat cortical tissue after a short duration of ischemia (i.e., ischemic preconditioning). The principle of the TaqMan real-time detection is based on the fluorogenic 5' nuclease assay that allows simple and rapid quantitation of a target sequence during the extension phase of PCR amplification. Using a cloned plasmid DNA as a standard and normalizing RNA samples with a housekeeping gene for the TaqMan real-time PCR, we detected the significant induction in absolute copy numbers of IL-1[beta] mRNA in the ipsilateral cortex after preconditioning, suggesting a potential role of this inflammatory cytokine in ischemic brain tolerance.


We describe a protocol for analysis of gene expression in single, acutely dissociated adult rat retinal ganglion cells using RT-PCR. Retrograde tracing of retinal ganglion cells from the superior colliculi was conducted using Fluorogold. Retinas were dissected and ganglion cells isolated using retinal layer separation (sandwiching). Single, fluorescently labelled retinal ganglion cells
were aspirated using a micropipette and used for PCR. Two PCR protocols are described where single cell cDNA was analysed for TrkB and GAPDH or TrkB, TrkC, Ret, Met, ErbB2 and Beta-actin by multiplex-PCR. All five tyrosine kinase receptors were amplified from single retinal ganglion cells. The method will prove useful for the molecular characterization of adult retinal ganglion cells.


http://www.sciencedirect.com/science/article/B6T3N-44HYF22C/2/2d65e5a10bf9a2d98792ebec7baae0ac

Aldose reductase (AR) and sorbitol dehydrogenase (SDH) are the enzymes constituting the polyol pathway, an alternate route of glucose metabolism. A wealth of experimental data has indicated the involvement of the polyol pathway in the pathogenesis of diabetic complications. However, there has been surprisingly little research on the relative abundance of SDH to AR in the tissues affected in diabetes. We therefore developed a competitive RT-PCR system to simultaneously determine the mRNA levels of these two enzymes in small amounts of samples, and studied their expression in Schwann cells isolated from adult rat sciatic nerves. Although both AR and SDH mRNA were expressed in the Schwann cells, the levels of SDH cDNA were much lower than those of AR cDNA. The induction of AR mRNA expression in the Schwann cells under hyperosmotic conditions was similarly detected by Northern blot analysis and our competitive RT-PCR method. The RT-PCR system developed in this study may be a useful tool in ascertaining the relative contributions of AR and SDH to the metabolic derangements resulting from the acceleration of polyol pathway activity in the target organ of diabetic complications.


http://www.sciencedirect.com/science/article/B6T3N-3XG1TBJ-1/2/a6a0df67f98ecd193c267149074bb2e8

The reverse transcription linked polymerase chain reaction (RT-PCR) is a powerful technique for detecting mRNAs of low abundance while enabling distinction between homologous mRNAs such as family members and between alternative splice variants. We utilized this technique for quantitative analysis of expression of nine fibroblast growth factor (FGF) and four FGF receptor (FGFR) family genes in mouse brain during development and adulthood. The primer sets and reaction conditions for each family member were optimized for efficient amplification, and the amplified products were detected by hybridization with specific probes to ensure specificity. To achieve quantitative measurement, serial concentrations of the cloned cDNAs were simultaneously amplified and the results were used to titrate the amount of mRNA in the samples. Since FGF family has been recently recognized to be important in various functions of central nervous system and the protocol described here is directly applicable for a variety of small tissue samples, this protocol is very helpful in understanding the involvement of FGF family in various physiological phenomena.

The P2X receptor is a receptor-gated cationic channel that responds to ATP. The quantification of P2X mRNA expression in dorsal root ganglion (DRG) provides important information for neuropathic pain studies. We developed a rapid and sensitive external-standard-based real-time quantitative PCR assay for the quantification of mRNA of P2X receptors in mouse tissue samples. The assay uses a double-stranded DNA fluorescent dye, SYBR Green I, to continuously monitor product formation with a GeneAmp 5700 Sequence Detection System (PE Applied Biosystems). To establish the quantitative PCR amplification in a wide range of target transcripts, optimum parameters of primer sequences, concentrations of primers and/or templates, and PCR thermal protocols were experimentally determined. We also tested the reliability of this method in established experimental murine models, which were made by ligation or cutting down of the sciatic nerve. The parameters defined in this assay should be applicable to the quantification of other types of pain models and other tissue samples of mouse.


Defining molecular repertoires within virally infected tissues of the nervous system may provide insight into the pathogenesis of, and immunity to, neurotropic viruses. Here we report the application of such a method, namely mRNA differential display (DD), to the identification of mRNAs that are expressed at different levels in herpes simplex virus (HSV) infected nervous tissue from immunocompetent and CD8+ lymphocyte depleted mice. Small amounts of input RNA can be used by DD, making the method ideal for experiments based on murine sensory ganglia (DRG), which on average yield less than 0.5 [mu]g of total RNA. In the current work, DD facilitated the identification of a mRNA whose abundance in HSV-infected ganglia, based on Northern blot analysis, was reduced in mice depleted of CD8+ cells. The cloned product of this mRNA was of particular interest to our research as sequence data strongly suggested that it represented the murine homologue of the [alpha] chain of a G protein termed Golf. This G protein had not previously been reported from dorsal root ganglial tissue. RT-PCR confirmed the presence of Golf in DRG and in situ hybridization studies localised this molecule to primary sensory neurons. These data indicate that DD is sufficiently robust to be applied to the study of virus pathogenesis within the nervous system.


Reverse-transcribed polymerase chain reaction (RT-PCR) can quantify gene transcripts at low levels and in small samples. Semi-quantitative and quantitative RT-PCR has significant advantages over traditional RNA assays, such as Northern blotting and ribonuclease protection assay. However, owing to the exponential nature of PCR, considerable effort is required to verify linearity of the reaction. Thus, care must be taken to detect small but physiologically relevant changes in gene expression. Using a rapid and highly sensitive RT-PCR method, TaqMan real-time RT-PCR, we determined agonist-induced changes in rat mu opioid receptor (MOR) mRNA
levels in cultured cells and compared our results with those obtained by radiolabeled quantitative RT-PCR, which is also highly sensitive but much more time-consuming than TaqMan RT-PCR. Both methods showed up-regulation of agonist-induced MOR. TaqMan RT-PCR showed a similar sensitivity to radiolabeled quantitative RT-PCR and is suitable for the measurement of large numbers of samples. Moreover, no need for radiolabeled compounds is also an advantage of TaqMan PCR. This protocol will probably be useful for quantifying MOR in animal and human tissues.

Brain Research Reviews (1)


http://www.sciencedirect.com/science/article/B6SYS-3YYTCYS-12/2/8f93439b1da9686ae92e18bbf492a47

Astrocytes are characterized by extensive gap junctional intercellular communication (GJIC) mediated primarily by channels composed of connexin43. In contrast, C6 glioma cells are deficient in connexin expression and gap junctional communication. Transfection of these glioma cells with connexin cDNAs results in changes in cellular phenotype following increased GJIC. Specifically, connexin expression correlates with reduced cellular proliferation and tumorigenicity. To characterize the role of gap junctions in this growth control, we have screened for changes in gene expression by differential display. We have observed that these changes in GJIC are associated with changes in expression of several genes, including those coding for a number of secreted factors which may play a role in modulating the tumor phenotype of these cells. These include the immediate early gene cyr61, ostoepontin and the KC gene (murine homologue of the human gro gene).

Brain, Behavior, and Immunity (3)


http://www.sciencedirect.com/science/article/B6WC1-470M3GB-7/2/d295fc00917da1acbe740d43c9e9a98f


http://www.sciencedirect.com/science/article/B6WC1-49J8TGN-
Several reports show that behavioural and physiological components of the acute phase reaction can be conditioned. However, the mechanisms responsible for these effects remain obscure. The underlying assumption that the changes observed in conditioned animals are dependent on a conditioned production of cytokines has never been demonstrated. In the present study, the possibility of conditioning the production of cytokines or molecules implicated in their signalling pathways was tested by submitting mice to conditioned taste aversion with a new saccharin taste paired with intraperitoneal (i.p.) injections of lipopolysaccharide (LPS, 0.83 [mu]g/g) or peptidoglycan (PGN, 20 [mu]g/g). After two conditioning sessions, conditioned mice developed a clear aversion to saccharine that was not associated with activation of genes of the cytokine network either at the periphery, or in the hypothalamus, as demonstrated by a macroarray approach and confirmed by real time RT-PCR. In contrast, there was an activation of the genes coding for nuclear factor kappa B (NF[kappa]B) and mitogen activated protein kinase (MAPK) signalling pathways in the spleen and to a lesser extent in the hypothalamus. This modulation of the NF[kappa]B and MAPK signalling pathways is interpreted in terms of a possible conditioned sensitisation of the immune system.


The beta-2-adrenergic receptor ([beta]2AR) is expressed by most lymphocyte populations and binds the sympathetic neurotransmitter norepinephrine (NE). Stimulation of the [beta]2AR is reported to be the primary mechanism by which signals from the sympathetic nervous system influence both cell-mediated and humoral immunity. We report here that body/organ weights, lymphoid organ cell number/phenotype/histology, the contact sensitivity response, and the amount, avidity, and isotype of antibody resulting from a T cell-dependent antibody response in [beta]2AR deficient mice ([beta]2AR/- mice) were all similar to measures made in [beta]2AR+/+ mice. Other members of the adrenergic receptor family did not appear to compensate for the absence in [beta]2AR expression. In contrast, [beta]2AR/- B cells cultured in vitro were unable to respond to NE in a manner similar to [beta]2AR+/+ B cells. Thus, mice in which expression of the [beta]2AR gene is defective from early development to adulthood may no longer require that NE stimulate the [beta]2AR to maintain immune homeostasis, and this may be due to a non-adrenergic mechanism that provides compensation in vivo.


Human T cell leukemias can arise from oncogenes activated by specific chromosomal
translocations involving the T cell receptor genes. Here we show that five different T cell oncogenes (HOX11, TAL1, LYL1, LMO1, and LMO2) are often aberrantly expressed in the absence of chromosomal abnormalities. Using oligonucleotide microarrays, we identified several gene expression signatures that were indicative of leukemic arrest at specific stages of normal thymocyte development: LYL1+ signature (pro-T), HOX11+ (early cortical thymocyte), and TAL1+ (late cortical thymocyte). Hierarchical clustering analysis of gene expression signatures grouped samples according to their shared oncogenic pathways and identified HOX11L2 activation as a novel event in T cell leukemogenesis. These findings have clinical importance, since HOX11 activation is significantly associated with a favorable prognosis, while expression of TAL1, LYL1, or, surprisingly, HOX11L2 confers a much worse response to treatment. Our results illustrate the power of gene expression profiles to elucidate transformation pathways relevant to human leukemia.


http://www.sciencedirect.com/science/article/B6WWK-4CM8XX0-8/2/eafb75e98b129ac92a872da45d265b52

R-etodolac, a nonsteroidal anti-inflammatory drug, inhibits the progression of CWRSA6 androgen-independent and LuCaP-35 androgen-dependent prostate cancer xenograft growth through downregulation of cyclin D1 expression via the PPAR[gamma] pathway. PPAR[gamma] protein degradation, observed post-R-etodolac treatment, resulted from phospho-MAP kinase (p44/42) induction by R-etodolac negatively regulating PPAR[gamma] function. Negative regulation of PPAR[gamma] was overcome by a combination regimen of R-etodolac with the HER-kinase axis inhibitor, rhuMab 2C4, which demonstrated an additive antitumor effect. We further show that the inhibition of HER-kinase activity by rhuMab 2C4 is sufficient to inhibit PPAR[gamma] protein degradation. This study introduces a novel concept of an in vivo crosstalk between the HER-kinase axis and PPAR[gamma] pathways, ultimately leading to negative regulation of PPAR[gamma] activity and tumor growth inhibition.


http://www.sciencedirect.com/science/article/B6WWK-48FC6DV-B/2/ce3955c94a141080a13e296530212a23

The mechanisms of drug resistance in cancer are poorly understood. Serial analysis of gene expression (SAGE) profiling of cisplatin-resistant and sensitive cells revealed many differentially expressed genes. Remarkably, many ECM genes were elevated in cisplatin-resistant cells. COL6A3 was one of the most highly upregulated genes, and cultivation of cisplatin-sensitive cells in the presence of collagen VI protein promoted resistance in vitro. Staining of ovarian tumors with collagen VI antibodies confirmed collagen VI expression in vivo and suggested reorganization of the extracellular matrix in the vicinity of the tumor. Furthermore, the presence of collagen VI correlated with tumor grade, an ovarian cancer prognostic factor. These results suggest that tumor cells may directly remodel their microenvironment to increase their survival in the presence of chemotherapeutic drugs.
A CpG island DNA methylator phenotype has been postulated to explain silencing of the hMLH1 DNA mismatch repair gene in cancer of the microsatellite mutator phenotype. To evaluate this model, we analyzed methylation in CpG islands from six mutator and suppressor genes, and thirty random genomic sites, in a panel of colorectal cancers. Tumor-specific somatic hypermethylation was a widespread age-dependent process that followed a normal Gaussian distribution. Because there was no discontinuity in methylation rate, our results challenge the methylator phenotype hypothesis and its hypothetical pathological underlying defect. We also show that the mutator phenotype dominates over the gradual accumulation of DNA hypermethylation in determining the genotypic features that govern the phenotypic peculiarities of colon cancer of the mutator pathway.

Cancer Detection and Prevention  (4)


The detection of circulating cancer cells in the bone marrow (BM) and peripheral blood (PB) of patients with solid tumors may be useful for disease staging. To this aim, we evaluated the expression of the mammaglobin gene by reverse transcriptase polymerase chain reaction (RT-PCR) in 60 patients with breast cancer. Moreover, several controls were examined to test the specificity of this marker. The positive cases included 23.6% of the patients with and 9% of those without metastasis. Only 4/60 negative controls analyzed were positive by PCR. Our results show high specificity and a good correlation with disease status.


To investigate the etiological association of allelic loss at chromosomal regions containing tumor suppressor genes (TSGs) in non-small cell lung cancer (NSCLC) in Taiwan, we examined 48 microdissected NSCLC samples for loss of heterozygosity (LOH) at nine loci where TSGs are localized nearby. The associations of LOH at each locus with clinicopathological features and prognosis were also examined. The frequent LOH was observed using markers, D3S1285 near the FHIT gene (58.3%), D17S938 near the p53 gene (56.7%), D9S925 near the p16 gene (54.5%), and D13S153 near the RB gene (47.6%). The occurrence of LOH at each TSG locus was compared
with the patients’ clinicoparameters. The incidence of LOH at D17S938 (p53 gene) and D3S4545 (VHL gene) was significantly higher in squamous carcinoma tumors than in adenocarcinoma tumors (P=0.003 and 0.024, respectively). LOH of these two loci also occurred frequently in tumors from smoker patients compared to that from nonsmoker patients (P=0.013 and 0.025, respectively). LOH at D13S153 (RB gene) was also associated with smoking (P=0.008). In addition, the prognostic analyses indicated that the patients with LOH at D18S535 (18q21, near the SMAD2/4 gene) had significantly longer post-operative survival time compared to those without LOH (P=0.03). Our results suggested that LOH at FHIT, p53, and p16 genes may occur frequently in NSCLC patients in Taiwan. In addition, LOH at p53, RB, and VHL may associate with smoking or squamous carcinoma patients and LOH at SMAD2/4 may be correlated with better prognosis.


Synovial sarcoma (SS) is characterized by the t(X;18)(p11.2;q11.2) chromosomal translocation, which results in generating either SYT-SSX1, SYT-SSX2 or, infrequently, SYT-SSX4 fusion gene. The ratio of SYT-SSX1:SYT-SSX2 fusions is close to 2:1 in the majority of studies, and SYT-SSX2 fusion has been only rarely observed in biphasic SS. In the present study, we compared two series of patients with SS, Slovenian (37 cases) and Dutch (14 cases), with respect to clinical, pathological and molecular findings. The two groups did not differ with regard to clinicopathological features. Whereas the frequency of different SYT-SSX fusions in the Dutch group was similar to that reported in the literature, we found an unexpectedly high number of tumors with SYT-SSX2 fusion in the Slovenian group. The ratio of SYT-SSX1:SYT-SSX2 fusion was 7:18 for monophasic and 2:7 for biphasic tumors in the Slovenian group. This distribution differs significantly from that observed in the Dutch group in the present study (P = 0.041) as well as from data reported in the recent large multi-institutional study on 243 patients (P = 0.0001). Our findings indicate possible geographical differences in the frequency of two SYT-SSX fusion transcripts in patients with synovial sarcoma.
transcripts in patients with synovial sarcoma.

**Cancer Epidemiol. Biomarkers Prev.** (37)


http://cebp.aacrjournals.org/cgi/content/abstract/13/12/2141

Objective: The progestagenic milieu of pregnancy and oral contraceptive use is protective against epithelial ovarian cancer. A functional single nucleotide polymorphism in the promoter of the progesterone receptor (+331A) alters the relative abundance of the A and B isoforms and has been associated with an increased risk of endometrial and breast cancer. In this study, we sought to determine whether this polymorphism affects ovarian cancer risk. Methods: The +331G/A polymorphism was genotyped in a population-based, case-control study from North Carolina that included 942 Caucasian subjects (438 cases, 504 controls) and in a confirmatory group from Australia (535 cases, 298 controls). Logistic regression analysis was used to calculate age-adjusted odds ratios (OR). Results: There was a suggestion of a protective effect of the +331A allele (AA or GA) against ovarian cancer in the North Carolina study [OR, 0.72; 95% confidence interval (95% CI), 0.47-1.10]. Examination of genotype frequencies by histologic type revealed that this was due to a decreased risk of endometrioid and clear cell cancers (OR, 0.30; 95% CI, 0.09-0.97). Similarly, in the Australian study, there was a nonsignificant decrease in the risk of ovarian cancer among those with the +331A allele (OR, 0.83; 95% CI, 0.51-1.35) that was strongest in the endometrioid/clear cell group (OR, 0.60; 95% CI, 0.24-1.44). In the combined U.S.-Australian data that included 174 endometrioid/clear cell cases (166 invasive, 8 borderline), the +331A allele was significantly associated with protection against this subset of ovarian cancers (OR, 0.46; 95% CI, 0.23-0.92). Preliminary evidence of a protective effect of the +331A allele against endometriosis was also noted in control subjects (OR, 0.19; 95% CI, 0.03-1.38). Conclusions: These findings suggest that the +331G/A progesterone receptor promoter polymorphism may modify the molecular epidemiologic pathway that encompasses both the development of endometriosis and its subsequent transformation into endometrioid/clear cell ovarian cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/11/11/1394

**Hybrid Capture 2 Test using probe B (HC2-B) is a clinical test for the detection of 13 human papillomavirus (HPV) types associated with cervical cancer (oncogenic types), but the potential clinical significance of HC2-B cross-reactivity with untargeted (nononcogenic) HPV types has not been fully evaluated. Thus, HC2-B test results on 954 clinical cervical specimens from a population-based natural history study of HPV in Costa Rica were compared with the data from testing of the same specimens twice by HPV type-specific MY09/MY11 L1 consensus primer PCR. Specimens positive by PCR for single HPV types not targeted by HC2-B were used for**
determining type-specific cross-reactivity. Effects of cross-reactivity on clinical performance were estimated by calculating sensitivity and specificity with and without cross-reactivity for the detection of high-grade cervical lesions. HC2-B tested positive for single infections by untargeted (cross-reactive) types 11, 53, 61, 66, 67, 70, 71, and 81. Cross-reactivity was strongly associated with PCR signal strength (PTrend = 0.0001) and cervical abnormalities (P = 0.0002, Pearson \( \chi^2 \)). We estimated that HC2-B cross-reactivity resulted in minor changes in screening performance. Clinical sensitivity increased from 84.3% to 87.9%, clinical specificity decreased from 89.6% to 88.1%, and referral rates increased from 11.7% to 13.2% for detection of \[ \geq \]cervical intraepithelial neoplasia grade 2. The clinical effect of cross-reactivity varied by cytologic interpretation. Among women with normal cytologic interpretations, cross-reactivity significantly improved the accuracy of identifying cytologically nonevident histology of \[ \geq \]cervical intraepithelial neoplasia grade 2 because of increased sensitivity with maintained specificity. However, among women with equivocal or mildly abnormal cytologic interpretations, cross-reactivity decreased the accuracy of HPV testing because of substantial decreases in specificity. In summary, cross-reactivity with nononcogenic HPV types had little effect on the overall clinical performance of HC2-B as a general screening test, but reduction of cross-reactivity might improve the performance of HPV testing for triage of equivocal or mildly abnormal cytologic interpretations.


http://cebp.aacrjournals.org/cgi/content/abstract/14/1/256

In large active cohort studies of women investigating human papillomavirus (HPV) and cervical neoplasia, many women will be HPV-negative at all time points and testing of all their cervical specimens is an inefficient use of laboratory resources. The aim of this pilot study was to evaluate whether pooling cervical specimens from the same woman might provide a useful pretest of specimens from women unlikely to have high-grade cervical neoplasia or significant HPV exposure. We selected women (n = 187) participating in the Guanacaste Project for whom we already had HPV testing data on all their specimens from multiple visits (median = 8 visits), who were HPV DNA-negative at enrollment and at their 5- to 7-year exit from the cohort, and had no evidence of high-grade cervical neoplasia. Equal aliquots of cervical specimens from these women were pooled to create a proportional pooled specimen. Aliquots of pooled specimens were tested in a masked fashion by MY09/11 L1 consensus primer PCR. Second aliquots of some pooled specimens (n = 83) were included to assess the reliability of pooled testing. Results were compared with the predicted (expected) results based on the obtained test results of the individual specimens collected at interim visits. There was good overall agreement between observed and expected HPV DNA positivity, with \( \kappa \) of 0.63 [95% confidence interval (95% CI), 0.51-0.75] and a percent agreement of 83.4% (95% CI, 77.3-88.5%) although the HPV DNA positivity in the pooled specimen was less than expected (P = 0.001). The agreement between observed and expected HPV DNA positivity was related to the number of aliquots pooled, suggesting that positivity was related to viral genome concentrations. The\( \kappa \) and percent agreement for intra-batch reliability of testing pooled specimens were 0.68 (95% CI, 0.53-0.84) and 84.3% (95% CI, 74.7-91.4%), respectively. We conclude that pooling specimens and testing by PCR may be useful for discriminating HPV DNA-positive from completely negative specimen sets in women who are likely to have been HPV DNA-negative.

The ideal technology for screening single-nucleotide polymorphisms requires high throughput with minimal cost per sample, minimal usage of valuable DNA resources, and maximal flexibility for assessment of new polymorphisms. We demonstrate here the feasibility of kinetic allele-specific PCR with DNA pooling (S. Germer et al., Genome Res., 10: 258-266, 2000) in a population study that satisfies all of the mentioned criteria and offers a powerful new tool for detecting meaningful polymorphic differences in candidate gene association studies and genome-wide linkage disequilibrium scans. Three individuals prepared pooled DNA samples from 269 individuals separated into three racial/ethnic groups: Caucasians (n = 56), African-Americans (n = 86), and Hispanics (n = 127). We used kinetic allele-specific PCR to determine the allele frequencies of the common paraoxonase 1 polymorphism, PON1 Q191R, in these pools. Paraoxonase 1 is a critical enzyme for inactivating neurotoxic intermediates in the metabolism of organophosphates. In a blinded test of the technology, these nine pooled DNA samples were sent to Roche for genotyping by kinetic allele-specific PCR. The allele frequencies found were 0.266 {+/-} 0.011, 0.386 {+/-} 0.011, and 0.617 {+/-} 0.010, respectively, which were comparable to the frequencies of 0.269, 0.403, and 0.622 determined by PCR-restriction fragment length polymorphism analysis. These same samples were genotyped on two kinetic PCR platforms from different manufacturers, using three different DNA polymerases. The results were comparable between both platforms and among all three polymerases. The results demonstrate a powerful new technology for determining frequencies of single-nucleotide polymorphisms in an epidemiological study.


5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, diverting metabolites toward methylation reactions or nucleotide synthesis. Using data from an incident case-control study (1608 cases and 1972 controls) we investigated two polymorphisms in the MTHFR gene, C677T and A1298C, and their associations with risk of colon cancer. All of the combined genotypes were evaluated separately, and the 1298AA/677CC (wild-type/wild-type) group was considered the reference group. Among both men and women, the 677TT/1298AA (variant/wild-type) genotype was associated with a small reduction in risk [men: odds ratio (OR), 0.7, 95% confidence interval (CI), 0.5-1.0; women: OR, 0.8, 95% CI, 0.5-1.2]. However, the 677CC/1298CC (wild-type/variant) genotype was associated with a statistically significant lower risk among women (OR, 0.6; 95% CI, 0.4-0.9) but not men. When the polymorphisms were considered individually, for A1298C a significant risk reduction associated with the homozygous variant CC genotype was seen among women only (OR, 0.6; 95% CI, 0.4-0.9), and nonstatistically significant reduced risks were observed for the variant 677 TT genotypes among both men and women. Stratification by nutrient intakes showed inverse associations with higher intakes of folate, vitamin B2, B6, B12, and methionine among women with the MTHFR 677CC/1298AA genotypes, but not those with 677TT/1298AA. We observed opposite risk trends for both MTHFR variants, depending on whether women used hormone-replacement therapy or not (P for interaction = <.01). In summary, this study supports recent findings that the MTHFR A1298C polymorphism may be a predictor of colon cancer risk and have functional relevance. The possible interaction with hormone-replacement therapy warrants additional investigation.

Microsatellite instability (MSI) occurs in 10-20% of the sporadic colon carcinomas and appears to be primarily due to alterations in hMLH1 and hMSH2. Little is known about the role of diet in MSI-related colon carcinogenesis. We used data from a Dutch population-based case-control study on sporadic colon carcinomas (184 cases and 259 controls) to evaluate associations between dietary factors previously reported as being associated with colon cancer risk and MSI, hMLH1 expression, and hMLH1 hypermethylation. Red meat intake was significantly differently related to microsatellite instability-high (MSI-H) tumors compared with microsatellite instability-low/microsatellite stable (MSI-L/MSS) \[odds ratio (OR), 0.3; 95\% confidence interval (CI), 0.1-0.9\]. It was inversely associated with MSI-H tumors when compared with the population-based controls (OR, 0.5; 95\% CI, 0.2-1.2) and positively associated with MSI-L/MSS tumors (OR, 1.5; 95\% CI, 0.9-2.6). A positive association was observed for alcohol intake with MSI-H tumors (OR, 1.9; 95\% CI, 0.8-4.7). Fruit consumption seemed to especially decrease the risk of MSI-H tumors with hypermethylated hMLH1 (Methyl+ tumors) \[Methyl+ versus controls: OR = 0.4 and 95\% CI = 0.2-0.9; MSI-H tumors without hypermethylated hMLH1 (Methyl- tumors) versus controls, OR = 1.2 and 95\% CI = 0.8-1.7; Methyl+ versus Methyl- tumors, OR = 0.2 and 95\% CI = 0.1-0.9\]. Most other evaluated dietary factors were not distinctively associated with a specific MSI or hMLH1 methylation status. Our data suggest that red meat consumption may enhance the development of MSI-L/MSS carcinomas in particular, whereas alcohol intake appears to increase the risk of MSI-H tumors. Fruit consumption may especially decrease the risk of MSI-H carcinomas exhibiting epigenetically silenced hMLH1.


Increased understanding of human papillomavirus (HPV) infection as the central cause of cervical cancer has permitted the development of improved screening techniques. To evaluate their usefulness, we evaluated the performance of multiple screening methods concurrently in a large population-based cohort of >8500 nonvirginal women without hysterectomies, whom we followed prospectively in a high-risk region of Latin America. Using Youden's index as a measure of the trade-off between sensitivity and specificity, we estimated the performances of a visual screening method (cervicography), conventional cytology, liquid-based cytology (ThinPrep), and DNA testing for 13 oncogenic HPV types. The reference standard of disease was neoplasia \[IMG]="cervical intraepithelial neoplasia grade 3 (CIN 3), defined as histologically confirmed CIN 3 detected within 2 years of enrollment (n = 90) or invasive cancer detected within 7 years (n = 20). We analyzed each technique alone and in paired combinations (n = 112 possible strategies), and evaluated the significance of differences between strategies using a paired Z test that equally weighted sensitivity and specificity. As a single test, either liquid-based cytology or HPV DNA testing was significantly more accurate than conventional cytology or cervicography. Paired tests incorporating either liquid-based cytology or HPV DNA testing were not substantially more accurate than either of those two test strategies alone. However, a possibly useful synergy was observed between the conventional smear and cervicography. Consideration of age or behavioral risk profiles did not alter any of these conclusions. Overall, we conclude that highly accurate screening for cervical cancer and CIN 3 is now technically feasible. The remaining vital issue is to extend improved cervical cancer prevention programs to resource-poor regions.


An increased occurrence of colorectal cancer and its adenoma precursor is observed among individuals with low intakes or circulating levels of folate, especially if alcohol intake is high, although results have not been statistically significant in all studies. We examined folate and alcohol intake and genetic polymorphisms in methylenetetrahydrofolate reductase [MTHFR 667[-&gt;]T (ala[-&gt;]val) and MTHFR 1298A[-&gt;]C (gln[-&gt;]ala)] (associated with reduced MTHFR activity) and in alcohol dehydrogenase 3 [ADH3 (2-2) associated with decreased alcohol catabolism] in relation to risk of colorectal adenoma in the Health Professionals Follow-Up Study. Among 379 cases and 726 controls, MTHFR genotypes were not appreciably related to risk of adenoma, but a suggestive interaction (P = 0.09) was observed between MTHFR 677C[-&gt;]T and alcohol intake; men with TT homozygotes who consumed 30+ g/day of alcohol had an odds ratio (OR) of 3.52 [95% confidence interval (CI), 1.41-8.78] relative to drinkers of [<=]5 g/day with the CC/CT genotypes. ADH3 genotype alone was not appreciably related to risk, but its influence was modified by alcohol intake. Compared with fast alcohol catabolizers [ADH3(1-1)] with low intakes of alcohol ([<=]5 g/day), high consumers of alcohol (30+ g/day) had a marked increase in risk if they had the genotype associated with slow catabolism [ADH3(2-2); OR, 2.94; 95% CI, 1.24-6.92] or intermediate catabolism [ADH3(1-2)] of alcohol (OR, 1.83; 95% CI, 1.03-3.26) but not if they were fast catabolizers [ADH3(1-1); OR = 1.27; 95% CI = 0.63-2.53]. In addition, an increased risk of colorectal adenoma (OR, 17.1; 95% CI, 2.1-137) was observed for those with the ADH3(2-2) genotype and high alcohol-low folate intake compared with those with low alcohol-high folate intake and the ADH3(1-1) genotype (P for interaction = 0.006). Our results indicate that high intake of alcohol is associated with an increased risk of colorectal adenoma, particularly among MTHFR 677TT and ADH3(2-2) homozygotes. The findings that alcohol interacts with a folate-related gene (MTHFR) and that the interaction between alcohol and ADH3 is stronger among those with low folate intake support the hypothesis that the carcinogenic influence of alcohol in the large bowel is mediated through folate status.


Studies investigating human papillomavirus (HPV) viral load as a risk factor in the development of squamous intraepithelial lesions (SILs) and cancer have often yielded conflicting results. These studies used a variety of HPV viral quantitation assays [including the commercially available hybrid capture 2 (HC 2) assay], which differ in their ability to account for differences in cervical cell collection, linear dynamic range of viral load quantitation, and determination of type-specific versus cumulative viral load measures. HPV-16 and HPV-18 viral quantitation using real-time PCR assays were performed to determine whether type-specific viral load measurements that adjust for specimen cellularity result in a different association between viral load and prevalent SIL and cancer, compared with HC 2 quantitation (which does not adjust for cellularity or multiple infections). In general, HPV-16 viral load as measured by real-time PCR increased linearly with increasing grade of SIL while HPV-18 measured using similar techniques increased through low-grade SIL (LSIL), with HPV-18 viral load among high-grade SIL and cancers near the level of cytologically normal women. HC 2 viral load, using the clinical 1.0 pg/ml cut point, differentiated cytologically normal women from women with any level of cytological abnormality (normal versus >=LSIL) but did not change as lesion severity increased. There was no evidence for plateau of HC 2 at high copy numbers, nor was significant variability in total specimen cellularity observed. However, cumulative viral load measurements by HC 2, in the presence of multiple coinfections, overestimated type-specific viral load. Multiple infections were more common among women with
no (32%) or LSIL (51%) [versus 23% in high-grade SIL/cancer], partially explaining the lack of a
dose response using a cumulative HC2 viral load measure. The nonrandom distribution of
multiple infections by case-control status and the apparent differential effect of viral load by
genotype warrant caution when using HC 2 measurements to infer viral load associations with
SIL and cancer.


http://cebp.aacrjournals.org/cg i/content/abstract/13/8/1407

The production of estrogen from androgen via the estrogen biosynthesis pathway is catalyzed by
aromatase P450 (CYP19). To assess the association between breast cancer risk and a
polymorphism at codon 39 Trp/Arg of the encoding gene, a case-control study was conducted at
Aichi Cancer Center Hospital in Japan. Subjects were 248 histologically confirmed breast cancer
patients and 603 hospital controls without cancer. Odds ratios (OR) and 95% confidence intervals
(95% CI) were determined by logistic regression analysis. The allele frequency among controls
was 3.8% for the C allele, and the OR (95% CI) of the polymorphism relative to TT genotype was
1.21 (0.69-2.14) for TC/CC genotypes combined. There was no association between CYP19
gene polymorphism and breast cancer risk in the study group as a whole, but homozygous and
heterozygous carriers of the variant Arg allele showed a significantly increased risk of breast
cancer among premenopausal women with a late age at first full-term pregnancy (OR 7.31, 95%
CI 1.88-28.5) or a high body mass index (OR 2.77, 95% CI 1.12-6.87). Additional larger studies
should be done to confirm that the rare CYP19 variant increases the risk of breast cancer among
premenopausal Japanese women.


http://cebp.aacrjournals.org/cgi/content/abstract/12/9/838

Extensive mammographic density is heritable, strongly associated with increased breast cancer
risk, and is influenced by sex hormone exposure. In a cross-sectional study of 181 pre- and 171
postmenopausal women without breast cancer, we examined the relationship of a functional
polymorphism in catechol-O-methyltransferase (COMT; VAL[-&gt;MET] to mammographic
density and other risk factors for breast cancer. We hypothesized that individuals who inherited
the low-activity form of COMT (COMT*2 allele) would have higher levels of breast density,
presumably because of reduced inactivation/ detoxification of catecholestrogens. Subjects were
recruited across five categories of breast density. Risk factor information, anthropometric
measures, and blood samples were obtained; sex hormone and growth factor levels were
measured, and COMT genotypes determined. Mammograms were digitized and measured using
a computer-assisted method. After adjustment for age and ethnicity, among pre- but not
postmenopausal subjects, each low-activity COMT*2 allele was associated with lower levels of
percentage breast density. The statistical significance of this association was lost after further
adjustment for serum growth factors [growth hormone, insulin-like growth factor-1 (IGF-1), and
insulin-like growth factor binding protein-3 (IGFBP-3)], hormones [follicle-stimulating hormone
(FSH) and progesterone], and body size (body mass index and waist:hip ratio). The low-activity
COMT*2 allele was also associated, after adjustment for age and ethnicity in premenopausal
women, with lower serum levels of IGF-1, higher levels of FSH and progesterone, and with a
larger waist:hip ratio, body mass index, and subscapular skinfold. After adjustment for body size,
the associations of genotype with IGFBP-3 and FSH were no longer significant. These findings indicate that COMT genotype is associated with several risk factors for breast cancer and suggest that the low-activity COMT*2 allele is associated with a reduced risk of breast cancer among premenopausal women.


http://cebp.aacrjournals.org/cgi/content/abstract/14/4/913

Somatic mutations of BRAF have been identified in both melanoma tumors and benign nevi. Germ line mutations in BRAF have not been identified as causal in families predisposed to melanoma. However, a recent study suggested that a BRAF haplotype was associated with risk of sporadic melanoma in men. Polymorphisms or other variants in the BRAF gene may therefore act as candidate low-penetrance genes for nevus/melanoma susceptibility. We hypothesized that promoter variants would be the most likely candidates for determinants of risk. Using denaturing high-pressure liquid chromatography and sequencing, we screened peripheral blood DNA from 184 familial melanoma cases for BRAF promoter variants. We identified a promoter insertion/deletion in linkage disequilibrium with the previously described BRAF polymorphism in intron 11 (rs1639879) reported to be associated with melanoma susceptibility in males. We therefore investigated the contribution of this BRAF polymorphism to melanoma susceptibility in 581 consecutively recruited incident cases, 258 incident cases in a study of late relapse, 673 female general practitioner controls, and the 184 familial cases. We found no statistically significant difference in either genotype or allele frequencies between cases and controls overall or between male and female cases for the BRAF polymorphism in the two incident case series. Our results therefore suggest that the BRAF polymorphism is not significantly associated with melanoma and the promoter insertion/deletion linked with the polymorphism is not a causal variant. In addition, we found that there was no association between the BRAF genotype and mean total number of banal or atypical nevi in either the cases or controls.


http://cebp.aacrjournals.org/cgi/content/abstract/11/12/1611

We evaluated polymorphisms in methylenetetrahydrofolate reductase (MTHFR), folate intake and alcohol consumption in relation to risk of colon cancer in a population-based case-control study in North Carolina. The study included 555 cases (244 African Americans and 311 whites) and 875 controls (331 African Americans and 544 whites). Total folate intake of <400 versus [≥400 (micro)g/ day showed a weak positive association with colon cancer among both African Americans [adjusted odds ratio (OR) = 1.4, 95% confidence interval (CI) = 1.0-2.0] and whites (OR = 1.6, 95% CI = 1.2-2.2). No association was observed with use of alcohol. Compared with wild-type genotypes, there was no association between the low activity MTHFR codon 677 TT genotype and colon cancer, but the low activity codon 1298 CC genotype was inversely associated with colon cancer in whites (OR = 0.5, 95% CI = 0.3-0.9). Unlike previous studies, we did not observe a strong protective effect of the codon 677 TT low-activity genotype when folate intake was high. Instead, we observed an increased risk of colon cancer when folate intake was low for participants with wild- type genotypes. Adjusted ORs for the combined effects of codon 677 CC and codon 1298 AA genotypes and folate intake <400 (micro)g/day were 1.9 (95% CI = 1.1-3.4) in African Americans and 2.5 (95% CI = 1.2-5.2) in whites. Our results suggest that variation at MTHFR codon 1298 (within the COOH-terminal region) may be more important for
colon cancer than variation at codon 677 (NH2-terminal region), and in populations where folate intake is low, wild-type MTHFR activity may increase risk for colon cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/11/1/127

p53 is a transcription factor for Waf-1/p21, a cyclin-dependent kinase inhibitor. Certain polymorphic variants of Waf-1 and p53 have been evaluated for their association with cancer risk. Previous studies indicated that certain p53 polymorphisms confer an increased risk of breast cancer [odds ratios (ORs) and 95% confidence intervals (CIs) = 2.9, 1.4-6.3 Carcinogenesis (Lond.), 17: 1313, 1996; 2.5, 1.3-4.8 Cancer Epidemiol. Biomark. Prev., 6: 105, 1997; and 1.5, 1.1-2.0, Anticancer Res., 18: 2095, 1998]. The primary objectives of this study were to test the hypotheses that the serine variant (codon 31 polymorphism) of Waf-1 is also involved in this process and that there is an interaction between Waf-1 and p53 polymorphisms. To do this, Waf-1 and p53 genotypes were determined for women enrolled in a breast cancer case-control study (Caucasians, African-Americans and Latinas: 487 Waf-1 and 504 p53 genotypes were obtained). Multivariate logistic regression was used to evaluate possible associations between Waf-1 and p53 polymorphisms, race, and menopause. The primary aim was to determine whether an interaction between Waf-1 and p531-2-1 existed. Whereas multivariate analysis suggested associations between breast cancer and inheritance of Waf-1ser31 in African-Americans (OR, 2.32; 95% CI = 0.66-5.60; n = 37 cases and 65 controls) and Latinas (OR, 2.22; 95% CI = 0.71-6.89; n = 30 cases and 75 controls), and inheritance of p53ser1-2-1 in Caucasians (OR, 3.15; 95% CI = 1.14-8.89; n = 93 cases and 187 controls), we did not see an interaction between Waf-1ser31 and p53ser1-2-1. Consistent with the finding that p53ser1-2-1 is a risk factor for Caucasian women was the observation of a strong interaction between race and p53 (P < 0.01).


http://cebp.aacrjournals.org/cgi/content/abstract/11/12/1684

An Arg/Pro polymorphism in codon 72 of the TP53 gene was analyzed in blood samples from 390 breast and 162 colorectal cancer patients previously investigated for TP53 mutations in their tumors. Among the breast cancer cases, 228 were homozygous for the Arg72 allele, of which, 65 (28.5%) also had a TP53 mutation in their tumors. In contrast, of 26 cases that were homozygous for the Pro72 allele, only 1 case (3.8%) had a TP53 mutation in the tumor (P = 0.004). Cloning the TP53 gene from tumor DNA followed by sequencing was performed in 14 heterozygotes with tumor mutation, and 9 of the mutations resided on the Arg72 allele. Among the colorectal cancer cases, no difference in mutation frequency was seen between the two different homozygotes, 40 TP53 mutations in 97 Arg72 homozygous cases (41.2%) versus 7 in 16 Pro72 homozygous cases (43.8%). These results suggest a selective growth advantage for cells carrying a type of TP53 mutation seen in breast carcinomas when the mutation resides on an Arg72 allele.

Introduction: The oral squamous cell carcinoma (OSCC) is the sixth most common malignant tumor worldwide. No significant better progress has been made in the treatment of OSCCs during the last decades. The heterodimeric CD97 protein is a epidermal growth factor seven-transmembrane family member and was identified as a dedifferentiation marker in thyroid carcinomas. Nothing is known about CD97 in OSCCs. Material and Methods: Employing UV-laser microdissection, CD97 and its ligand CD55 were investigated in normal oral mucosa and OSCCs (n = 78) by multiplex reverse transcription-PCR. Frozen sections were investigated by immunohistochemistry. The effects of retinoic acid and sodium butyrate on the CD97/CD55 expression in OSCC cell lines were determined by quantitative PCR, immunocytochemistry, and flow cytometry. Results: Weak CD97 transcripts were expressed in normal mucosa and normal basal epithelial cells revealed specific CD97 immunostaining. Strong CD97 transcripts were detected in pT3/T4 and G3/G4 OSCC tissues, whereas pT1/T2 and G1/G2 carcinomas revealed weak CD97 transcript levels. A weak CD97 immunostaining was observed in pT1/T2 and G1/G2 tumors. By contrast, intensive CD97 immunostaining was detected in pT3/T4 OSCCs and G3/G4 lesions. CD55 gene expression was low in normal mucosa. All OSCCs, irrespective of stage and grading, displayed strong CD55 immunostaining. Sodium butyrate and retinoic acid inhibited CD97 mRNA and protein in OSCC cell lines. Interestingly, CD55 was up-regulated by both substances. Conclusion: We identified CD97 as a novel marker of dedifferentiated OSCC. Interaction of CD97 and CD55 may facilitate adhesion of OSCC cells to surrounding surfaces that would result in metastases and bad prognosis.


JC virus (JCV) is an ubiquitous human polyomavirus that frequently resides in the kidneys of healthy individuals and is excreted in the urine of a large proportion of the adult population. Polyomaviruses are associated with disease largely in immunocompromised individuals (progressive multifocal leukoencephalopathy). Colorectal cancers can show chromosome instability and it was hypothesized that JCV may account for some of this instability. We screened urine from 45 healthy donors and 233 colorectal cancer/normal tissue pairs for the presence of JCV sequences using a Taqman assay. This assay could detect 1 virus genome in 10 human genomes. In the urine samples, we found an infection rate of approximately 70%. The JCV isolates in these samples could be categorized into four JCV types (2B, 4, 7, and 8), none of which had a rearranged regulatory region. Among the colon tissues, one normal tissue (<0.5%) and none of the matched tumors tested positive for JCV. There is no evidence in these data to indicate that JCV is the cause of genetic instability in colorectal cancer.


Endogenous sex hormones play an important role in the etiology of breast cancer. Polymorphisms in genes encoding for enzymes involved in steroidogenesis may therefore play a role in breast cancer risk. Cytochrome P450c17(alpha) (Cyp17) functions at key branch points in human steroidogenesis. A T[&gt;C] transition (A1 and A2 allele) in the 5’ untranslated region may be associated with increased expression of Cyp17. Using a case-cohort design, we studied the
effects of the A2 allele on endogenous sex hormone levels and breast cancer risk within a large population-based cohort (n = 9,349) in the Netherlands (the DOM-cohort). Cyp17 genotype was determined in 335 incident postmenopausal breast cancer cases, which occurred after follow-up (median time to follow-up, 19 years) of the entire cohort, and in a random sample of 373 women (subcohort). Concentrations of estrone (E1), estradiol (E2), testosterone, 5(alpha)-androstane-3(alpha), 17(beta)-diol (3(alpha)D), and creatinine were measured in first-morning urine samples. Only among women with body mass index (BMI) < 25 kg/m2 was the A2A2 genotype associated with higher levels of E1, E2, and 3(alpha)D compared with a group of women with either the A1A1 or the A1A2 genotype (e.g., geometric means of E1 in ng/mgcreatinine: A2A2, 2.23; A1A1/A1A2, 1.47; P = 0.03). Adjusted breast cancer rate ratios for women with the A1A2 or A2A2 genotype compared with women with the A1A1 genotype were 0.96 (0.68-1.37) and 0.80 (0.47-1.35), respectively. These results did not differ between women with low and high BMI. In conclusion, this paper shows that women with low BMI and the A2A2 genotype had higher endogenous sex steroid levels compared with women with the A1A1 genotype. However, these increased sex steroid levels are not translated into an increased breast cancer risk in these women.


http://cebp.aacrjournals.org/cgi/content/abstract/14/4/809

Multiple conflicting findings have been presented which indicate that EBV may be found anywhere from 0% to 51% of breast carcinomas. When EBV has been found causally associated with other human cancers, its DNA and one or more of its viral products have been detected in most tumor cells of a given biopsy. To test whether EBV has such an association with breast cancer, we measured the number of viral DNA molecules per cell in matched normal and tumor biopsies from 45 patients using real-time quantitative PCR. In no case could EBV DNA consistently be detected, with either of two different probes, at levels above 0.1 molecules per cell in two sections of the tumor samples. These levels of detection match those detected in EBV-negative cell lines and therefore likely represent noise in the assays. Equally importantly, the distribution of these low signals was the same between tumors and their matched normal controls. We conclude that EBV does not contribute to the development of breast cancers as it does to epithelial cancers such as nasopharyngeal and gastric carcinomas or to Burkitt's and Hodgkin's lymphomas.


http://cebp.aacrjournals.org/cgi/content/abstract/13/5/795

We have evaluated the use of allele-specific PCR (AS PCR) on DNA pools as a tool for screening inherited genetic variants that may be associated with risk of adult acute myeloid leukemia (AML). Two DNA pools were constructed, one of 444 AML cases, and another of 823 matched controls. The pools were validated using individual genotyping data for GSTP1 and LT(alpha) variants. Allele frequencies for variants in GSTP1 and LT(alpha) were estimated using quantitative AS PCR, and when compared to individual genotyping data, a high degree of concordance was seen. AS primer pairs were designed for nine candidate genetic variants in DNA repair and cell cycle/apoptotic regulatory genes, including Cyclin D1 [codon 870 splice site variant (A>G)]; BRCA1, P871L; ERCC2, K751Q; FAS -1377 (G>A); hMLH1 -93 (G>A) and V219I; p21, S31R; and the XRCC1 R194W and R399Q variants. For six of these assays, there was at least 95%
concordance between AS PCR genotyping and an alternative approach carried out on individual samples. Furthermore, these six AS PCR assays all accurately estimated allele frequencies in the pools that had been calculated using individual genotyping data. A significant disease association was seen with AML for the -1377 variant in FAS (odds ratio 1.76, 95% confidence interval 1.26-2.44). These data suggest that quantitative AS PCR can be used as an efficient screening technique for disease associations of genetic variants in DNA pools made from case-control studies.


http://cebp.aacrjournals.org/cgi/content/abstract/13/9/1515

Estrogen unopposed by progestins is a key factor in endometrial cancer etiology. Cytochrome P450 1B1 (CYP1B1), responsible for the 4-hydroxylation of estrogen, may be important in endometrial carcinogenesis, either as a regulator of estrogen availability or as a producer of potentially genotoxic estrogen metabolites. We investigated the association of CYP1B1 genotype and endometrial cancer risk in a population-based case-control study of postmenopausal Swedish women. We used the Expectation-Maximization algorithm to estimate the haplotype frequencies in the population and calculated odds ratios and 95% confidence intervals from conditional logistic regression models. In stratified analysis, we investigated the possible effects of CYP1B1 genotype on endometrial cancer risk in subgroups defined primarily by menopausal hormone use and also by body mass index, smoking, use of combined oral contraceptives, and family history. We genotyped 689 cases and 1,549 controls for the CYP1B1 single nucleotide polymorphisms m2, m3, and m4 and estimated the haplotype frequencies among controls to 0.086, 0.291, 0.452, and 0.169 for the CYP1B1*1, CYP1B1*2, CYP1B1*3, and CYP1B1*4 alleles, respectively. We found no evidence for an overall association between CYP1B1 genotype and endometrial cancer risk, nor was there any clear indication of gene-environment interaction.


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The population of Linxian in north central China is at high risk for gastric cardia adenocarcinoma (GCC) and esophageal squamous cell carcinoma (ESCC), and chronic inflammation may contribute to this risk. Interleukin-8 (IL8), a potent chemoattractant, has three well-characterized single nucleotide polymorphisms (SNP), one (-251) of which alters transcriptional activity. Four well-described SNPs in the two IL8 receptors, IL8RA and IL8RB, have been associated with inflammation. We conducted a case-cohort study in the Nutrition Intervention Trials (Linxian, China) to assess the association between these SNPs and incident GCC (n = 90) and ESCC (n =
IL8, IL8RA, and IL8RB SNPs were analyzed using a multiplex assay system, haplotypes were constructed, and risks were estimated using Cox proportional hazards models. The homozygous variants of IL8-251 and +396 were associated with 2-fold increased relative risks for GCC, but the highest risk observed was for the AGT/AGC haplotype of IL8-251/+396/+781 (relative risk, 4.14; 95% confidence interval, 1.31-13.1). Variation within IL8 was not associated with ESCC. Few subjects had variation at the IL8RA SNP and no significant associations were observed for IL8RB SNPs or haplotypes with either GCC or ESCC. We conclude that variation in IL8 seems to increase the risk for GCC but not ESCC in this high-risk population. These variants could confer an altered IL8 expression pattern or interact with environmental factors to increase the risk for inflammation and GCC.


http://cebp.aacrjournals.org/cgi/content/abstract/13/11/1805

A polymorphism at codon 72 of the human tumor suppressor p53 determines translation into either arginine or proline. Yet, the impact of this amino acid variability on the risk to develop malignant tumors, particularly carcinomas associated with human papilloma virus (HPV) infections, remains unresolved because of contradictory results. To address a potential correlation between the different genotypes and the manifestation of squamous cell carcinomas of the head and neck (SCCHN), we determined the p53 codon 72 in 193 healthy subjects and 122 unselected SCCHN with known HPV status. Furthermore, loss of allele-specific transcription was analyzed in p53 codon 72 heterozygous (Arg/Pro) SCCHN and correlated with HPV 16 and/or 18 E6 transcript expression. We found a moderately increased risk (odds ratio, 1.86; 95% confidence interval, 1.0-3.3) for individuals with germ line heterozygosity to develop SCC of the pharynx. On the other hand, p53 codon 72 polymorphic variants, most notably the Arg/Arg genotype, showed no association with the presence of HPV 16 and/or 18 E6 transcript. Moreover, there was no evidence for HPV-driven selection in SCCHN with allele-specific loss of transcription. Our data suggest that the p53 codon 72 polymorphism has a minor impact on the development of SCCHN.


http://cebp.aacrjournals.org/cgi/content/abstract/13/7/1230

Ras proto-oncogene mutations have been implicated in the pathogenesis of many malignancies, including leukemia. While both human and animal studies have linked several chemical carcinogens to specific ras mutations, little data exist regarding the association of ras mutations with parental exposures and risk of childhood leukemia. Using data from a large case-control study of childhood acute lymphoblastic leukemia (ALL; age <15 years) conducted by the Children's Cancer Group, we used a case-case comparison approach to examine whether reported parental exposure to hydrocarbons at work or use of specific medications are related to ras gene mutations in the leukemia cells of children with ALL. DNA was extracted from archived bone marrow slides or cryopreserved marrow samples for 837 ALL cases. We examined mutations in K-ras and N-ras genes at codons 12, 13, and 61 by PCR and allele-specific oligonucleotide hybridization and confirmed them by DNA sequencing. We interviewed mothers and, if available, fathers by telephone to collect exposure information. Odds ratios (ORs) and
95% confidence intervals (CIs) were derived from logistic regression to examine the association of parental exposures with ras mutations. A total of 127 (15.2%) cases had ras mutations (K-ras 4.7% and N-ras 10.68%). Both maternal (OR 3.2, 95% CI 1.7-6.1) and paternal (OR 2.0, 95% CI 1.1-3.7) reported use of mind-altering drugs were associated with N-ras mutations. Paternal use of amphetamines or diet pills was associated with N-ras mutations (OR 4.1, 95% CI 1.1-15.0); no association was observed with maternal use. Maternal exposure to solvents (OR 3.1, 95% CI 1.0-9.7) and plastic materials (OR 6.9, 95% CI 1.2-39.7) during pregnancy and plastic materials after pregnancy (OR 8.3, 95% CI 1.4-48.8) were related to K-ras mutation. Maternal ever exposure to oil and coal products before case diagnosis (OR 2.3, 95% CI 1.1-4.8) and during the postnatal period (OR 2.2, 95% CI 1.0-5.5) and paternal exposure to plastic materials before index pregnancy (OR 2.4, 95% CI 1.1-5.1) and other hydrocarbons during the postnatal period (OR 1.8, 95% CI 1.0-1.3) were associated with N-ras mutations. This study suggests that parental exposure to specific chemicals may be associated with distinct ras mutations in children who develop ALL.


http://cebp.aacrjournals.org/cgi/content/abstract/13/7/1206

Introduction: Insulin, insulin-like growth factor (IGF), and IGF binding protein (IGFBP) are involved in cell growth and proliferation and are thought to be important in the etiology of colorectal cancer. We hypothesize that genetic polymorphisms of insulin receptor substrates (IRS-1 and IRS-2), IGF-1, and IGFBP-3 alter colorectal cancer risk because of their roles in the insulin-related signaling pathway. Methods: Data from a population-based incident case-control study of 1,346 colon cancer cases and 1,544 population-based controls and 952 rectal cancer cases and 1,205 controls were used to evaluate associations. Genetic polymorphisms of four genes were investigated: an IGF1 CA repeat, the IGFBP3 -202 A > C, the IRS1 G972R, and the IRS2 G1057D. Results: Having at least one R allele (GR or RR) for IRS1 G972R was associated with an increased risk of colon cancer [odds ratio 1.4, 95% confidence interval (95% CI) 1.1-1.9]. The IRS2 G972R heterozygote GD genotype significantly reduced risk of colon cancer (odds ratio 0.8, 95% CI 0.6-0.9). Neither the IGF1 nor the IGFBP3 variants was associated independently with colon cancer, but there was an association when examined with IRS1. Individuals with an IRS1 R allele and IGF1 non-192 allele were at a 2-fold increased risk of colon cancer (95% CI 1.2-4.4). There was a 70% (95% CI 1.02-2.8) increased risk of colon cancer with an IRS1 R allele and the IGFBP3 AC or CC genotype. The IRS2 GD genotype reduced risk of colon cancer, except among those with an IRS1 R allele. No significant associations were seen in analyses of main effects or interactions of these variants and rectal cancer risk. Conclusions: Both IRS1 and IRS2 variants were associated with colon cancer risk independently. Associations were slightly stronger when polymorphisms in multiple genes were evaluated in conjunction with other genes rather than individually. These data suggest that the insulin-related pathway may be important in the etiology of colon cancer but not rectal cancer.


http://cebp.aacrjournals.org/cgi/content/abstract/13/4/538

Introduction: Aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to reduce risk of colorectal cancer. Although inhibition of cyclooxygenase (COX)-2 is generally thought to be the relevant mechanism, aspirin-like drugs apparently are involved in other pathways and mechanisms. We explore the associations between aspirin/NSAIDs, the insulin-
related pathway, and the risk of colorectal cancer. Methods: Genetic polymorphisms of five genes identified as being involved in an insulin-related pathway were genotyped using data collected in a case-control study of 1346 incident colon cancer cases and 1544 population-based controls and 952 incident rectal cancer cases and 1205 controls. Genotypes assessed were the 3' untranslated region poly(A) and the intron 8 BsmI polymorphisms of the VDR gene, a CA repeat polymorphism of the IGF1 gene, the A/C polymorphism at nucleotide -202 of the IGFBP3, the Gly972Arg polymorphism of the IRS1 gene, and the Gly1057Asp polymorphism of the IRS2 gene. Results: Use of aspirin and NSAIDs was associated with a decreased risk of colorectal cancer, with slightly greater protection from NSAIDs than aspirin for rectal cancer. We observed a significant interaction between IRS1 genotype and aspirin/NSAIDs use and risk of colorectal cancer. Relative to the GR/RR IRS1 genotype, a protective effect from the GG IRS1 genotype was seen in those who did not use NSAIDs; use of NSAIDs was protective for all genotypes. These associations were especially strong for those diagnosed prior to age 65 (P interaction = 0.0006). We also observed a significant interaction between aspirin/NSAIDs use and the VDR gene. Having the SS or BB VDR genotypes reduced risk of colorectal cancer among non-aspirin/NSAID users; however, aspirin/NSAIDs reduced risk for all VDR genotypes. Conclusions: These data support the protective effect of aspirin and NSAIDs on colorectal cancer risk. In addition, the observed interactions for aspirin/NSAIDs and IRS1 and VDR genotypes suggest that mechanisms other than COX-2 inhibition may be contributing to the protective effect of aspirin and NSAIDs on colorectal cancer risk.


http://cebp.aacrjournals.org/cgi/content/abstract/11/10/1004

Cigarette smoking is the main risk factor for bladder cancer, accounting for at least 50% of bladder cancer in men. Cigarette smoke is a rich source of arylamines, which are detoxified by the NAT2 enzyme and activated by the NAT1 enzyme to highly reactive species that can form bulky adducts on DNA. DNA damage from such adducts is mainly repaired by the nucleotide excision repair pathway, in which the XPD protein functions in opening the DNA helix. We hypothesized that an XPD codon 751 polymorphism (Lys-to-Gln amino acid change) could affect the repair of smoking-induced DNA damage and could be associated with bladder-cancer risk. We also hypothesized that allelic variants of the NAT1 and NAT2 genes might modify the effect of the XPD codon 751 polymorphism on smoking-associated bladder-cancer risk. We determined the XPD codon 751 genotype for 228 bladder-cancer cases and 210 controls who were frequency-matched to cases by age, sex, and ethnicity, and we used our previously published data on the NAT1 and NAT2 genotypes for these same individuals (J. A. Taylor et al., Cancer Res., 58: 3603-3610, 1998). We found a slight decrease in risk for the XPD codon 751 Gln/Gln genotype (adjusted odds ratio: 0.8; 95% confidence interval: 0.4-1.3) compared with subjects with the Lys/Lys or Lys/Gln genotypes. The analysis with smoking showed that smokers with the Lys/Lys or Lys/Gln genotypes were twice as likely to have bladder cancer than smokers with the Gln/Gln genotype (test of interaction P = 0.03). The combined presence of the NAT1/NAT2 high-risk genotype and the XPD Lys/Lys or Lys/Gln genotypes ignoring smoking had an odds ratio that was only slightly higher than expected, assuming no genotype-genotype interaction (P = 0.52). We found little evidence for a gene-gene-exposure, three-way interaction among the XPD codon 751 genotype, smoking, and the NAT1/NAT2 genotype.

DNA repair efficiency varies among individuals, with reduced repair capacity as a risk factor for various cancers. This variability could be partly explained by allelic variants for different DNA repair genes. We examined the role of a common polymorphism in the XRCC3 gene (codon 241: threonine to methionine change) and bladder cancer risk. This gene plays a role in the homologous recombination pathway, which repairs double-strand breaks. The functional consequences of the XRCC3 codon 241 polymorphism are still unknown. We hypothesized that this polymorphism could affect repair of smoking-associated DNA damage and could thereby affect bladder cancer risk. We genotyped 233 bladder cancer cases and 209 controls who had been frequency matched to cases on age, sex, and ethnicity. We observed little evidence of a positive association between subjects who carried at least one copy of the codon 241 Met allele and bladder cancer (odds ratio: 1.3; 95% confidence interval: 0.9-1.9). Among heavy smokers, individuals with the Met allele had about twice the risk of those without it; however, a test of interaction was not statistically significant (P = 0.26). Previously, we observed in these subjects an association between bladder cancer risk and allelic variants of the XRCC1 gene, which is involved in the repair of base damage and single-strand breaks. In this study, we found some evidence for a gene-gene interaction between the XRCC1 codon 194 and XRCC3 codon 241 polymorphisms (P = 0.09) and some support for a possible gene-gene-smoking three-way interaction (P = 0.08).


Lung adenocarcinoma has replaced squamous cell lung carcinomas as the most frequent histological subtype in lung cancers. However, genetic factors that affect cancer susceptibility are much less understood in adenocarcinoma than in squamous cell carcinoma. In this study, polymorphisms in five genes involved in the metabolism of carcinogens or in the repair of damaged DNA in lung cells, NQO1-Pro187Ser, GSTT1-positive/null, GSTM1-positive/null, CYP1A1-Ile462Val, and OGG1-Ser326Cys, were examined for association with lung adenocarcinoma risk in a case-control study of 198 patients and 152 control subjects. The NQO1 and GSTT1 polymorphisms were associated with lung adenocarcinoma risk with adjusted odds ratio of 2.15 for the NQO1-Pro/Pro genotype versus the Ser/Ser genotype and adjusted odds ratio of 1.61 for the GSTT1-null genotype versus the positive genotype, respectively. Furthermore, individuals with the combined genotype of NQO1-Pro/Pro and GSTT1-null showed greater risk compared with those of NQO1-Ser/Ser and GSTT1-positive. In contrast, significant association was not observed for the GSTM1, CYP1A1, and OGG1 polymorphisms with lung adenocarcinoma risk, although several studies have shown their implication in the risk for squamous cell lung carcinoma. The result indicates that the NQO1-Pro/Pro and GSTT1-null genotypes are risk factors for lung adenocarcinoma development, and that the genetic factors for susceptibility to adenocarcinoma are different from those to squamous cell carcinoma. The enhanced risk of the NQO1-Pro/Pro genotype combined with the GSTT1-null genotype was more evident in smokers than in nonsmokers. Therefore, carcinogens in tobacco smoke, which are activated by NQO1 and detoxified by GSTT1, could have a role in lung adenocarcinoma development.

In large studies and under field conditions common to epidemiological research, factors outside of and inside the laboratory can introduce misclassification of genetic susceptibility markers. Few reports have been made on the accuracy of genotyping individuals using DNA extracted from frozen urine that was stored for ~20 years. This study was performed to determine the reproducibility and accuracy of N-acetyltransferase 2 (NAT2) genotyping by RFLP analysis using DNA from stored urine. To obtain long-term frozen urine and blood samples from the same person, the databases of two large prospective studies were linked by name and date of birth. Six polymorphisms within the coding region of NAT2 were determined in 65 urine and blood samples after which, genotypes and imputed phenotypes (rapid, slow) were derived. To test reproducibility, all of the six polymorphisms were determined twice in 47 urine-blood pairs. Reproducibility of imputed phenotypes was 91.5% in urine samples and 97.9% in blood samples. To test accuracy, results for all six polymorphisms were compared between urine and blood DNA. All of the {kappa}'s were at least 0.85 except one. Identical results for all six polymorphisms were seen in 78.5% of urine-blood pairs. Taking blood samples as a reference standard, rapid acetylators were classified as rapid in 97% of subjects (95% confidence interval, 90-100%), and slow acetylators were classified as slow also in 97% of subjects (95% confidence interval, 91-100%), when using urine. This study shows that stored urine samples can be used for DNA genotyping in large cohort studies, when blood samples are not available.


Whether antibodies to human papillomavirus (HPV) capsids, elicited by natural infection, are protective is unknown. This question was addressed in a population-based cohort of 7046 women in Costa Rica by examining the association between baseline seroreactivity to HPV-16, HPV-18, or HPV-31 virus-like particles and the risk of subsequent HPV infection at a follow-up visit 5-7 years after enrollment. Seropositivity to HPV-16, HPV-18, or HPV-31 was not associated with a statistically significant decreased risk of infection with the homologous HPV type [relative risk (RR) and [95% confidence interval (CI)], 0.74 (0.45-1.2), 1.5 (0.83-2.7), and 0.94 (0.48-1.8), respectively]. Seropositivity to HPV-16 or HPV-31 was not associated with a decreased risk of infection with HPV-16 or its genetically related types [RR (95% CI), 0.82 (0.61-1.1) and 0.93 (0.68-1.2), respectively]. Seropositivity to HPV-18 was not associated with a decreased risk of infection with HPV-18 or its genetically related types (RR 1.3; 95% CI 1.0-1.8). Thus, we did not observe immunity, although a protective effect from natural infection cannot be excluded because of the limits of available assays and study designs.


In this paper, we present evidence that alleles of several polymorphisms in the chromosomal region 19q13.2-3, encompassing the genes RAI and XPD, are associated with occurrence of basal cell carcinoma in Caucasian Americans. The association of one of these, RAI-intron1, is sufficiently strong to make mass significance unlikely (P = 0.004, \( \chi^2 \)). We interpret our combined data to indicate that a specific haplotype partly defined by the alleles of three single nucleotide polymorphisms, RAI intron1G, RAI exon6T, and XPD exon 6C, is associated with a protective gene variant in a region spanning from XPD to ERCC1.


There have been few studies of the associations of genetic polymorphisms with precancerous gastric lesions. We conducted a cross-sectional study to compare the prevalences of several genetic polymorphisms in 302 subjects with mild chronic atrophic gastritis with prevalences in 606 subjects with deep intestinal metaplasia or dysplasia. This stratified random sample of 908 subjects was selected and analyzed for genetic polymorphisms from 2,628 individuals who had gastric biopsies with histopathology in 1989 in Linqu County, Shandong Province, China. In subjects with mild chronic atrophic gastritis, the frequencies of the variant (less common) alleles of CYP2E1 Rsal, CYP2E1 Dral, GSTP1, ALDH2, and ODC were, respectively, 0.156, 0.201, 0.189, 0.190, and 0.428. The frequencies of the null genotypes of GSTM1 and GSTT1 in the mild chronic atrophic gastritis group were 0.509 and 0.565, respectively. Comparing mild chronic atrophic gastritis with deep intestinal metaplasia or any degree of dysplasia, we found no statistically significant associations with any genotype from these loci for dominant, additive, or recessive inheritance models. There was no statistically significant evidence of multiplicative interactions between any pair of genotypes based on CYP2E1 Rsal, CYP2E1 Dral, GSTP1, GSTM1, or GSTT1; nor between Helicobacter pylori status and any of these five loci; nor
between smoking status and GSTP1, GSTM1, or GSTT1; nor between alcohol consumption and ALDH2. Statistically significant interactions were noted between salt consumption and GSTP1 and between sour pancake consumption and CYP2E1 RsaI. There was, moreover, a statistically significant interaction (odds ratio, 1.78; 95% confidence interval, 1.03-3.08) between CYP2E1 Dral and smoking at least one cigarette per day. A positive but not statistically significant interaction was also seen between CYP2E1 RsaI and smoking status. These polymorphisms do not seem to govern progression from mild chronic atrophic gastritis to advanced precancerous gastric lesions, but the effects of smoking may be accentuated in individuals carrying variants of CYP2E1.


http://cebp.aacrjournals.org/cgi/content/abstract/13/5/709

The CYP11A gene encodes the cholesterol side-chain cleavage enzyme (P450scc) that catalyzes the first and rate-limiting step for the biosynthesis of sex hormones. A pentanucleotide repeat [(TAAAA)n] polymorphism in the 5' of the CYP11A gene has been reported to be related to the risk of polycystic ovary syndrome, an inherited endocrine disorder characterized by hyperandrogenemia. We investigated the association of this polymorphism with breast cancer risk in a population-based case-control study conducted among Chinese women in Shanghai. Genotype assays were completed for 1015 incident breast cancer cases and 1082 community controls. Three common alleles with 4, 6, or 8 TAAAA repeats were identified in the study population. The frequency of the 8 repeat allele was more common in cases (12.6%) than controls (8.5%) (odds ratio = 1.6, 95% confidence interval = 1.3-1.9; P < 0.0001). Compared to subjects who did not carry this allele, adjusted odds ratios were 1.5 (95% confidence interval = 1.2-1.9) and 2.9 (1.3-6.7) (P for trend, <0.001), respectively, for those who carried one and two copies of this allele. This positive association was observed in both pre- and postmenopausal women and all strata defined by major breast cancer risk factors, including years of menstruation, body mass index, and waist-to-hip ratio. The results from this study indicate that the TAAAA repeat polymorphism near the promoter region of the CYP11A gene may be an important susceptibility factor for breast cancer risk.


http://cebp.aacrjournals.org/cgi/content/abstract/14/4/830

Most esophageal adenocarcinomas arise within Barrett's esophagus but the cause of this increasingly prevalent condition remains unknown. Early detection improves survival and discriminant screening markers for Barrett's esophagus and cancer are needed. This study was designed to explore the natural history of eyes absent 4 (EYA4) gene methylation in the neoplastic progression of Barrett's esophagus and to evaluate methylated EYA4 as a candidate marker. Aberrant promoter methylation of EYA4 was studied by methylation-specific PCR using bisulfite-treated DNA from esophageal adenocarcinomas, Barrett's esophagus, and normal epithelia, and then confirmed by sequencing. Eight cancer cell lines were treated with the demethylation agent 5-aza-2'-deoxycytidine, and EYA4 mRNA expression with and without treatment was quantified by real-time reverse-transcription PCR. EYA4 hypermethylation was detected in 83% (33 of 40) of esophageal adenocarcinomas and 77% (27 of 35) of Barrett's tissues, but only in 3% (2 of 58) of normal esophageal and gastric mucosa samples (P < 0.001). The unmethylated cancer cell lines had much higher EYA4 mRNA expression than the methylated cancer cell lines. Demethylation caused by 5-aza-2'-deoxycytidine increased the
mRNA expression level by a median of 3.2-fold in methylated cells, but its effect on unmethylated cells was negligible. Results indicate that aberrant promoter methylation of EYA4 is very common during tumorigenesis in Barrett's esophagus, occurs in early metaplasia, seems to be an important mechanism of down-regulating EYA4 expression, and represents an intriguing candidate marker for Barrett's metaplasia and esophageal cancer.

Cancer Genetics and Cytogenetics (51)


http://www.sciencedirect.com/science/article/B6T53-4902B4S-8/2/a4d6217bff1d730b737644fe30848a38

Promoter hypermethylation represents a primary mechanism in the inactivation of tumor suppressor genes during tumorigenesis. To determine the frequency and timing of hypermethylation during carcinogenesis of nonastrocytic tumors, we analyzed promoter methylation status of 10 tumor-associated genes in a series of 41 oligodendrogliomas (22 World Health Organization [WHO] grade II; 13 WHO grade III; 6 WHO grade II-III oligoastrocytomas) and 7 WHO grade II-III ependymomas, as well as 2 nonneoplastic brain samples, by a methylation-specific polymerase chain reaction. Aberrant CpG island methylation was detected in 9 of 10 genes analyzed, and all but one sample displayed anomalies in at least one gene. The frequencies of hypermethylation for the 10 genes were as follows, in oligodendrogliomas and ependymomas, respectively: 80% and 28% for MGMT; 70% and 28% for GSTP1; 66% and 57% for DAPK; 44% and 28% for TP14ARF; 39% and 0% for THBS1; 24% and 28% for TIMP3; 24% and 14% for TP73; 22% and 0% for TP16INK4A; 3% and 14% for RB1; and 0% in both neoplasms for TP53. No methylation of these genes was detected in normal brain tissue samples. We conclude that a high frequency of aberrant methylation of the 5' CpG island of the MGMT, GSTP1, TP14ARF, THBS1, TIMP3, and TP73 genes is observed in nonastrocytic neoplasms. This aberration seems to occur early in the carcinogenesis process (it is already present in the low-grade forms), although in some instances (DAPK, THBS1, and TP73) it appears also associated with the genesis of anaplastic forms.


http://www.sciencedirect.com/science/article/B6T53-3Y4C5FB-7/2/f48917b9ec524d9e7f0624d857e00b73

We analyzed eight samples of xenografted human pancreatic tumors and two metastases developed in mice by comparative genomic hybridization (CGH). The most recurrent changes were: gains on chromosomes 8 (8q24~qter; 7/8 cases), 15 (15q25~q26; 6/8 cases), 16 (16p in 6/8 cases; 16q in 5/8 cases), 20 (20q; 6/8 cases), and 19 (19q; 5/8 cases); and losses on chromosomes 18 (18q21; 6/8 cases), 6 (6q16~q21 and 6q24~qter; 5/8 cases each), and 9 (9p23~pter; 5/8 cases). The two metastases maintained the aberrations of the original pancreatic tumor plus gain of 11q12~q13 and 22q. Loss of heterozygosity analysis was carried out for
10p14–pter, a region that was lost in 3/8 samples. All of them presented allelic imbalance for all the informative loci. Fluorescence in situ hybridization and Southern analysis were performed to test some candidate oncogenes in 8q24 (MYC) and 15q25–pter (IGF1R and FES). Two of seven tumors showed high-level amplification of MYC relative to the centromere (>3-fold), another two tumors had low-level amplification (1.5- to 3.0-fold), and one displayed 5.5 MYC signals/cell. In relation to the FES gene, low-level amplification was found in three tumors. Southern analysis showed five cases with a low-level amplification of IGF1R. Our data suggest that either few extra gene copies may be enough for cancer progression or other genes located in these regions are responsible for the amplifications found by CGH.


http://www.sciencedirect.com/science/article/B6T53-4BSW1SS-F/2/f3449bcac96d55c0a7820a0678a25cd6

FLT3 gene internal tandem duplication (ITD) and activating loop mutations (D835) were determined in 22 cases of therapy-related acute myelocytic leukemia/myelodysplastic syndrome (t-AML/MDS) and 102 cases of de novo AML/MDS. In t-AML/MDS, FLT3 ITD was absent, and D835 was found in only one case of therapy-related acute promyelocytic leukemia (APL). In de novo AML/MDS, however, FLT3 ITD and D835 were significantly more frequent (28 of 102 cases, P = 0.024) and were associated with high peripheral blood and marrow blast counts. Our results suggest that different pathogenetic pathways might be involved in t-AML/MDS and de novo AML/MDS.


http://www.sciencedirect.com/science/article/B6T53-3W787FV-4/2/979a370750bc17275569e0b980ba774f

To evaluate the potential cytogenetic heterogeneity in breast carcinoma, several small cell groups (each consisting of 20 to 50 cells) were investigated within paraffin sections. By laser-microdissection, three to seven cell groups were taken per case. The DNA was amplified by degenerate oligonucleotide primed PCR (DOP-PCR), and the samples were analyzed by CGH for chromosomal gains and losses. Two ductal invasive breast carcinomas, one of them with two lymphnode metastases, were investigated. To compare the results from the small samples, CGH was also performed on DNA isolated from the tumorous regions of three to five serial sections (107 to 106 cells). The aberrations observed in the microdissected tumor samples were multiple and involved up to 14 different chromosomal or subchromosomal regions. The most frequent changes were gains on chromosomes 12q (14/20) and 20q (16/20), and loss on 13q (12/20). Some aberrations have rarely been detected (e.g., loss on 2p, gain on 8q). Comparing chromosomal imbalances in primary tumors and lymph node metastases, more consistent changes were found between the primary tumor and its corresponding metastases than between both primary tumors. The laser-microdissected samples in general showed more chromosomal aberrations than DNA isolated from several tumor sections. Our CGH results were confirmed by fluorescence in situ hybridization (FISH) for the chromosomal regions of centromere 1 and 20, and 20q13. In addition, microsatellite analyses on 31 samples confirmed our CGH findings for selected chromosome regions 2p and 11q. It can be concluded that there is a distinct intratumoral heterogeneity in primary breast tumors as well as in the corresponding lymph node metastases.
The combination of microdissection and CGH enabled us to detect cytogenetic aberrations from important clones which are missed when analyzing DNA extracted from large cell numbers.


http://www.sciencedirect.com/science/article/B6T53-49HM0SG-B/2/db74e767f55c50e2dd30150d50cf36a7

Endometrial cancer is the second most common malignancy in patients with hereditary nonpolyposis colorectal cancer (HNPCC). This cancer is caused by germline mutations in one of the DNA mismatch repair (MMR) genes. The present study was undertaken to analyze the relation between microsatellite instability (MSI) and germline mutations of MMR genes. We analyzed MSI in 38 cases of endometrial cancer. MSI was present in one or more (out of 5 examined) regions in 11 (29%) cases. Furthermore, alterations in MLH1 and MSH2, two culprit genes representative of HNPCC, were examined in the 11 MSI-positive patients using polymerase chain reaction-single-strand conformation polymorphism and sequencing. Germline mutations, namely, 1) a missense mutation at codon 688 (ATG->ATA, Met->Ile) and 2) a missense mutation at codon 390 (CTT->TTT, Leu->Phe) of the MSH2 gene, were found in 2 of the 11 patients (18%). Although these two cases do not fulfill the new Amsterdam criteria, they had strong family histories of colorectal and endometrial carcinoma. Our results show that genetic testing is important in cases of endometrial cancer with a history suggestive of HNPCC even if the new Amsterdam criteria are not fulfilled.


http://www.sciencedirect.com/science/article/B6T53-407GJ2G-9/2/c41ad4baae853cf35357e36bf2875b1a

Comparative genomic hybridization (CGH) was used to identify chromosomal imbalances in 19 samples of squamous cell carcinoma of the head and neck (HNSCC). The chromosome arms most often over-represented were 3q (48%), 8q (42%), and 7p (32%); in many cases, these changes were observed at high copy number. Other commonly over-represented sites were 1q, 2q, 6p, 6q, and 18q. The most frequently under-represented segments were 3p and 22q. Loss of heterozygosity of two polymorphic microsatellite loci from chromosome 22 was observed in two tongue tumors, in agreement with the CGH analysis. Gains of 1q and 2q material were detected in patients exhibiting a clinical history of recurrence and/or metastasis followed by terminal disease. This association suggests that gain of 1q and 2q may be a new marker of head and neck tumors with a refractory clinical response.


http://www.sciencedirect.com/science/article/B6T53-4471GH6-B/2/879a54aab4cb374e18b19c6eaa933276
The occurrence of secondary chromosome changes is frequent in Ewing tumors, in particular trisomies for chromosomes 8 and 12, and unbalanced (1;16) translocations leading to gains of 1q and losses of 16q. The prognostic value of these secondary aberrations has not been statistically demonstrated. We report here a CGH analysis of a series of 43 primary tumors corresponding to 21 localized and 22 metastatic tumors. For five of them, a sufficient amount of DNA for the CGH analysis was available from the frozen samples. For 19 samples, a preliminary step of DOP-PCR amplification of the DNA was necessary. For the last 19 tumors, DNA was obtained after DOP-PCR amplification of small amount of DNA contaminating the RNA. As a whole, the main chromosome imbalances previously described, such as trisomies for 1q, 8, and 12, were observed. It is noteworthy that the mean number of imbalances was more frequent in localized versus metastatic tumors. Gain of 1q was more frequent in metastatic than in localized tumors. Nevertheless, these two results do not reach statistical significance. Conversely, a statistically significant excess of copy number of chromosome 2 was observed in non-metastatic tumors, suggesting that this imbalance, which has never been previously reported, could be associated with more favorable tumor behavior.


http://www.sciencedirect.com/science/article/B6T53-4BYJ6CW-6/2/1ead1dd79c3b729973f4560d124a7ac7

Turner syndrome (TS) is a disorder caused by partial or complete X-chromosome monosomy. Studies in TS patients with different karyotypes have demonstrated the presence of Y-chromosome-derived sequences (4-61%). Early detection of Y-chromosome sequences in TS is of great importance because of the high risk of gonadal tumor development. We investigated the presence of Y-chromosome sequences in TS patients with a 45,X karyotype. One hundred seven unrelated 45,X Mexican TS patients recruited between 1992 and 2003 were included. Y-chromosome-derived sequences were found by polymerase chain reaction in 10 (9.3%) patients. Six subjects underwent gonadectomy and in one of them a gonadoblastoma was found; another developed a gonadoblastoma with dysgerminoma. Because of the high proportion (33%) of gonadal tumors in patients with Y-chromosome sequences found among our patients of mestizo origin, adequate counseling regarding a gonadectomy should be given.


http://www.sciencedirect.com/science/article/B6T53-4418YNS-7/2/a9ab54f3c78a673af4d3b689c1ca3ba2

To determine the pathogenic role of chromosomes 11 and 17 in the carcinogenesis of human ovarian cancers, neoR-tagged chromosome 11 or 17 was transferred from cell lines A9H11 or A9H17, respectively, into the ovarian carcinoma cell line SKOV-3 using microcell-mediated chromosome transfer. The chromosome transfer was verified by polymerase chain reaction detection of the neoR gene, fluorescence in situ hybridization detection of an extra chromosome 11, and microsatellite polymorphism detection of an exogeneous chromosome 11. Five SKOV-3/A9H11 hybrids and five SKOV-3/A9H17 hybrid clones were generated. For the chromosome 11 transfer, complete suppression of tumorigenicity was observed in four clones, (11)9-8 and 11(H)7-2, 11(H)8-3, and 11(H)7-2, 100 days post implantation. For the chromosome 17 transfer, no complete suppression of tumorigenicity was observed. However, an increased latency period ranging from 25 to 49 days in contrast to 7 days for the SKOV-3 parental line, and a significant
reduction in tumor size was observed. There was no correlation between the in vitro growth rate and the tumorigenicity or length of latency period. Our results demonstrate functionally that chromosome 11 may carry a tumor suppressor gene(s) while chromosome 17 may carry a tumor growth-inhibitor gene(s) for the ovarian carcinoma cell line, SKOV-3.


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http://www.sciencedirect.com/science/article/B6T53-47RB04H-1D5/2/2833c50c2a536f8fa5ebaddfccc28da46

Cytogenetic analysis of 184 adipose tissue tumors, 175 lipomas, and nine liposarcomas (LPS) showed the presence of a ring chromosome and/or a long marker chromosome in 10 cases with common histologic features such as atypical stromal cells with or without lipoblasts. In five of the cases, this appeared to be the sole cytogenetic abnormality. Fluorescence in situ hybridization (FISH) analysis with a microclone library specific for chromosome region 12q13-q15 showed extensive staining of the ring and long marker chromosomes, indicating that genetic sequences of this particular region of chromosome 12 are present in these marker chromosomes, most likely in an amplified form.


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http://www.sciencedirect.com/science/article/B6T53-4FB8J0J-K/2/c2520baba4b72de096be19246ebeca6

The malignant transformation that characterizes the development of Barrett esophagus-associated adenocarcinomas is a multi-step process in which genetic alterations in various tumor-associated genes accumulate. Defective mismatch repair (MMR) is the cause of microsatellite instability (MSI) pathway that characterizes a subset of gastrointestinal tumors and is specifically associated with tumor development within the hereditary nonpolyposis colorectal cancer (HNPPC) syndrome. The few studies that have assessed MMR defects in Barrett-associated adenocarcinomas have reached different results. We therefore assessed the expression of the MMR proteins MLH1 and MSH2 in a series of 59 Barrett adenocarcinomas and found a loss of MMR protein immunostaining in 2/59 (3%) tumors; one tumor showed a loss of MSH2 expression, the other tumor showed a loss of MLH1, and both tumors displayed an MSI-high phenotype. Our findings suggest that only a small subset of Barrett adenocarcinomas develop because of defective MMR, but demonstrate that MLH1 and MSH2 are the primary targets for defective MMR also in this tumor type.


Cancer Genetics and Cytogenetics 120(1): 37.

http://www.sciencedirect.com/science/article/B6T53-40NMS6M-7/2/22a307508bcb38b445896d2bfa85606
We have examined the sequence of the cDNA encoding the sodium/hydrogen exchanger isoform 1 (NHE1), from 23 bases upstream of the start codon to 28 bases downstream of the stop codon. Template was prepared from (1) peripheral blood mononuclear cells (PBMC) isolated from 10 healthy unrelated Caucasian volunteers; (2) PBMCs isolated from 6 leukemic patients (acute lymphoblastic leukemia [ALL], n = 3; chronic lymphocytic leukemia [CLL], n = 1; chronic myelogenous leukemia [CML], n = 2); and (3) samples of 4 leukemic cell lines (ALL: CEM, MOLT4; AML: KG1a; CML: K562). NHE1 cDNA in normal PBMCs showed silent polymorphism of nucleotides 112 (N1: T, frequency 0.70; C, frequency 0.30; prevalence of heterozygosity 0.42); 2248 (N2: G, frequency 0.90; A, frequency 0.10; prevalence of heterozygosity 0.18); and 2493 (N3: G, frequency 0.90; A, frequency 0.10; heterozygosity 0.18). Deduced primary structure of NHE1 protein in all normal volunteers was identical to that previously published for NHE1 from renal and cardiac tissue. Similar to normal PBMCs, NHE1 cDNA from leukemic cells showed polymorphism of nucleotides N1, N2, and N3, but failed to demonstrate leukemia-specific sequence differences. We conclude that the coding region of NHE1 cDNA shows a greater level of polymorphism than is currently recognized, but that sequence mutation of NHE1 is not a key event in the pathogenesis of leukemia.


http://www.sciencedirect.com/science/article/B6T53-4CB7MY0-7T/2/cfe77b93d11776f1a39e6411b6221e39

Since exposure to ionizing radiation, a risk factor for thyroid cancer, may produce genotoxins potentially eliminated by glutathione-S-transferases, we conducted a case control study to evaluate the role of the GSTM1- and GSTT1-null genotypes and GSTP1 polymorphisms in thyroid cancer. The frequency of GSTP1 Ile/Ile, GSTM1-, and GSTT1-null genotypes was increased in cancer patients when compared with control population. Considering the genotypes over-represented in thyroid cancer patients as potential risk genotypes, we carried out an odds ratio (OR) analysis considering the presence of none, one, two, or three risk genotypes. The results obtained showed that the presence of three potentially risk alleles (GSTM1 null, GSTT1 null, and GSTP1 Ile/Ile) lead to a significant OR increase for all the cases, irrespective of the type of tumor (OR = 2.91), for papillary (OR = 3.64) but not for follicular tumors. The presence of GSTP1 Ile/Ile leads to a significant later age of tumor onset when compared with GSTP1 Ile/Val and Val/Val (PGSTP1 Ile/Ile and the age of disease manifestation. These results suggest that combined GST polymorphisms lead to a moderate increased risk for thyroid cancer, especially for the papillary type, and GSTP1 polymorphisms might modulate the age of onset of the disease.


http://www.sciencedirect.com/science/article/B6T53-3RJG2YY-G/2/f3d72bd65db19e1311b609c51c5b3977

Recently, the high mobility group protein gene, HMGIC, was identified as a common genetic denominator in benign tumors with chromosome 12q13-15 aberrations, such as lipomas, uterine leiomyomas, pleomorphic adenoma of the salivary glands, hamartomas of breast and lung, angiomyxomas, and endometrial polyps. In most cases, the rearrangements resulted in the separation of the three HMGIC DNA-binding motifs from the acidic carboxy-terminal tail. Here, we report about the molecular characterization of a case of pleomorphic adenoma carrying a t(11;12)(p22;q15). Studies were performed on a cell line derived from the primary tumor, i.e., cell
Although the chromosome 12 breakpoint was initially mapped more than 1 Mb distal to the HMGIC gene by fluorescence in situ hybridization (FISH) analysis, the present molecular studies reveal a more complex chromosomal rearrangement that directly affects the HMGIC gene. Using 3'-RACE analysis, a HMGIC fusion transcript was detected that contained the complete HMGIC coding region but lacked the putative mRNA destabilizing AUUUA motifs that are normally present in the 3'-UTR of HMGIC. Wild-type HMGIC transcripts were also detected in the tumor cells. The results suggest that alterations in the 3'-noncoding region of HMGIC may have to be considered as pathogenetically relevant.


Deletion of chromosome 11q23 is a common alteration in parathyroid adenomas and hyperplasias. A new potential suppressor gene PPP2R1B encoding the [beta] isoform of the A subunit of the serine/threonine protein phosphatase 2A was recently identified and localized to chromosome 11q23. We performed polymerase chain reaction-based single-strand conformation polymorphism and direct sequencing on six parathyroid hyperplasias and 12 adenomas to evaluate the role of PPP2R1B in the pathogenesis of parathyroid lesions. A previously identified germine G-A transition (GGC-GAC) in codon 90, changing glycine (Gly) to aspartic acid (Asp), was detected in one adenoma. Both the common Gly allele and the variant Asp allele were detected by direct sequencing in the patient's somatic cells. We conclude mutations of PPP2R1B are not frequent in parathyroid lesions, and that other genes located at 11q23 may be more closely associated with pathogenesis of parathyroid hyperplasia and adenoma.


Extraskeletal myxoid chondrosarcoma (EMC) is a rare mesenchymal tumor cytogenetically characterized by reciprocal translocations, such as t(9;22)(q22;q12) and t(9;17)(q22;q11), which result in EWSR1/NR4A3 and TAF15/NR4A3 fusion genes (alias EWS/NOR1, TAF2N/NOR1), respectively. NOR1 is an orphan nuclear receptor and acts as a transcription factor that can bind to its putative coactivator, SIX3. Although the NOR1 fusion protein has been implicated in oncogenesis of EMC, a small fraction of EMC lacks detectable rearrangements of the NR4A3 gene or 9q22. We report a case of EMC with no detectable NR4A3 gene alterations, as assessed with various molecular techniques including reverse transcription-polymerase chain reaction (RT-PCR), Southern blotting, interphase fluorescence in situ hybridization, and PCR single-strand conformation polymorphism--but with coexpression of native NOR1 and SIX3. In our survey of another 18 EMCs, we identified one more case expressing both NOR1 and SIX3 but lacking NR4A3 fusion. Fourteen tumors with detectable NR4A3 fusion genes (EWSR1-NR4A3; TAF15-NR4A3) expressed neither native NOR1 nor SIX3. SIX3 expression is normally confined specifically to the developing eye and fetal forebrain, although the expression of NR4A3 is largely ubiquitous. Our data suggest that aberrant coexpression of NOR1 and SIX3 is a potential alternative mechanism underlying the development of EMC.
The HMGIC gene, which codes a protein that acts as an architectural transcription factor, is frequently rearranged in a variety of benign or locally aggressive mesenchymal tumors. In tumors of smooth muscle differentiation, only uterine leiomyoma and lipoleiomyoma are known to be associated with the altered HMGIC. We investigated molecular and genetic alterations of the HMGIC in 36 benign and malignant smooth muscle tumors arising at various anatomical sites, including 13 uterine leiomyomas, two leiomyomas of the kidney with a t(12;14), one pelvic lipoleiomyoma, one vascular leiomyoma of the foot, two uterine leiomyosarcomas, six retroperitoneal leiomyosarcomas, one leiomyosarcoma of the urinary bladder, and 10 leiomyosarcomas of external soft tissues. HMGIC gene expressions were detected in both uterine (73.3%) and extrauterine (57.1%) smooth muscle tumors by reverse transcriptase polymerase chain reaction (RT-PCR), and benign tumors (70.5%) more frequently expressed the HMGIC than leiomyo-sarcomas (57.8%). Variant transcripts of the HMGIC containing cryptic exonic sequences previously described were found in one renal and three uterine leiomyomas and four leiomyosarcomas arising in the uterus and soft tissues by RT-PCR. Southern blot analysis identified a rearranged HMGIC in one soft tissue leiomyosarcoma. Thus, the HMGIC alterations in smooth muscle tumors are not confined only to uterine leiomyoma or lipoleiomyoma. Our data expand the variety of mesenchymal tumors associated with HMGIC alterations.


We conducted a hospital-based prevalent case-control study in a Japanese population (cases = 103, controls = 487) to ascertain the previous report about the association between the polymorphism in exon 13 of the hMSH2 gene (gIVS 12-6T->C) and the risk of non-Hodgkin lymphoma in an Ecuadorian population. When the TT genotype was defined as the reference, none of the CT genotypes (OR = 1.52; 95% CI, 0.97-2.37), CC genotypes (OR = 1.06, 95% CI, 0.44-2.54), or CT and CC genotypes combined together (OR = 1.44, 95% CI, 0.94-2.23) demonstrated significant OR. Further investigations with sufficiently larger populations and in other ethnicities are required to verify this association.


In this study we used polymorphic DNA markers to examine 38 patients with gastric carcinoma for loss of heterozygosity (LOH) on five chromosomal arms. The aims were to compare LOH genotyping with the clinicopathologic variables and to identify some genetic differences between
early (EGC) and advanced gastric carcinoma (AGC). The frequency of LOH was found in 27 of 38 (71.1%) cases with a low-level LOH in 17 (44.7%) and a high-level LOH (LOH-H) in 10 (26.3%). There was statistical significance found in the differentiation of cells (WD/MD vs. PD [well or moderately differentiated vs. poorly differentiated]), metastasis (absent vs. present), and tumor-node-metastasis stage (I/II vs. III/IV) based on LOH genotyping. The frequency of LOH in the markers of chromosome 6 revealed a significant difference between the early and advanced stages (P = 0.043). However, there were no differences in each chromosome or in the number of affected chromosomes with an allelic loss between the histologic types EGC and AGC, except for the frequency of the markers on chromosome 22. These findings suggest that LOH genotyping may be another independent prognostic indicator in gastric carcinoma, that LOH-H, particularly the LOH on chromosome 6, could be associated with an unfavorable prognosis, while the LOH on chromosome 22 may be related to the histologic progression of gastric carcinoma.


http://www.sciencedirect.com/science/article/B6T53-47S16GS-3X/2/ce0414a5dc4a9f8e2a5393218ecfbe3f

A patient with bilateral retinoblastoma and subsequent multiple primary osteosarcomas has been described previously. Osteosarcoma cell lines established from this patient were shown to express a shortened RB1 mRNA transcript and no detectable normal Rb protein. We now show that the osteosarcoma cell lines have lost one TP53 allele and contain a mutation in exon 8 codon 286 [GAA to AAA (Glu to Lys)] in the remaining allele. Consequently, the osteosarcoma cell lines have no normal Rb protein and no normal p53 protein. Neither constitutional DNA nor DNA extracted from a retinoblastoma of the left eye of the patient contained the TP53 mutation, suggesting that the TP53 mutation in the osteosarcoma cells may represent a tumor-promoting mutation, which confers a selective growth advantage. If both RB1 and TP53 are involved in the initiation of osteosarcoma, the mechanisms for development of the retinoblastoma and osteosarcoma tumors are different.


The deletion of chromosome 1p is one of the frequent genetic alterations found in testicular germ cell tumors (GCTs), suggesting the presence of a tumor suppressor gene. BCL10, which was identified as a gene altered in mucosa-associated lymphoid tissue lymphoma, has been mapped at 1p22. The gene has been reported to be mutated in a variety of human cancers. In this study, we investigated the allelic deletions on 1p and the mutation of BCL10 in 51 GCTs comprising 30 seminomas and 21 non-seminomatous germ cell tumors. Loss of heterozygosity (LOH) on 1p was tested using three microsatellite markers. The search for BCL10 mutations in each of the three exons was screened by a single-stranded conformation polymorphism (SSCP) analysis and samples with abnormal bandshifts were directly sequenced. LOH at at least one locus tested was found in 42% (21/49) of the tumors (43% of seminomas and 38% of NSGCTs). SSCP and direct sequence analyses revealed that there were single nucleotide polymorphisms at codon 5, 8, 162, and intron 1. However, there were no somatic mutations of BCL10 in the 51 tumors. In support of the previous studies, our results demonstrated that LOH on 1p is frequent in both seminomas and NSGCTs, indicating that there is an important tumor suppressor on 1p in GCT. However, the
results indicate that BCL10 is not a candidate target gene of the 1p deletion.


http://www.sciencedirect.com/science/article/B6T53-3W9KWJ1-G/2/1c6e988c7d12564905678f17a954701d

Esophageal cancer ranks among the 10 most common cancers in the world, and is almost uniformly fatal. The genetic events leading to the development of esophageal carcinoma are not well established. To identify genomic regions involved in esophageal carcinogenesis, we performed a systematic screening for loss of heterozygosity (LOH) in 24 samples of squamous cell carcinomas, initially focusing the analysis on chromosome 18. Thirteen short tandem repeat markers spanning 18p and 18q were used. We found a broad peak of LOH spanning 18p11.2 and 18q21.1 with the most frequent LOH (72%) at D18S978 on 18q12.2, which coincides with a known fragile site FRA18A. This region is 4 cM proximal to known tumor suppressor genes and therefore suggests the possible existence of a yet undiscovered tumor suppressor gene.


http://www.sciencedirect.com/science/article/B6T53-3RKYV1V-1/2/7e8e0d9a5ef6d5279ee4f2723d372b4fd

A new cell line designated CUME-1 has been established from a poorly differentiated endometrial adenocarcinoma of the uterus. This cell line grew well without interruption for more than 88 months and 110 serial passages were successively carried out. The cells were highly tumorigenic in nude mice (85%). Repeated karyotype analyses from early (4th) to late (55th) passages of this cell line revealed a diploid stable clone in each passages without any noticeable structural or numerical aberrations. But from the 80th passage, a subpopulation with reciprocal translocation between chromosomes 1q and 9q consistently appeared and was observed in about 30% of the cells. This cell line is one of the rare examples of experimentally proved tumorigenic cells of human solid tumor origin that retains the diploid karyotype in vitro. HLA typing indicated the presence of DR4, DR13, DQ3, and DQ6. Cytosol estrogen and progesterone receptors were found both in fresh primary tumor and in this cell line. Gonadotropin-releasing hormone (Gn-RH) receptor mRNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in cultured cells. Using the single-strand conformation polymorphism (SSCP) technique, we have screened CUME-1 cells for p53 mutation in exons 4 to 9. No mobility shift was observed. This cell line may be useful in studying the in vitro chromosomal evolution of the cell line and the in vivo properties of human endometrial adenocarcinoma.


http://www.sciencedirect.com/science/article/B6T53-49NFKC6-8/2/70f78fa8f408da2064166b6e7379f76e

A series of 20 hepatocellular carcinomas and 8 intrahepatic cholangiocarcinomas was screened
from the Korean population for microsatellite alterations, including a loss of heterozygosity and replication errors using nine microsatellite markers containing several genes. The microsatellite results and our previous comparative genomic hybridization results of two tumors were compared at each locus, and the correlations between these and clinicopathologic variables were examined. The most characteristic findings were found at 13q. Replication errors were prevalent at D13S160 (13q21.2~q31) and D13S292(13q12). The incidence of loss of heterozygosity, however, was higher at D13S153 (13q14.1~q14.3) and D13S265(13q31~q32). In contrast, there were higher deletion frequencies observed in hepatocellular carcinoma (HCC) and higher amplification frequencies observed in intrahepatic cholangiocarcinoma at 13q in our previous comparative genomic hybridization (CGH) study. Higher frequencies of replication errors were observed at D16S408 (13q12~q21) and D16S504(13q23~q24) in the HCC. This study found that significant differences in the patterns of genetic instability of microsatellites were dependent on the chromosomal loci. It is believed that certain genes at altered CGH regions, which are relevant to the development and/or progression of these cancers, are activated by different mutation mechanisms.


http://www.sciencedirect.com/science/article/B6T53-3W2V4SX-1/2/1b5be92ea4d68d5fc3da66197acdd49c

Recently the high-mobility group protein gene HMGIC has been found to be rearranged in a variety of benign mesenchymal tumors with 12q13-q15 aberrations, such as angiomyxoma, fibroadenomas of the breast, lipomas, pleomorphic salivary gland adenomas, polyps of the endometrium, pulmonary chondroid hamartomas, and uterine leiomyomas. Here we report on HMGIC aberrations in the osteosarcoma cell line OsA-CI. In Northern blot studies, aberrant HMGIC transcripts were detected. Analysis of cDNA sequence data, obtained after 3' rapid amplification of cDNA ends, indicated these to consist of 5' HMGIC sequences encoding the three DNA binding domains fused to ectopic sequences apparently derived from part of the human lumican (keratan sulphate proteoglycan) gene (LUM), which we mapped by fluorescence in situ hybridization (FISH) to chromosome 12q22-q23. Moreover, Southern blot analysis revealed amplification of this fusion gene but not of the 3' HMGIC sequences. This observation was independently confirmed by FISH analysis using yeast artificial chromosome (YAC) and cosmid clones, which furthermore indicated that the amplified 5' HMGIC sequences were contained within an amplicon of about 200 kb. Our results indicate that aberrations in HMGIC might not be restricted to benign mesenchymal tumors.


http://www.sciencedirect.com/science/article/B6T53-4379NF8-3/2/1fd35ff091b05139894dde28db130d95

Chromosome 17q is highly susceptible to rearrangement mutations in breast cancer. c-erbB-2 at 17q11.2~q21.1 is frequently amplified, as is a region at 17q22~q24. As a step in the search for the target gene(s) of the 17q22-q24 amplification we determined whether the placental lactogen (PL) genes at 17q23 were amplified in 59 breast carcinomas. These genes were selected as their upregulation could theoretically be involved in breast cancer tumorigenesis. Amplification of the PL genes, and also of c-erbB-2, was detected using semi-quantitative PCR. The reliability of this method was confirmed since c-erbB-2 results obtained using PCR, Southern blotting and
immunohistochemistry were in good agreement. The PL genes were amplified in 13 (22%) of the
tumors. Furthermore, the PL and c-erbB-2 genes were frequently co-amplified although there is a
non-amplified region between them. Expression of PL was investigated in 26 tumors and was
detected in 16 of these cases including all 10 tumors with amplification of the PL genes. The
tumors with PL gene amplification were all aneuploid. A trend was seen towards an increased
incidence of lymph node involvement for tumors with amplification of the PL genes and for tumors
with co-amplification of PL and c-erbB-2, which suggests a possible association with high
malignancy.

chromosome 2 rearrangement in a case of human papillary thyroid carcinoma with radiation

http://www.sciencedirect.com/science/article/B6T53-3RH6Y4M-7/2/092243ea66a8a27ba4456dd6dc56a14c

Karyotype analysis of a primary culture from a case of papillary thyroid cancer (PTC) showed an
abnormal short arm of one homologue of chromosome 2 as sole abnormality in 4 of 16
metaphases. Based on G-banding analysis, two different aberration types on chromosome 2
could be assumed representing either a del(2)(p22-23) or a pericentric inversion. Further
comparative genomic hybridization (CGH) analysis as well as fluorescence in situ hybridization
(FISH) analysis were performed to confirm the assumed alterations. While CGH analysis showed
no loss of chromosome 2 material, FISH with yeast artificial chromosome (YAC) probes
homologous to the region 2p22-23 demonstrated two pericentric inversions of chromosome 2
involving different breakpoints on 2p in 6.8% and 4.2% of the metaphases, respectively.
Polymerase chain reaction (PCR) analysis with degenerated oligonucleotide primers that bind
within the conserved catalytic domain of tyrosine kinase (tk) genes resulted in amplification
products with DNA of YAC 851D11 suggesting the presence of such genes at or near the
translocation breakpoint.

Loh, M. L., Y. Samson, et al. (2000). "Translocation (2;8)(p12;q24) associated with a cryptic
t(12;21)(p13;q22) TEL/AML1 gene rearrangement in a child with acute lymphoblastic leukemia." Cancer Genetics and Cytogenetics 122(2): 79.

http://www.sciencedirect.com/science/article/B6T53-41TN42J-3/2/3eb8c2b6c5f4e1c4e5cbc37034c6ccfc

We report a case of childhood acute lymphoblastic leukemia with the simultaneous occurrence
of a t(2;8)(p12;q24) typically associated with mature B cell or Burkitt leukemia, and a
t(12;21)(p13;q22) exclusively associated with pre-B cell ALL. The lymphoblasts were
characterized as L2 morphology by the French-American-British classification. However, there
were atypical morphologic findings for L2 ALL, including vacuolization in some cells. The
lymphoblasts were periodic acid-Schiff positive and myeloperoxidase negative.
Immunophenotypic analysis revealed that the majority of lymphoblasts were TdT+, CD10+, CD19+, CD20-, and cytoplasmic [mu]+. These features were consistent with an immature pre-B
cell leukemia phenotype with some characteristics of a mature B-cell leukemia. A
t(2;8)(p12;q24)(p12;q24), characteristic of mature B-cell leukemia or Burkitt type leukemia, was
detected by conventional cytogenetics with no other cytogenetic abnormalities. However,
diagnostic peripheral blood and bone marrow specimens demonstrated simultaneous occurrence
of a cryptic t(12;21)(p13;q22) by both FISH and RT-PCR. The simultaneous occurrence of these
translocations in a pediatric patient have implications for the pathogenesis of leukemias with
t(2;8)(p12;q24) as well as t(12;21)(p12;q22). Analysis of additional cases of leukemia with
translocations involving the MYC locus on 8q24 will be required to determine the frequency of association with the cryptic t(12;21)(p13;22), and the prognostic significance of the simultaneous occurrence of the translocations.


http://www.sciencedirect.com/science/article/B6T53-3W787FV-6/2/4fe0210393e07992160a9065a4c3b186

The inv(16) and t(16;16) characterize a subgroup of acute myelomonocytic leukemia (AML) with distinct morphological features and a favorable prognosis. Both cytogenetic abnormalities result in a fusion of CBF[beta] at 16q22 and MYH11 gene at 16p13, whose detection by PCR and fluorescence in situ hybridization (FISH) is useful for diagnosis and monitoring of the disease. Variant translocations of inv(16)/t(16;16) are very rare and whether they are also associated with a favorable prognosis is unknown. We report a patient presenting with typical AML-M4Eo and a three-way translocation of inv(16) involving 16p13, 16q22, and 3q22. FISH studies on bone marrow (BM) chromosomes using CBFB and MYH11 DNA probes revealed a fusion of CBFB and MYH11 on 16q of the der(16), as well as a signal from MYH11 on 16p but not from CBFB; normal signals for both probes were present on the normal 16. Neither of these labeled probes was on the der(3), but the translocation between the der(3) and der(16) was confirmed by using a chromosome 16 painting probe. Molecular analysis of BM cells using RT-PCR identified a CBFB-MYH11 fusion transcript type D. After achieving complete remission, the patient relapsed. We conclude that FISH and PCR are feasible tools to distinguish cases with variant abnormalities of inv(16) from cases with other chromosome 16 abnormalities. Variant abnormalities of inv(16) may be not associated with favorable prognosis.


http://www.sciencedirect.com/science/article/B6T53-4D43PNN-8/2/1a71f549c7e14be85a8e77flee38322e

We have performed a cytogenetic analysis of 23 myelodysplastic syndromes (MDS) with complex karyotypes (CK) using GTG-banding and spectral karyotyping techniques. Fifty-five percent of cases were hypodiploid, 34% were hyperdiploid, and 11% were pseudodiploid. The most recurrent alterations were monosomy of chromosomes 18, 5, and 7; trisomy of chromosome 8; and deletion of 5q, 11q, and 12p. Ninety-two structural alterations were mostly identified as unbalanced. The chromosomes and regions more frequently affected were 16q12, 17p11, and 20q11. Eight of 92 structural alterations were reciprocal translocations. Two translocations were recurrent, t(X;20)(p11.4;q11.2) and der(17)t(5;17)(p11.2); each one was present in about 10% of cases (2 cases, t[X;20] and 3 cases, t[5;17]). Mutations of TP53 were observed in five cases (22%), all with rearrangements affecting 17p. Total or partial inactivation of TP53 was detected in six cases (26%) as a result of loss of either both copies (four cases) or just one copy (two cases). Fluorescence in situ hybridization analysis showed amplification of genes previously identified in myeloid and/or hematological processes, such as HER2neu, MLL, and AML1, which could represent frequent events in MDS with CK.

http://www.sciencedirect.com/science/article/B6T53-453WKK3-9/2/5b64d65bd1edba92407183b43add575a

Synovial sarcoma is the most common nonrhabdomyosarcomatous soft-tissue sarcoma in children and young adults. It is characterized by the common t(X;18)(p11.2;q11.2) that results in the fusion of SYT on chromosome 18 to one of two closely related and adjacent genes on the X chromosome, SSX1 or SSX2. Here we describe a poorly differentiated, monophasic synovial sarcoma in a 17-year-old adolescent boy. Hyperdiploidy, a t(X;18)(q13;q11), and other structural abnormalities were detected by conventional cytogenetic analysis. Fluorescence in situ hybridization with the PAC probe RP3-519N18, which is specific for the Xp11 region, resulted in a signal on the der(Xq), a finding consistent with a pericentric inversion of the X chromosome that resulted in a t(X;18)(p11.2;q11.2)inv(X)(p11.2q13). Real-time polymerase chain reaction using primer sets specific for SYT-SSX1 and SYT-SSX2 confirmed the presence of an SYT-SSX1 fusion transcript. Our finding of this unique and complex translocation in synovial sarcoma demonstrates the utility of molecular methods in confirming the diagnosis of synovial sarcoma.


http://www.sciencedirect.com/science/article/B6T53-4031TSR-1/2/48f15a0db6a6b9586a56f5c1dd1225d

Deletions of the long arm of chromosome 20 are associated with several myeloid malignancies. We have analyzed the structure of the del(20q) in 30 patients and two cell lines. Twenty-one of the patients presented with a myeloproliferative disorder and nine with a myelodysplastic syndrome. Two categories of deletions were identified. Eighteen patients had a large deletion with loss of both G(+) bands from the long arm of chromosome 20. Twelve patients had small deletions with loss of one G(+) band from the long arm of chromosome 20. A chromosome paint was generated from a del 20q marker carrying a small deletion. This probe was hybridized to normal metaphases (reverse chromosome painting) and also to metaphases from patients with a del 20q (comparative reverse chromosome painting). All six small deletions analyzed were characterized by loss of the proximal G(+) band (q12) and retention of the distal G(+) band (813.2). These data define a minimal deleted region extending from 20811.2-20g13.1.


http://www.sciencedirect.com/science/article/B6T53-41189W7-9/2/0046cba6df517ec985732ac92a518af6

A 43-year-old female with a peripheral white cell count of 118.0 x 10^9/L and 96% blasts was diagnosed with acute myeloid leukemia (AML), FAB M4. Cytogenetics, performed on a bone marrow sample, revealed the following abnormal karyotype: 46,XX,ins(16)(q22p13.1p13.3). Fluorescence in situ hybridization (FISH) confirmed the inter-arm insertion using a probe for 16p. The result of this structural rearrangement was the fusion of CBF[beta] to MYH11 seen commonly
in inv(16)(p13q22). The patient commenced high-dose intensive combination chemotherapy (big ICE; Idarubicin, Cytarabine, and Etoposide). Five days post chemotherapy, she developed febrile neutropenia. Despite broad spectrum intravenous antibiotics and antifungal therapy, the patient died at day nine post chemotherapy. This case demonstrates a previously unreported structural abnormality of chromosome 16 in a patient with AML M4, which represents a third mechanism to inv(16)(p13q22) and t(16;16)(p13q22) in producing the CBFB[beta]-MYH11 fusion. CBFB[beta]-MYH11 fusions masked by cryptic translocations at the cytogenetic level have been detected by FISH and PCR techniques. Due to the improved prognosis associated with CBFB[beta]-MYH11 fusions compared to the standard risk group for AML, its detection remains important.


http://www.sciencedirect.com/science/article/B6T53-41189W7-2/2/46b8deb97467dea5d031e0edb959394e

A continuously growing human hepatocellular carcinoma (HCC) cell line was established from a Chinese male, carrier of the hepatitis B virus (HBV). This cell line, designated HKCI-1, grows as an adhering monolayer of polygonal epithelial cells that embody one or more nuclei. HKCI-1 secretes [alpha]-fetoprotein but shows no evidence of HBV carriage. Conventional banding analysis of the short-term cultured primary tumor and the propagated HKCI-1 revealed a chromosome modal number of near-triploidy. It was, however, impossible to derive their complete karyotype due to the complex nature of chromosomal rearrangements and many marker chromosomes of uncertain origin. Spectral karyotyping (SKY) is a newly developed molecular cytogenetic technique that allows the unprecedented discernment of chromosomal abnormalities. Spectral karyotyping analysis on HKCI-1 and the primary tumor elucidated all aberrant chromosomes and revealed complex karyograms. Recurring aberrations detected in both primary tumor and HKCI-1 included der(X)t(X;11)(q10;p10), der(1)t(1;10)(q10?qp), der(4)t(4;16)(p10;q10), i(5p), del(5)(q13), der(7)t(7;21)(q32q10;q10), der(8)t(8;17)(q10;p10), and der(9)t(9;22)(q34?qp). Comparative genomic hybridization (CGH) was employed to monitor the culture evolution in vitro. Genomic imbalances in HKCI-1 involved chromosomal losses on 4q, 5q13-qter, 8p, 9pter-q33, 10q, 11q, 13q, 16q, 17q12-qter, and 22, and low-level gains on 6pter-q22, 7p, 8q, 9q34, 10p, 11p, 12, 17pter-q11.2, 18, 19, 20, 21, and Y. High-level amplifications were also detected on 5pter-q12, 7q11.2-qter, and Xq. The corresponding CGH finding on the primary tumor indicated similar imbalances. TP53 mutational analysis showed that both HKCI-1 and the primary tumor had the aflatoxin-associated mutation in codon 249 and an additional TP53 polymorphism in codon 72. Our present study demonstrates the value of combined SKY and CGH study in defining complex rearrangements and identifying cryptic translocations, and provides a comprehensive analysis on the chromosomal abnormalities in HKCI-1.


http://www.sciencedirect.com/science/article/B6T53-47VPWY9-2/2/3f2df290a4620f660a2f202e5f9f0c4d

The INK4a/ARF locus on human chromosome band 9p21 carries two tumor suppressor genes, TP14ARF and TP16INK4a, and both are frequently inactivated in nonsmall cell lung carcinoma (NSCLC). TP14ARF and TP16INK4a play important roles in the TP53 and RB tumor suppressor pathways, respectively. To elucidate the genetic and epigenetic status of the TP14ARF and TP16INK4a genes in NSCLC, we comprehensively analyzed mutations, homozygous deletions,
methylations in the CpG regions, and expression of the TP14ARF and TP16INK4a genes in 31 NSCLC cell lines. TP16INK4a (84%) was inactivated more frequently than TP14ARF (55%). Moreover, p16INK4a was inactivated in all 17 cell lines with TP14ARF inactivation. Three cell lines with base substitutions in exon 2 resulted in missense mutations of TP16INK4a but silent mutations of TP14ARF. There was a case of mutation in exon 1[alpha] unique to TP16INK4a, but not a mutation in exon 1[beta] unique to TP14ARF. The TP16INK4a gene was methylated in 6 cell lines, but the TP14ARF gene was not methylated in any cell line. Unlike a mutually exclusive relationship for inactivation between TP16INK4a and RB, TP14ARF and TP53 did not show such a relationship (P = 0.61, Fisher exact test). Thus, the present results indicate the TP16INK4a gene to be the primary target of INK4a/ARF locus alterations. Transient TP14ARF expression induced G1 arrest in the cells with wild-type TP53, but not in the cells with mutated TP53. Thus, the pathogenetic and biologic significance of TP14ARF inactivation is different between NSCLC cells with wild-type TP53 and those with mutated TP53.


http://www.sciencedirect.com/science/article/B6T53-45GWHMM-1/2/3856bc6a0aac9c232aa331507c602573

Two different proteins, p16INK4a and p14ARF, encoded by the INK4a/ARF locus play important roles in the RB and p53 pathways, respectively. This study was performed to determine genetic and epigenetic alterations in the INK4a/ARF locus and their effects on the growth of osteosarcoma. Among six cell lines examined, both p16INK4a and p14ARF exons were homozygously deleted in two cell lines, MG63 and HOS, and both p16INK4a and p14ARF promoters were methylated in one cell line, U2OS. Wild-type mRNA and proteins for p16INK4a and p14ARF were expressed in three other cell lines, SaOS2, HuO9, and G292. Transfection studies were performed using two cell lines, U2OS and MG63. Both the RB and p53 genes were wild types in U2OS, whereas p53 but not RB was mutated in MG63. Both p16INK4a and p14ARF suppressed the growth of U2OS, whereas p16INK4a but not p14ARF suppressed the growth of MG63. p53 only did not suppress the growth of MG63 either; however, coexpression of p14ARF with p53 increased the fraction of the G0/G1 phase in MG63 cells. The data presented here demonstrate the importance of genetic and epigenetic alterations in the INK4a/ARF locus for the growth of osteosarcoma and thus will be useful to further understand the biologic behavior of osteosarcoma in association with the defects in the p53 and RB pathways.


http://www.sciencedirect.com/science/article/B6T53-45B5DHS-3/2/01e7d02cb815fa0b9ad7fcd04b1198a

DNA common variants may significantly contribute to genetic risk for common diseases. Because of its biological function in DNA repair, hMSH2 gene polymorphisms are candidates for influencing cancer susceptibility and overall genetic stability. Twenty-two individuals with non-Hodgkin lymphomas (NHL) and 50 normal individuals were screened for polymorphic variants in exon 13 of the hMSH2 mismatch repair gene in order to determine if there is any association with development of lymphomas. The polymorphism screening was carried out by single strand conformation polymorphism analysis and DNA sequencing. We found a single nucleotide polymorphism: a T to C substitution at the -6 intronic splice acceptor site of exon 13 (gIVS12-6T>C). This polymorphism was present in 7.5% of normal individuals (allele FREQUENCY = 0.05) and in 22.73% of lymphomas (allele FREQUENCY = 0.11) (P<0.01). These results suggest
that the polymorphism may be associated with an increased risk to develop NHL and that probably there are differences in the effect of the polymorphisms among populations.


http://www.sciencedirect.com/science/article/B6T53-45PRT4M-B/2/918642ed8ee7bd470b468ae1fb0cde51

The majority of tumors from patients affected by hereditary nonpolyposis colorectal cancer (HNPCC) exhibit a mutator phenotype characterized by widespread microsatellite instability (MSI) and somatic mutations in repeated sequences in several cancer-associated genes. An inverse relationship between MSI and chromosomal instability (CIN) has been demonstrated and HNPCC-associated tumors are generally characterized by diploid or near-diploid cells with few or no chromosomal rearrangements. We have studied MSI, somatic mutations in repeat-containing genes, DNA-ploidy, and cytogenetic aberrations in a colon carcinoma from a patient with a germline MLH1 mutation. Mutations in coding repeats were assessed in 10 macroscopically separate areas of the primary tumor and in two lymph nodes. Some of the genes studied (E2F4, MSH3, MSH6, TCF4, and TGFBRII) showed a consistent lack of mutations, whereas others (BAX, Caspase-5 and IGFIIR) displayed alterations in some tumor regions but not in others. The tumor had DNA-index 1.1-1.2 and a stable, aberrant karyotype with extra copies of chromosomes 7 and 12 and the structural aberrations i(1q), der(20)t(8;20), and der(22)t(1;22). The finding of CIN, MSI, and somatic mutations in coding repeats in this tumor suggests that these phenomena may act together in HNPCC tumorigenesis. Furthermore, the observed intratumoral heterogeneity of mutations in coding repeats implies these changes occur late in tumorigenesis and, thus, probably play a role in tumor progression rather than initiation.


http://www.sciencedirect.com/science/article/B6T53-42RDSHB-5/2/c83afc33677a40700bc2e212af06b60f

A novel tumor suppressor gene, PTEN/MMAC1, on 10q23, displayed a number of mutations in solid tumors as gliomas and breast cancer. Aberrations of the long arm of chromosome 10 have been frequently detected in tumor progression of malignant melanoma of the skin by a variety of methods including cytogenetic analysis, fluorescence in situ hybridization and loss of heterozygosity analysis. Compared to previous studies, which propose an involvement of PTEN/MMAC1 in malignant melanoma mostly on the basis of data derived from cell lines and metastases, we analyzed a broader spectrum of exclusively patient derived tumor tissue by PCR and direct sequencing analysis of PTEN/MMAC1. Here, we present data of 25 primary melanomas (8 superficial spreading melanomas, 17 nodular melanomas) and 25 metastases of 41 patients. Neither loss of the complete gene nor a whole exon nor any nonsense mutations could be demonstrated. However, we detected several polymorphisms and some mutations in the introns, and in two metastatic tumors mutations with an amino acid change. Our results obtained from tissue samples underline that mutations of PTEN/MMAC1 are not an essential event in the onset of malignant melanoma of the skin, but could have an impact on tumor progression.

http://www.sciencedirect.com/science/article/B6T53-44WYJ7T-3/2/84234f13b4eddeeff748cf36f811db2c

Alterations of the candidate tumor suppressor gene PTEN/MMAC1 and the cell cycle control gene p16(CDKN2/MTS-1/INK4a) have been detected in many types of human cancer. Here, we wanted to study the role of PTEN/MMAC1 in head and neck squamous cell carcinomas (HNSCC) in correlation to mutation and methylation of p16 and to previous in situ hybridization results concerning loss of chromosomes 9 and 10. We screened for alterations of PTEN/MMAC1 and p16 in 52 HNSCC of different sites. Mutations of PTEN/MMAC1 were found in 23% of tumor samples (missense mutations in 7 carcinomas, 13%). A loss of chromosome 10 was detected in five carcinomas with missense PTEN/MMAC1 mutations (71%). The missense mutations of PTEN/MMAC1 occurred in exons 5 (five different mutations in the neighborhood of the protein tyrosine phosphatase domain), 6, 7, and 8. Only one of these mutations had been described before. In addition, in three laryngeal carcinomas (6%), missense mutations of p16 (in exon 2) were detected and 14% of carcinomas showed a methylation of p16. Our results focus on the essential but not solitary role of PTEN/MMAC1 in the tumorigenesis or progression of a subset of HNSCC.


http://www.sciencedirect.com/science/article/B6T53-48TCBDC-3/2/4da2c8ffdf73055e279e1d4238a631e80

Cytogenetic and molecular studies of radiation-induced meningiomas (RIM) are rare and controversial. While comparative genomic hybridization (CGH) analysis identified monosomy 22 as the predominant change in RIM, occurring in frequencies comparable to those found in spontaneous meningioma (SM), molecular genetic analysis shows infrequent loss of chromosome 22 DNA markers. We have performed CGH analysis of six additional cases of RIM and detected an unbalanced genome in five of 6 cases. Loss of 1p and 7p was identified in the majority of RIM with an abnormal karyotype (4/5 cases), whereas loss of 6q occurred in three of five cases. Only one of five RIM had monosomy for chromosome 22. Loss of 7p is not frequently reported in SM and yet it was detected in four of 5 RIM with an abnormal karyotype in our study. Molecular and cytogenetic studies of chromosome 7 copy number should be attempted on a larger number of RIM to further investigate the role of 7p loss in RIM.


http://www.sciencedirect.com/science/article/B6T53-47VPWY9-5/2/1cbbd937a305176ce508386946fe38a

In this study we aimed at investigating the incidence and the role of 3p deletions, particularly at the 3p25–pter region, in follicle cell-derived thyroid neoplasms, by using loss of heterozygosity (LOH) analysis. We analyzed 12 follicular adenomas (FA), 13 follicular thyroid carcinomas (FTC), and 15 papillary thyroid carcinomas (PTC) with 11 microsatellite markers for chromosome 3. One additional marker on 3q25.2 was also investigated for assessment of deletion extent on 3q.
Microsatellite instability was detected at one locus in 1 of 15 PTC (7%) and at four loci in 1 of 13 FTC (8%). Loss of heterozygosity was found in 8 of 12 cases of FTC (67%), in 6 of 15 cases of PTC (40%), and in 2 of 12 FA (17%). We identified three minimal common deleted regions (CDR) involving significant sites of LOH: two in FTC (a new terminal region, of approximately 8 cm distal to D3S1620 at 3p25.3~pter and the D3S1573-D3S1595 region at 3p21.2~p12) and one in PTC (D3S1304-D3S1263 region at 3p25.3~p24.2). The newly identified 3p25.3~pter CDR seems to be specific for FTC. Our results suggest the existence of at least three distinct regions on 3p that might harbor tumor suppressor genes involved in the carcinogenesis processes of FTC and PTC.

http://www.sciencedirect.com/science/article/B6T53-4FB8J0J-F/2/b8df44ba5a7c68bb629d63069f21a350

Uterine leiomyomata are benign, smooth-muscle tumors. The tumors are very common, affecting [not, vert, similar]10-15 million women in the United States annually. Uterine leiomyomata are often asymptomatic, but may cause symptoms that range in severity from mild abdominal discomfort to uterine prolapse. Several different chromosomal aberrations have been found in the tumor tissue. Because of the common occurrence of this tumor and the potential severity of associated sequelae, research delineating the different molecular subtypes is needed. Deletions on the long arm of chromosome 7 are believed to be the most common genetic anomaly in uterine leiomyoma. The size of the deletion varies, which makes it difficult to identify the genes that, upon deletion, contribute to tumor growth. The smallest previously defined interval was >12,000 kb. We have narrowed a minimal region to an interval of <500 kb.

http://www.sciencedirect.com/science/article/B6T53-4379NF8-D/2/0ba7e172525a5adc0447f2365afdeaf

Loss of heterozygosity and allelic imbalance in tumors are usually detected by either radioactive labeling of PCR products with subsequent scoring of autoradiographs or by a semi-quantitative fluorescence-based protocol. Polymorphic microsatellite loci are the most common marker type used in these studies. Even though no consensus exists as to how to evaluate such data, results are often compared directly between studies applying the two different protocols. In the present study, we analyzed twice by each protocol three loci in 60 blood/tumor pairs, finding good correlation between the results obtained by the two methods. However, a higher sensitivity and the possibility to correct for stutter peaks were among several advantages inherent in the fluorescence labeling approach. In addition, we determined the cut-off level for allelic imbalance scoring by the fluorescent primer protocol, by repeated analysis of 485 constitutional heterozygous genotypes at 20 different dinucleotide repeat loci. Based on the standard deviation, we found that allelic imbalance should be scored whenever the peak height of one allele in tumor DNA is reduced to less than 0.84 of its value in constitutional DNA, relative to the other allele. Applying this cut-off value, more imbalances are detected than by the visual scoring of autoradiographs. Our data therefore suggest that a lower threshold value (0.75) must be used when results from both fluorescent and radioactive assays are compared.

http://www.sciencedirect.com/science/article/B6T53-3XSJXSS-3/2/96d0df2ea779d987348fc7be39eccc71

Cytogenetic analysis was conducted on tumor biopsy material from two pediatric, small, round, blue-cell tumors whose histology failed to give a clearcut diagnosis. The first case showed a complex composite karyotype within which there were two normal chromosomes 11 and one abnormal chromosome 22 present. The composite karyotype in the second case was similarly complex but this time included an abnormal chromosome 11 but no corresponding abnormal chromosome 22. Analysis of tumor mRNA from both cases using a Reverse Transcriptase PCR test with primers derived from a Ewing's sarcoma t(11;22)(q24;q12) breakpoint sequence showed both to have abnormal, chimeric transcribed messengers, each of different lengths. Further analysis of case 2 using chromosome painting and centromeric probing confirmed the abnormal chromosome 11 to be a der(11)t(11;22)(q24;q12) and also revealed two additional minor clones containing a der(22), which may be the karyotypic locations of the t(11;22) fusion sequences. Taken into consideration with clinical and histologic information, the results of these investigations indicated that both were neuroectodermal tumors (Ewing sarcomas of the chest wall/Askin tumors). The comparative values of both cytogenetic and molecular analysis in the diagnosis of neuroectodermal tumors and the detection of covert chromosome rearrangements are discussed.


http://www.sciencedirect.com/science/article/B6T53-3YRVKMX-9/2/dc6cbcdc57e9d46dac77dadbc14aeeac

We analyzed the loss of heterozygosity (LOH) for 1p in 18 Wilms tumors using a panel of 11 polymorphic markers. Loss of heterozygosity was identified in 56% of the tumors. The smallest region of overlap was defined for marker D1S247, underlying the 1p35-1p36.1 locus. This is the highest LOH frequency for 1p, or for the well-defined 11p13 and 11p15.5 loci. Based on the fact that tumors of all stages, with both favorable and unfavorable histology, exhibited LOH, we suggest that the 1p35-1p36.1 locus is involved in the etiology of Wilms tumor.


http://www.sciencedirect.com/science/article/B6T53-47S16R7-5F/2/83f9c9fbb4d636cfa1bf7839a5f5f19

Thirteen male patients affected by different hematologic diseases who underwent bone marrow transplantation (BMT) with female donors were investigated by cytogenetic analysis and polymerase chain reaction (PCR) amplification of a DNA sequence specific for the Y chromosome. In six of these patients, PCR showed the presence of the Y chromosome-related sequence; in only three of these did cytogenetic analysis confirm the presence of mixed chimerism. In the remaining three patients, the results of the PCR were confirmed by in situ hybridization on cell nuclei with a probe for the [alpha]-satellite of the Y chromosome. We compare results obtained with the two methods and discuss the meaning of the minimal residual disease detected by PCR in patients submitted to BMT.
The molecular events leading to the development and progression of serous ovarian carcinoma are not completely understood. We performed a large scale survey for the identification of differentially expressed genes in serous ovarian carcinoma by using cDNA array analysis. Differences in gene expression between serous adenocarcinoma and benign serous adenoma, and between advanced and/or moderately or poorly differentiated and local, highly differentiated serous adenocarcinoma were assessed. The most striking difference between adenocarcinoma and benign adenoma was upregulation of RHOGDI2 in the carcinomas irrespective of the clinical tumor stage. Other changes in carcinoma were upregulation of MET and Ne-dlg, and downregulation of HGFAC, desmin, and PDGFA. The most prominent differences between advanced and local adenocarcinoma were upregulation of COL3A1, CFGR, and MET in advanced carcinoma, and downregulation of HGFAC, FZD3, and BFL1 in the same tumors. In conclusion, significant differences were found in the gene expression between benign and malignant serous ovarian tumors, and between local, highly differentiated and advanced and/or moderately or poorly differentiated serous adenocarcinomas. The differentially expressed genes may be related to the carcinogenesis and progression of the malignant growth.

cDNA microarray analysis was used to screen for gene expression alterations in human osteosarcoma cell lines. The analysis using three cell lines revealed changes in the expression of several genes in comparison with normal human osteoblasts. Among the 5,184 sequences that were analyzed, 35 showed aberrant expression in all the cell lines. Eight of these showed overexpression and 27 underexpression compared to their expression levels in osteoblasts. The most highly up-regulated genes included heat shock protein 90[beta] and polyadenylate-binding protein-like 1. Commonly down-regulated genes included fibronectin 1 and thrombospondin 1. RT-PCR was used to verify these changes in the cell lines and in three primary osteosarcoma samples. This study shows that (1) gene expression pattern in osteosarcoma cell lines differs considerably from normal osteoblasts, (2) osteosarcoma cell lines can be used as a model system to detect novel gene expression alterations present in primary tumors, (3) the overexpression of heat shock protein 90[beta] and polyadenylate-binding protein-like 1, and (4) the down-regulation of fibronectin 1 and thrombospondin 1 may play a role in the development and/or progression of osteosarcoma. This study indicates that microarray-based expression surveys may be used to establish the molecular fingerprint of osteosarcoma, however, larger cDNA chips and more tumor specimens are required to define the clinically relevant gene expression patterns.
In this study, 27 malignant cell lines, including leukemias, gliomas, and lung and bladder carcinomas were screened for homozygous deletions of the putative tumor suppressor gene p16 (MTS1/CDK4/CDKN2) and other markers within the chromosome 9p21 region; these include the genes for interferon-[alpha]1 (IFNA1), interferon-[beta]1 (IFNB1), methylthioadenosine phosphorylase (MTAP), and two microsatellite markers, D9S171 and D9S169. The purpose of this study was to determine the incidence of codeletion of these markers. Screening for homozygous deletions was carried out using direct polymerase chain reaction of genomic DNA, or, in the case of MTAP, a functional enzyme assay. Of these cell lines, 14 (52%) were found to have homozygous deletions of the p16 gene. Two of the 14 p16-negative cell lines (14%) were found to have homozygous deletions within the p16 domain but no other 9p21 marker. MTAP was codeleted in 12 of the 14 p16-negative cell lines (86%), whereas IFNA1 was codeleted with p16 in eight of these lines (57%); IFNB1 was codeleted in five (36%) of the p16-deleted cell lines. The D9S171 marker, which may lie greater than 3 cM centromeric to p16, is codeleted in three cell lines (21%); the D9S169 marker, which maps even further toward the centromere, was codeleted in only one cell line (7%). Loss of any 9p21 marker, e.g., MTAP or IFNA1, were invariable predictors of the loss of the p16 gene. In addition, loss of IFNA1 always predicted a loss of MTAP (eight of eight cell lines), although loss of MTAP did not always predict a loss of IFNA1 (four of 12 MTAP-deleted cell lines did not have homozygous deletions of IFNA1). Thus loss of nearby genes occurs in a high percentage of cell lines that bear homozygous deletions of the p16 locus. Codeletion of MTAP or IFN in p16-negative malignant cells is of interest, as loss of these genes may influence the biologic behavior of these cells and render them susceptible to certain therapeutic approaches.


In this study, we used comparative genomic hybridization to provide an overview of chromosomal imbalances in a series of 20 adult and 8 childhood ependymomas. All tumors displayed multiple genomic imbalances. Loss of genetic material was observed in chromosomes 22q (71%), 16 (57%), 17 (46%), 6 (39%), 19q (32%), 20q (32%), and 1p (29%), with the overlapped deletion regions determined at 16p13.1-13.3, 16q22-q24, 19q13.1-13.4, 20q13.1-13.2 and 1p36.1-36.3. Gain of DNA was commonly detected on chromosomes 5q (46%), 12q (39%), 7q (36%), 9q (36%), and 4q (32%), with overlapped regions of gain mapped to 5q21-22, 12q15-24.1, 7q11.2-31.2, 9q12-32, and 4q23-28, respectively. These findings suggest a greater degree of genomic imbalance in ependymomas than has been recognized previously and highlight chromosomal loci likely to contain oncogenes or tumor suppressor genes that may contribute to the molecular pathogenesis of this tumor. Our study also confirmed previous findings on frequent losses of 17 and 22q in ependymomas and further identified chromosome 16 loss as a common recurrent genetic aberration in ependymomas.
We examined lung cancer cell lines for the presence of [beta]-tubulin gene alteration based on a previous report of a relationship between frequent [beta]-tubulin gene mutation in non-small-cell lung cancer and clinical response to taxanes as well as the prognosis. The mutation was defined by analyzing genomic DNA from 31 lung cancer cell lines by direct genomic sequencing using specific primers for the [beta]-tubulin class I gene. We detected only three genetic alterations at nonsplice sites in two introns, and a silent genetic alteration at codon 217 in exon 4. The mutation of the [beta]-tubulin gene was rare; moreover, these genetic alterations were predicted to evoke no biological alteration of the cancer cells. Our data suggest that the [beta]-tubulin gene mutation does not play a major role in the genetic mechanism of resistance to taxanes. In addition, the presence of a closely related family of [beta]-tubulins or pseudogenes was thought to hinder accurate evaluation of the [beta]-tubulin gene.

Loss of heterozygosity (LOH) on 8p is a frequent event in many cancers and is often associated with more aggressive disease. Tumour necrosis factor-related apoptosis inducing ligand (TRAIL) receptor 2 (TRAIL-R2) also known as TNFRSF10B (tumour necrosis factor receptor (TNFR) super family 10b) or KILLER/DR5, a member of the TNFR family, is a promising candidate tumour suppressor gene at 8p21-22. Mutations in this gene have been identified in non-small cell lung cancer, head and neck cancer, breast cancer and non-Hodgkin's lymphoma. We carried out mutation analysis of TRAIL-R2 in bladder cancer cell lines and in primary bladder tumours. One novel protein truncating mutation was identified in a bladder cancer cell line. Our results suggest that if TRAIL-R2 is the target of LOH events in these cancers, inactivation of the remaining allele is by a mechanism other than mutation.

Mammaglobin B is a recently-isolated gene speculated to belong to the uteroglobin gene family and is overexpressed in primary breast cancers. We investigated mammaglobin B mRNA expression in various cancers of the digestive system. Given the absence of mammaglobin B expression in normal lymph nodes, we also assessed the usefulness of mammaglobin B as a marker for lymph node micrometastases in cancer patients. Mammaglobin B gene transcripts were frequently detected by reverse transcriptase-polymerase chain reaction (RT-PCR) assay in primary tumors of the esophagus (2/3), stomach (7/7), colon (15/15), pancreas (4/6), common
bile duct (6/6), cholangioma (2/2) and gall bladder (1/1). Mammaglobin B overexpression was observed in three of 15 cases (20%) of colon cancer, suggesting its possible contribution to colon carcinogenesis. Down-regulated mammaglobin B expression was observed in hepatoma cells in comparison with corresponding non-cancerous livers (3/3). RT-PCR assay of mammaglobin B detected 14 of 15 histologically positive lymph nodes from patients with gastric cancer, colon cancer and cholangioma. Seven of 32 (22%), three of nine (33%), and three of seven (43%) histologically negative nodes from patients with gastric, colon and cholangiocellular carcinoma, respectively, were found to express mammaglobin B mRNA. Our results showed that expression of mammaglobin B was frequently detected in cancers originating in digestive organs, especially adenocarcinomas, and that mammaglobin B gene detected by RT-PCR may be a potentially useful molecular marker for lymph node micrometastases of various digestive organ cancers.


http://www.sciencedirect.com/science/article/B6T54-3SV3NWP-2/2/e152c00787aa69f48f2f6c749c09c7a6

Deletion of 9p21 has frequently been observed in human bladder carcinomas. A candidate target suppressor gene, p16, was recently identified within this deleted region. In this study, we therefore investigated the loss of heterozygosity (LOH) of the p16 gene which is located on mouse chromosome 4, as well as its expression in mouse bladder carcinomas. We also studied the effects of normal cell contamination on LOH analysis using xenografts in CD-1(ICR) nude mice from B6C3F1 bladder carcinomas. We could not detect any LOH at the p16 locus in the mouse primary bladder carcinomas and xenografts. Surprisingly, overexpression of p16 was found in all primary mouse bladder carcinomas. Using microsatellite polymorphisms, a distinction could be made between PCR products derived from B6C3F1 and CD-1(ICR) nude mice. It was thereby confirmed that effects of normal cell contamination on LOH analysis are negligible when only tumor tissue is carefully sampled. The results suggest that abnormalities of p16 expression may be involved in mouse bladder carcinogenesis, but that gene deletion is not involved.


http://www.sciencedirect.com/science/article/B6T54-3VXHKS3-B/2/58035dc1e1267d68587a37de69ea065f

Amplification of the c-myc oncogene occurs in a variety of solid tumors, including pancreatic adenocarcinomas. The MXI1 gene, located at 10q24-q25, may serve to negatively regulate c-myc oncogene activity, and potentially has tumor suppressor function. As such, altered MXI1 function might contribute to tumorigenesis. We examined 40 primary human pancreatic adenocarcinomas for MXI1 mutations. Single-strand conformation variant analysis and direct sequencing of the variants revealed a MXI1 polymorphism in 1 of 40 tumors. No MXI1 mutations were identified. Southern blot analyses did not reveal any gross rearrangements of MXIL These results suggest that MXI1 is unlikely to play a role in human pancreatic adenocarcinoma tumorigenesis.


http://www.sciencedirect.com/science/article/B6T54-4DW90TS-
Germline mutations within the mismatch repair (MMR) genes are generally found in colorectal cancer (CRC) patients with a positive family history for the presence of the neoplasia. Clinical standard criteria have been established to define hereditary-non-polyposis-colorectal cancer (HNPCC)-prone families. Interestingly, the number of MMR gene mutations found in kindreds not fulfilling these criteria is still increasing. In this work we report the identification of a novel germline mutation of the hMSH2 gene, in two CRC-bearing subjects. The two probands belong to a large kindred from South Italy with no history suggestive for cancer aggregation. On the other hand, the early-onset of the neoplasia as well as the presence of a high number of tumor infiltrating lymphocytes (TILs) in the histological specimens of both patients, prompted us to perform a comprehensive genetic analysis. This analysis included the evaluation of the microsatellite instability (MSI) status with five markers according to the National Cancer Institute recommendations, followed by direct sequencing of the hMLH1 and hMSH2 genes. Both probands were found to carry a germline missense (277 C>T) mutation leading to the change (L93F) of an amino acid residue in a highly conserved domain of the MSH2 protein. This mutation is accompanied by the loss of expression of the hMSH2 gene in the tumor tissue. Our findings suggest that in the presence of the above-mentioned criteria it may be useful to perform a molecular analysis of the MMR genes, even if the pedigree does not show marked aggregation of cancers.


We have established two murine cell lines derived from Small Cell Lung Carcinomas (SCLCs) developed by HPV-E6/E7 transgenic mice. These cells named PPAP-9 and PPAP-10 were isolated from mice bearing tumors, 9 and 10 months old, respectively. The cells, 5 μm in diameter, express HPV oncoproteins and sustain tumor formation after subcutaneous injection in syngenic mice. A detailed analysis indicated the epithelial origin and the neuroendocrine differentiation of these cells. We showed by confocal immunofluorescence the expression of the epithelial marker cytokeratin 5, whose gene promoter was used to direct the expression of HPV E6/E. Cells express several neuroendocrine markers such as CGRP, MAP-2, Ash1, CgrA, Scg2. The neuroendocrine differentiation of these cells was further confirmed by electron microscopy demonstrating neuropeptides secreting granules in their cytoplasm. Furthermore, in agreement with the altered expression observed in the majority of human SCLC we showed in these cells the absence of both p53 and pRB and a dramatic reduction in the expression of Caveolin-1.

Charlton, M. A., B. J. Merry, et al. (1999). "Differential display analysis can reveal patterns of gene expression in immortalised hepatoma cells which are similar to those observed in young adult but not old adult liver cells." Cancer Letters 143(1): 45.

http://www.sciencedirect.com/science/article/B6T54-3X10V5J-7/2/01220df5776a672372735aa22a20632a

We used the differential display technique to examine whether there were any patterns of gene expression which were characteristic of both young adult rat liver and of immortalised rat hepatoma cell lines, but not of old adult rat liver. No genes were detected which appeared to be clearly expressed in young liver and immortalised cell lines, but not in old liver. However, 14 genes were detected in old liver which were down-regulated in young liver and the hepatoma cell lines. This observation lends support to the idea that immortalisation of malignant cells may
involve, at least in some aspects, a reversal of the ageing process in these cells and that the genes involved have a recessive action.


The resistance of cancer cells to chemotherapeutic agents is a major clinical problem and an important cause of treatment failure in cancer. Mechanisms that have developed to guard cancer cells against anti-cancer drugs are major barriers to successful anti-cancer therapy. Therefore, the identification of novel mechanisms of cellular resistance holds the promise of leading to better treatments for cancer patients. In the present study, we used human MCF-7 breast adenocarcinoma cell line and its doxorubicin-resistant variant MCF-7/R to determine the role of alterations of DNA methylation of chemoresitance-related genes, such as multidrug resistance 1 (MDR1), glutathione-S-transferase (GST[pi]), O6-methylguanine DNA methyltransferase (MGMT), and urokinase (Upa), in the development of drug resistance. The promoter regions of MDR1, GST[pi], MGMT, and Upa genes were highly methylated in MCF-7 cell line but not in its MCF-7/R drug resistant variant. The hypomethylated status of MDR1 gene was associated with overexpression of P-glycoprotein. We hypothesize that acquirement of doxorubicin resistance of MCF-7 cells is associated with DNA hypomethylation of the promoter regions of the MDR1, GST[pi], MGMT, and Upa genes.


http://www.sciencedirect.com/science/article/B6T54-3XD41GK-7/2/341f0ba3bd3c17debo28e1ef052f89

Variation in the frequency of microsatellite instability (MSI) has been reported in different kinds of human malignant tumors, with less than one-third of invasive urinary bladder carcinoma cases estimated to be affected. Here we investigated the MSI for 27 microsatellite sequences in invasive urinary bladder carcinomas of the NON/Shi mouse induced by N-butyl-N-(4-hydroxybutyl)nitrosamine. A total of 28 urinary bladder carcinomas of both transitional cell and squamous cell types were studied. All were invasive (greater than pT3) and high-grade and 10 of them had metastasis. Only two (11%) of 18 primary bladder carcinomas without metastasis foci showed alterations in one or two loci. None of 10 pairs of urinary bladder carcinomas and metastasis foci demonstrated any alterations. In conclusion, MSI which represents a defect in the DNA mismatch repair system is infrequent and therefore unlikely to be a critical step in genesis of invasive mouse urinary bladder carcinomas.


http://www.sciencedirect.com/science/article/B6T54-3WH642X-D/2/1062cc2a7e88394c3ab3a91d8d3589a77

The presence of mRNA of individual members of the CYP1 gene family in normal and neoplastic
kidney has been investigated by RTPCR. CYP1B1 mRNA was consistently expressed in both normal and neoplastic kidney while CYP1A1 was present in the majority of normal and neoplastic whereas CYP1A2 was infrequently expressed. Expression of the Ah receptor and Arnt which are involved in the transcriptional activation of the CYP1 genes was also studied. The Ah receptor mRNA and Arnt mRNA were consistently expressed both in kidney tumours and normal kidney. These results indicate differential expression of individual members of the CYP1 gene family in normal and neoplastic kidney and suggest that several mechanisms including the Ah receptor complex could be involved in their regulation.


http://www.sciencedirect.com/science/article/B6T54-4D98XYX-1/2/5dbf2b41a52c6a75da77fb4a15abe078

NOTCH1 is involved in the pathogenesis of T-acute lymphoblastic leukemia (T-ALL) carrying the very rare translocation t(7;9)(q34;q34.3). We analyzed the expression of genes belonging to NOTCH pathway, in acute leukemia primary samples and lymphoblastoid cell lines. NOTCH1 pathway activation represents a common feature of T-ALL when compared to acute myelogenous leukemia (AML) and B-cell precursor acute lymphoblastic leukemia. The contemporary expression of NOTCH1 and its ligands on cell surface contributes to high levels of pathway activity. AML primary samples show high levels of JAGGED1 expression despite the low NOTCH1 pathway activation, consistent with an autonomous JAGGED1 signaling in myeloid leukemogenesis.


http://www.sciencedirect.com/science/article/B6T54-42R0SBN-3/2/80b403e4b58691271040be8ef739b97

Platycodon grandiflorum has been claimed to have a wide range of health benefits, which include immunostimulation and antitumor activity. The associated biological mechanisms are unclear; however, of the wide diversity of effects, it is believed that their activities may be exerted through several potent effector cells such as macrophages. Therefore, the effects of an aqueous extract from the root of P. grandiflorum (Changkil: CK) on mouse peritoneal macrophage function were investigated. It was found that CK stimulated macrophage proliferation, spreading ability, phagocytosis, cytostatic activity, and nitric oxide production in a dose-dependent manner, and that the production of cytokines such as TNF-[alpha], IL-1[beta] and IL-6 were similarly increased. CK significantly affected secretion at concentrations greater than 10 [mu]g/ml; its maximal effects were at the concentration of 100 [mu]g/ml. Reverse transcription-polymerase chain reaction showed that CK increased the appropriate cytokine mRNAs. These results suggest that CK is a potent enhancer of macrophage function.


http://www.sciencedirect.com/science/article/B6T54-46MJ2Y9-9/2/f2403ecd3b31a4b54c4c335886f0e61a
Sperm protein 17 (Sp17) is a highly antigenic, testes-specific protein whose known function is to bind sperm to the zona pellucida. However, the Sp17 gene has been recently detected in normal non-testes tissues and malignant neoplasias. As the role of Sp17 in non-testes tissues is unknown, the characterization of the Sp17 gene in highly proliferating tissues may provide further insight into the regulation and alternative function of Sp17. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to amplify the Sp17-1 transcript in multiple normal human tissues and cancer cell lines. Similarly, the Sp17-2 gene was examined by PCR. In addition, Northern and Western blot analyses were used to detect Sp17 mRNA and protein expression. The Sp17-1a and Sp17-1b transcripts were amplified from cancer cell lines. Similarly, an Sp17-2 transcript was also detected in cancer cell lines. Furthermore, Northern blot analysis revealed Sp17 mRNA expression in all cancer cell lines examined. However, Sp17 protein expression was not detected. The differential detection of the Sp17 transcripts in cancer cell lines as compared to normal non-testes tissues, suggests a potential pathogenic role for Sp17 in diseased cells. Moreover, the Sp17-2 transcript may be a marker for highly proliferating cells. Collectively, these data implicate Sp17 as a cancer testis antigen.


http://www.sciencedirect.com/science/article/B6T54-43S5SNF-3/2/b7f6ec0eab5d5992286e86b18b0d404d

The human colon adenocarcinoma cell line HT29 can be adapted to 10-7- 10-4 M concentrations of methotrexate (MTX). Cells adapted to 10-4 M MTX have an enterocyte-like phenotype with DHFR gene amplification. Presently, we hypothesized that an increased expression of folate binding protein (FBP) may participate to the MTX resistance of 10-4 MTX HT29 cells. The cDNA FBP[alpha]/[beta]-actin ratio of amplified transcripts was 4.8- and 1.5- fold higher in 10-4 and in 10-7 M MTX HT29 respectively, than in standard type HT29 cells. An increase of transcript level was observed when decreasing folic acid concentration. PI-PLC cleaved 7.7 times more membrane FBP in 10-4 M than in 10-7 M MTX and wild type HT29 cells. In contrast to 10-7 M MTX cells, growth of 10-4 M MTX cells was dependent on folic acid concentration and abolished at a concentration lower than 0.9 [mu]M. In conclusion, the adaptive mechanism of HT29 cells resistant to 10-4 M MTX is the result of the synergistic overexpression of both DHFR and FBP[alpha]. Overexpression of FBP[alpha] may be related to the enterocyte-like phenotype of the cells.


http://www.sciencedirect.com/science/article/B6T54-3XCFJRK-7/2/a0a5a61b8a7e86546366f0a6260e8d1e

The expression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in samples of normal gastric mucosa and gastric cancer were examined by reverse transcriptase-polymerase chain reaction (RT-PCR) and semiquantitative Western blot. In normal gastric mucosa, eNOS protein was found in all samples examined (mean, 70.2+/−60.1), relative to a standard protein. In gastric cancer specimens, eNOS protein was also detected in all samples, but the quantity (86.5+/−76.6) was not different from that found in samples of normal mucosa. The quantity of eNOS in gastric cancer tissues was negatively correlated with serosal invasion. iNOS mRNA, detected in nine of 18 cases, was slightly related to massive lymph node metastasis (n1-3 vs. n4). Neither tumor necrosis factor alpha (TNF-alpha) mRNA nor interleukin-6 (IL-6) mRNA
was related to the expression of iNOS mRNA. These results suggest that iNOS not eNOS plays a role in gastric cancer tumor extension, but iNOS mRNA appears not to be induced by either TNF-alpha or IL-6.


http://www.sciencedirect.com/science/article/B6T54-4CYNSJP-2/2/554294d9eb920e1c8b30af3ad5cac0c0

We recently identified a heterozygous mutation in the [beta]1 integrin subunit of a squamous cell carcinoma (SCC) that maps to the I-like domain and activates ligand binding. To investigate the frequency of such mutations we screened 124 human oral SCCs. We identified six single nucleotide changes, all of which were also present in normal tissue, suggestive of polymorphisms. Two were in non-coding intronic sequences. Three were silent changes in exons. One caused a change in amino acid (A239V) that is unlikely to disturb integrin structure. We conclude that mutations in the [beta]1 I-like domain are uncommon in SCCs. However, population based studies of the polymorphisms we found may reveal an association with SCC development or prognosis.


http://www.sciencedirect.com/science/article/B6T54-3YKKB7Y-J/2/372f2b776cc3bb279a2bc1d75a95a53c

pS2, a member of the trefoil peptide family, has been suggested to be a gastric-specific tumor suppressor. We examined the expression of pS2 in gastric carcinomas, adenomas and non-neoplastic mucosa and analyzed the DNA methylation in the pS2 promoter. Reduced expression of pS2 was frequently associated with well-differentiated adenocarcinomas. The CpG sites within the promoter region of the pS2 gene were methylated in pS2-negative gastric carcinoma cell lines whereas it was not in pS2-positive cell line. The promoter methylation was detected in gastric carcinoma tissues and intestinal metaplasia with reduced pS2 expression whereas none of the carcinomas with preserved pS2 expression showed the promoter methylation. These findings suggest that reduced expression of pS2 due to the promoter methylation may participate in an early stage of stomach carcinogenesis, especially of well differentiated type.


http://www.sciencedirect.com/science/article/B6T54-402TP0K-J/2/4f486dadede095c8395bb40e01b147f1

Reverse transcriptase-polymerase chain reaction (RT-PCR) techniques have been widely employed as an ultra-sensitive method for detection of micrometastases in patients with various types of malignancies. Messenger RNA of a specific marker gene is a target for RT-PCR amplification to examine the presence of micrometastases in body fluids or tissues obtained from human. We developed the RT-PCR assay specific for rat [beta]-actin mRNA, which cannot detect human counterpart and assessed how much contamination of rat tissues can influence the result
of RT-PCR assay and how to avoid the influence of the contamination in RT-PCR assay.


http://www.sciencedirect.com/science/article/B6T54-46VBNX-H/2/4ea4564931b07e68ac9d3b3b5739e30

Thymidine phosphorylase (TP) regulates intracellular thymidine metabolism. It has been reported to be a prognostic factor for tumor angiogenesis and to activate some prodrugs of 5-fluorouracil (5-FU) to 5-FU. There is also evidence that TP is induced by interferons (IFNs) and xenobiotics, such as cyclophosphamide and taxanes, in experimental human cancer cells and xenografts. We investigated the induction of TP expression by IFN[alpha] and Paclitaxel in vitro and in vivo in human tumor cells with low and with high TP activity. TP activity in KB, NUGC-3, and KOC2S cells, which had low TP activity, was increased 2 to 4 fold by IFN[alpha], but was still lower than in non-treated SHIN-3 and HRA cells, which have high TP activity. IFN[alpha] did not promote TP activity in SHIN-3 and HRA cells, but expression of TP mRNA increased 2 to 4 fold in response to IFN[alpha] in all cells tested. These results suggest that the expression of TP protein would be regulated post-transcriptionally by another factor after IFN-induced amplification of TP mRNA. A single dose of Paclitaxel to nude mice xenografted with KB and KM20C tumors, expressing low TP activity, increased TP activity about 4 to 7 fold compared to non-treated tumors. In contrast, TP expression in MX-1 and H-31 tumors was originally high and did not change by the treatment of Paclitaxel. The activities of uridine phosphorylase in all tumors used showed no changes in response to IFN[alpha] or Paclitaxel. We determined the level of STAT1[alpha], an IFN-inducible transcription factor of the TP gene, and found that it was low in low TP expressing tumor cells and markedly increased to about 4 fold by IFN, almost reaching the level in high TP expressing cells whose STAT1[alpha] level was unchanged by IFN. When TP activity and STAT1[alpha] expression in clinically resected colorectal cancers were simultaneously measured, almost all tumors had high expression of both TP and STAT1[alpha]. In conclusion, our results suggest that IFN and Paclitaxel affect human cancer cells with low TP activity but not those with high TP activity and that the STAT1[alpha] expression may reflect TP activity, at least in experimental human cancer cells.


http://www.sciencedirect.com/science/article/B6T54-46VBNX-P/2/4b1a766e66a3fae0a69d15fd2c3d4dd4

Interactions between recombinant mistletoe lectin (rViscum) and anticancer drugs were investigated in vitro. rViscum enhanced the cytotoxic effects of vincristine, mafosfamide, idarubicin and cisplatin in the human leukemia cell lines K562 and KG1a. In human marrow progenitor cells, rViscum inhibited colony formation and did not exert any protective activity against cisplatin-induced inhibition of clonogenicity. Quantitative real-time reverse transcription polymerase chain reaction analysis revealed that cisplatin treatment of K562 cells resulted in a 1.9-fold increase in mRNA expression of the nucleotide excision repair gene ERCC-1. This upregulation was not prevented when cells were post-incubated with rViscum. Our study provides evidence that rViscum is capable of enhancing cytotoxicity of anticancer agents in vitro. This synergism appears to be independent of transcriptional activity of DNA repair relevant genes.

http://www.sciencedirect.com/science/article/B6T54-4CVRM5X-2/2/d58cd723d2c33ca1bba0cc191c8343f6

Dysregulation of total estrogen receptor [beta] (ER[beta]) expression has been implicated in breast tumorigenesis. The ER[beta] gene yields five exon 8 alternatively spliced transcripts (ER[beta]1-5), which encode proteins with different C-terminal amino acids. Individual expression analysis of these transcripts may provide new insights into estrogen signaling in breast cancer. We measured mRNA levels of total ER[beta] and its five isoforms in normal tissues, breast carcinomas from post-menopausal patients, and breast cancer cell lines by means of real-time reverse transcription-polymerase chain reaction and fluorescent fragment analysis. In various normal human tissues, ER[beta]1-5 isoforms displayed different qualitative and quantitative expression patterns that were consistent with previous reports. Total ER[beta] mRNA levels were significantly lower in breast tumors than in normal breast tissues (38-fold lower, PER[beta]1 and ER[beta]2 (ER[beta]5 expression was similar in the two tissue types). This altered expression pattern of ER[beta] isoforms in breast cancer should be taken into account in future ER[beta]-based clinical applications.


The study was set out to determine whether characteristic changes in the gene expression profile in papillary thyroid carcinoma (PTC) discovered by microarray assays can be used for conventional molecular diagnosis. Expression levels of five reported to be overexpressed and three underexpressed genes were examined in PTC and normal human tissues by real-time PCR and semi-quantitative duplex PCR. Stepwise logistic regression analysis, duplex PCR data evaluation with recursive partition machine algorithm and hierarchical cluster analysis identified SFTPB (upregulated) and TFF3 (downregulated) gene combination as most favorable for differential molecular diagnosis of PTC. Sensitivity, specificity and accuracy obtained in a series of histologically characterized thyroid tumor and normal tissue samples were 88.9, 96.7 and 94.9%, respectively. Applicability of the method to fine needle aspiration biopsy (FNAB) samples was demonstrated using a collection of needle washouts. In spite individual thyroid tumor and normal tissues as well as FNAB samples displayed a substantial degree of variability in the expression levels of analyzed genes, simultaneous molecular analysis of a panel of optimal markers allows making a high probability predictive estimate and may be considered as an informative method of preoperative PTC diagnosis.


http://www.sciencedirect.com/science/article/B6T54-454HKD7-6/2/d91c9b68af197f35a8b0253655831de5

A case-control study was conducted to examine the possible association between digestive tract
cancers and p73 G4C14-to-A4T14 at exon 2 and p53 Arg72Pro polymorphisms in Japanese. Cases were 102 esophageal cancer patients, 144 stomach cancer patients, and 147 colorectal cancer patients, and controls were 241 non-cancer outpatients. The genotype frequencies among controls were 55.3% for p73 GG at position 4, 40.4% for GA, and 4.3% for AA, and 37.7% for p53 ArgArg, 44.4% for ArgPro, and 18.0% for ProPro. No significant differences in the genotype frequencies were observed between the controls and each case group or cases as a whole.


http://www.sciencedirect.com/science/article/B6T54-44XJ8WS-5/2/0d5de61fdeb2666d4116e7def100d51

Insulin-like growth factor binding protein-3 (IGFBP-3) is postulated to be a mediator of growth suppression signals. Reduced expression of the IGFBP-3 was observed in nine out of 12 human hepatocellular carcinomas (HCC) (75%). Promoter hypermethylation of the IGFBP-3 was detected in four out of 12 HCCs (33%) although mutations were not identified. The expression of IGFBP-3 was restored by the demethylating agent 5-aza-2'-deoxycytidine in HCC cell line with promoter hypermethylation (HepG2). As IGFBP-3 functions like a tumor suppressor gene, it may be used as a therapeutic target for HCC.


http://www.sciencedirect.com/science/article/B6T54-4D1DKSK-4/2/1edf3f202ba3a5b93c38733e1cd762ee

Alteration of the p16 tumor suppressor gene has been implicated as a critical lesion in the molecular pathogenesis of esophageal adenocarcinoma. The aim of this study was to characterize the spectrum of p16 alterations in surgically resected esophageal tissues, comprising histologically normal esophageal squamous and gastric epithelia, premalignant Barrett's epithelia, and associated esophageal adenocarcinomas, and to explore associations between p16 mRNA expression and p16 mutations, deletions, promoter hypermethylation, p16 protein expression, and clinicopathologic features for the same tissues. We have shown that while p16 mutations are uncommon (2%; 1/54), hypermethylation of the p16 promoter is detected in 43% (9/21) of histologically normal epithelia, in 77% (14/18) of associated Barrett's epithelia, and in 85% (18/21) of esophageal adenocarcinomas. However, p16 mRNA levels (relative to matched normal epithelia) were variable in Barrett's epithelia and adenocarcinomas, having no clear correlation with methylation status or other molecular and clinicopathologic parameters. These findings are consistent with a role for the p16 tumor suppressor gene early in the molecular progression of Barrett's epithelium to invasive esophageal adenocarcinoma, but do not support the notion that the detection of hypermethylation is systematically associated with low levels of expression.

To explore the relation of the MMP-1 1G/2G and MMP-3 5A/6A promoter polymorphisms with head and neck squamous cell carcinoma (HNSCC), DNA specimens extracted from peripheral blood cells of HNSCC patients and healthy controls were genotyped. The frequency of the MMP-1 2G/2G genotype was significantly higher in HNSCC patients (n=140) than in age- and sex-matched controls (n=223) (P=0.042; OR, 1.56). In the MMP-3 polymorphism, there was no significant difference in the genotype distribution between patients and controls. These data suggest that the MMP-1 promoter polymorphism may be associated with HNSCC.


Glutathione S-transferases (GSTs) M1 and T1 are known to be polymorphic in humans. Both polymorphisms are due to gene deletions, which are responsible for the existence of null genotypes. The gene defect of GSTT1 has been reported to be associated with an increased risk of myelodysplastic syndromes, astrocytoma and meningioma. A lack of GSTM1 was associated with tobacco smoke-induced lung and bladder cancer. In this study we examined whether the GSTT1 and/or GSTM1 homozygous null genotypes were associated with an increased risk of ovarian cancer using a multiplex polymerase chain reaction protocol. The GSTT1 null genotype was observed in 14% of the control subjects that had never suffered from neoplastic disease (n=115) and in 16% of the patients affected with ovarian cancer (n=103, OR 0.87, 95% CI 0.39-1.92, P=0.73). A lack of GSTM1 was observed in 38% of the control subjects and in 46% of the patients (OR 0.77, 95% CI 0.44-1.32). This difference was not significant (P=0.34). Similarly, no significant differences were obtained if GSTT1 and/or GSTM1 null genotypes were analyzed in subgroups of control subjects and ovarian cancer patients between the ages of 20-40, 41-70 and 71-90 years and in individuals with a positive family history of neoplastic disease. GSTT1 and/or GSTM1 null genotypes were not significantly associated with the histologic type and grade or FIGO (International Federation of Gynecology and Obstetrics) stages of the ovarian carcinomas. In conclusion, GSTT1 and/or GSTM1 null genotypes are not markers for an increased risk of ovarian cancer.


AIE-75 has been known as a 75-kDa autoantigen detected in the serum of autoimmune enteropathy (AIE) and as a colon cancer-related antigen, and now designated as a gene causative of Usher syndrome type 1C hereditary syndromic hearing loss. It binds to a novel putative tumor suppressor MCC2 that is homologous to MCC (mutated in colon cancer) through a PSD-95/Dlg/ZO-1 (PDZ) domain. To clarify the functional role in colon cancer cells, we transfected AIE-75 gene into SW480 colon cancer cells which do not express AIE-75. Expression of AIE-75 suppressed growth of SW480 cells in vitro in correlation with the expression levels. It was due mainly to G2/M phase cell cycle arrest associated with mitotic slippage, resulting in emergence of hyperploid giant-nucleated or multi-nucleated cells. Screening of proteins that
bound to PDZ domains of AIE-75 by a yeast two hybrid system showed that three
serine/threonine phosphatase catalytic subunits (PP2AC-[alpha], PP2AC-[beta], and PPP6C)
could bind to AIE-75. Since PP2AC is known to regulate G2/M checkpoint, we suggest that AIE-
75 interacts with PP2AC and prevent cells to transit mitotic phase.


http://www.sciencedirect.com/science/article/B6T54-4891BXV-
1/2/50397ddef4ce3163e0f4beb6e99289bf

We identified two novel deletion variants of the thymidylate synthase transcript in gastric cell
lines. Sequence analyses indicate that none of these variants results in introduction of a
premature stop-codon or a frame shifts. In 39 gastric cancer samples, both the full-length and
one-deletion variant messages were detected in cancerous as well as non-cancerous tissues.
However, another isoform was found in only seven of 39 cancerous tissues. Our results provide
important information to assist more detailed studies on the regulation of thymidylate synthase
activity.

Iizuka, N., S. Hazama, et al. (1999). "Interleukin-1 receptor antagonist mRNA expression and the

http://www.sciencedirect.com/science/article/B6T54-3WX1HTB-
7/2/94d0b9ed35a825898b6d1236200afa6

Interleukin-1 receptor antagonist (IL-1ra), an endogeneous inhibitor of IL-1, plays an
immunosuppressive role in vivo by blocking the proinflammatory effects of IL-1. In the present
study, we examined whether IL-1ra expression in human gastric carcinoma correlates with tumor
progression and/or metastatic potential. The reverse transcription-polymerase chain reaction was
used to compare the expression of the secreted form of IL-1ra (sIL-1ra) and the intracellular form
of IL-1ra (iCL-1ra) mRNA in tumor and corresponding benign tissue obtained from 38 patients
with gastric carcinoma. The incidence of sIL-1ra mRNA expression was significantly higher in
tumor (52%) than in corresponding benign tissue (18%) (P=0.002). On the contrary, iCL-1ra
mRNA was detected in all tumors and benign tissues. The expression of sIL-1ra mRNA by
malignant tissue correlated positively with both lymph node metastasis (P=0.008) and liver
metastasis (P=0.015). There was no association between tumor sIL-1ra mRNA expression and
other clinicopathologic factors. The degree of regional lymph node reaction, such as sinus
histiocytosis, in tumors expressing sIL-1ra mRNA was significantly weaker than that in tumors
without sIL-1ra mRNA expression (5/20 vs. 12/18, P=0.010). These results demonstrate that the
altered expression of sIL-1ra by malignant tissue may be related to the progression of gastric
carcinoma via modulating host immune response.


http://www.sciencedirect.com/science/article/B6T54-405KDJW-
2/2/4f2e94b688937e988b0be31030ed0

The molecular pathogenesis of various categories of breast cancer (BC) has been well described,
but surprisingly few reports have appeared on analysis of somatic mutations in bilateral BC. We have performed a polymerase chain reaction (PCR)-driven investigation of chromosomal regions showing common loss of heterozygosity (LOH) in 23 cases (46 tumors) from patients diagnosed with bilateral BC. LOH was observed in 15/46 (33%) informative tumors for chromosome 1p, 5/32 (16%) for 5q, 12/44 (27%) for 11q, 15/40 (38%) for 13q and 4/24 (17%) for 17p. These values are within the range of interlaboratory variations reported for unilateral BC. There was no strong evidence for concordance of LOH within the same patient for any of the chromosomal loci tested. Atypical for breast carcinomas, 7/46 (15%) tumors accumulated a high frequency (ranging from 11 to 29%) of shortened dinucleotide CA repeats, implying microsatellite instability (MI). Further analysis with the highly informative BAT-26 marker allowed for the classification of two of these tumors as having a replication error positive (RER+/MSI-H) phenotype, whereas the remaining five carcinomas harbored so-called borderline MI. Thus an involvement of both RER+ and borderline MI appears to be a distinct feature of bilateral breast carcinomas compared to unilateral lesions.


http://www.sciencedirect.com/science/article/B6T54-3WNVP3J-W/2/e02967d1c0173d86ce680f7c55a0b11

Telomerase activity has been reported in cancer cells after treatment with antineoplastic agents. Assessment of telomerase activity could be a valuable tool to measure the reduction of aggression caused by chemotherapy. This study was designed to investigate the significance of telomerase for chemotherapy with respect to Adriamycin (ADM)-resistance. MCF-7 and its ADM-resistant line (AdrR) were treated with ADM, 5-fluorouracil (5FU) or taxotere (TAXO). Telomerase activity and human telomerase RNA component (hTR) were quantitatively measured by the telomeric repeat amplification protocol assay and RT-PCR, respectively. Cell counting and MTT assay were also performed. In MCF-7, enzyme activity was significantly reduced by ADM and 5FU treatments. In AdrR, 5FU and TAXO reduced enzyme activity, while ADM significantly increased the activity. No significant changes in hTR were seen in these two cell lines after treatment with any of these drugs. When Bcl-2 expression was examined after drug treatments, ADM increased Bcl-2 expression in AdrR cells, while not changing it in MCF-7 cells. We conclude that an unusual reaction of telomerase activity in AdrR may explain, at least in part, one of the mechanisms of the malignant biological behavior related with the drug-resistance to ADM.


http://www.sciencedirect.com/science/article/B6T54-49CRJJ4-1/2/a6ff784d6d54772ecbe48b17a79bb1f8

We investigated the frequency of BRAF mutations in human pancreatic cancer specimens to determine its role in the development of pancreatic cancer. Nine pancreatic cancer samples without a K-ras codon 12 mutation and 19 with a K-ras mutation were included in the study. Analyses of the BRAF sequence revealed mutations in exon 15 (V599E) in two cases, both of which also exhibited a K-ras codon 12 mutation. No BRAF mutation was found in cases without a K-ras mutation. The BRAF V599E mutation was not found to be a major mutation in pancreatic cancers that had no K-ras codon 12 mutation.

http://www.sciencedirect.com/science/article/B6T54-4DHVY8P-2/2/fd06ba507d57b28db3cd4b4ba55f84e

In the present study, Tbx3, a member of the T-box family of transcription factors, was identified as an up-regulated gene by mRNA differential display in the regression (apoptosis) stage after uracil-induction of papillomatosis in the rat urinary bladder. Immunohistochemical analysis revealed that apoptosis cells are negative and apoptosis resistant cells are positive for Tbx3 expression. That suggests that Tbx3 is an apoptosis resistant gene rather than an apoptosis induced gene. We have found the rat bladder carcinoma cell line, BC31, to feature Tbx3 expression detectable by RT-PCR. To investigate its role in such cancer cells, they were transfected with an anti-sense Tbx3 expression vector. The obtained stable transfectant clones showed reduced expression of Tbx3 and much slower cell growth, as compared with mock transfectants, and many apoptotic cells were observed under normal culture conditions. These results indicate that Tbx3 is a negative regulator of apoptosis in bladder normal epithelial cells and suppression of Tbx3 expression causes inhibition of cell proliferation and induction of apoptosis in BC31, a rat bladder carcinoma cells.


http://www.sciencedirect.com/science/article/B6T54-3VYTYH5-F/2/aec1dfb4ce288309654e80c41cc91

Deletions on the short arm of chromosome 9 (9p21 region) have been reported in a number of hematopoietic and solid tumors. These aberrations on 9p have been previously associated with the loss of the interferon gene cluster and the gene for methylthioadenosine phosphorylase (MTAP), localized to the 9p21-22 region. Recently, two putative tumor suppressor gene(s) CDKN2 and MTS2 have been mapped to the 9p21 region, and shown to be deleted in a large number of tumors including leukemias, melanomas, bladder cancers and brain tumors. We have previously reported a similar 9p21 abnormality and deletions of the CDKN2 and MTS2 genes in a myxoid chondrosarcoma cell line and its subclones. In this study we report consistent abnormalities of chromosome 9 in additional chondrosarcomas examined by a detailed cytogenetic and molecular analysis. Seven chondrosarcoma cell lines, one primary chondrosarcoma, and a benign chondroma were examined. Four of the seven tumor cell lines examined showed grossly visible aberrations of chromosome 9. Molecular analysis of these chondrosarcoma cell lines revealed hemizygous deletions of the interferon genes, and the absence of the MTAP gene, protein or activity. In addition, four of the seven chondrosarcoma cell lines also showed deletions of the CDKN2 and/or MTS2 putative tumor suppressor genes, or the absence of the CDKN2 protein product. No such chromosome 9 related aberrations were detected in the benign chondroma. These data suggest that chromosome 9p21 abnormality, and deletions of the CDKN2 and MTS2 tumor suppressor genes may be a significant event in the development of chondrosarcomas.

Recently, cloning of the gonadotropin-releasing hormone (GnRH) receptor from the human breast tumor cell line (MCF-7) and from an ovarian tumor, and its expression in various other human tumors, tumor cell lines and reproductive organs have been reported (Kakar et al., Mol. Cell. Endocrinol., 106 (1994) 145-149). In the present studies, we investigated the expression of GnRH and GnRH receptor mRNAs in normal human non-reproductive tissues. Using reverse transcriptase-polymerase chain reaction (RT-PCR) techniques and specific oligonucleotide primers derived from the placental GnRH cDNA sequence, PCR products of the expected size were obtained from human liver, heart, skeletal muscle, kidney, placenta, and pituitary. The authenticity of the PCR products was confirmed by Southern blot analysis with an internal oligonucleotide primer as probe. Similarly, using specific oligonucleotide primers for the GnRH receptor selected from the human pituitary GnRH receptor cDNA sequence, PCR products of the expected size were amplified from human liver, heart, skeletal muscle, kidney, placenta, and pituitary, and these strongly hybridized with the human GnRH receptor cDNA on Southern blot. Cloning and nucleotide sequencing of the PCR products for the GnRH and GnRH receptor from heart revealed identical sequences when compared to the human placental GnRH and pituitary GnRH receptor cDNAs, respectively. These data demonstrate for the first time the existence of GnRH and GnRH receptor mRNAs in normal human non-reproductive tissues and suggest that GnRH and its receptor may play an important role in the regulation of cellular functions in an autocrine or paracrine manner, in addition to regulating the secretion of gonadotropins from the anterior pituitary.


Infection of high-risk human papillomaviruses (HPVs), particularly the HPV types 16 and 18 and mutation or aberrant expression of the p53 tumour suppressor gene, has strongly been implicated in human esophageal carcinoma, which shows a great variation in geographic distribution. Neither the reason(s) for such a variation nor the etiopathogenesis of the disease is clearly understood. The present study has been carried out to determine prevalence of high-risk HPV types 16 and 18 and the p53 gene mutation in patients from three distinctly different endemic geographic regions of India, viz. Kashmir, Dibrugarh, and New Delhi where esophageal cancer is most prevalent. The people from each of these regions differ considerably in their food, drinking, smoking and chewing habits (tobacco and betel nut) and ethnic background. While PCR was employed to detect high-risk HPV types 16 and 18 DNA sequences, PCR-SSCP and direct nucleotide sequencing was used for analysis of p53 mutation. Out of a total of 101 biopsy specimens of carcinoma esophagus analysed, the frequency of HPV was found to be the highest 14/32 (44%) in Dibrugarh followed by 33% (11/33) in Kashmir, but, interestingly, no high-risk HPV could be detected in New Delhi patients who showed the highest frequency (30.6%) of p53 mutation as against only 12.5% in Dibrugarh and 6.1% in Kashmir. The difference in the frequency of p53 mutation between the three regions was statistically highly significant (0.018). Out of a total of 21 nucleotide alterations observed, 12 missense, five frameshift and four were silent changes. The p53 exon 7 appears to be the 'hot-spot' for esophageal cancer as it alone was responsible for more than 76% (13/17) of mutations and more than 95% (20/21) of the patients with p53 mutation were smokers. The results demonstrate differential distribution of HPV infection and p53 mutation in esophageal cancer from different geographic regions of India and this could be due to variation in diet, drinking, and tobacco habit, including ethnic, socio-cultural and genetic variation.

http://www.sciencedirect.com/science/article/B6T54-44MGNKR-1/2/bb193e297ea6b69a47732a278f93af27

Matrix metalloproteinases (MMPs) have been implicated in tumor invasion, metastasis, and angiogenesis. We have recently shown that MMI-166, a new orally active MMP inhibitor specific for MMP-2 and -9, suppressed experimental metastasis of Lewis lung cancer, C-H1 human colon cancer, and pancreatic cancer without affecting tumor growth in vitro. In the present study, we determined whether oral administration of MMI-166 reduces tumor growth not only in such tumors but also in squamous cell carcinoma of head and neck (SCCHN). MMI-166 inhibited both activity of MMP-2 and -9 without affecting steady state levels of their mRNAs in SCCHN. Interestingly, protein levels of MMP-2 and -9 from the cultures were drastically diminished by culturing with MMI-166. This was also observed in xenografts of MMI-166-administered mice. In addition, daily oral administration of MMI-166 (100 mg/kg) inhibited local tumor growth accompanied by the reduction of blood vessel density and Ki-67-positivity and increase in terminal deoxynucleotidyl transferase-mediated cUDP nick-end labeling (TUNEL)-positivity. These results suggested that orally administered MMI-166 reduced in vivo tumor growth of SCCHN through inhibition of angiogenesis and induction of apoptosis accompanied by the reduction of MMP productions and activities. Therefore, MMI-166 seems to be useful for tumor dormancy therapy of SCCHN.


http://www.sciencedirect.com/science/article/B6T54-4C8NR8F-1/2/19c0681856a28930cea660db1ac6883e

Mutations in the p53 tumor suppressor gene are frequent in breast tumors but the implication of p53 mutations in breast cancer development remains poorly understood. In this study, we applied laser capture microdissection (LCM) microscope to histologically review and sample cells from paraffin-embedded breast tissue sections obtained from six cases of ductal carcinoma in situ (DCIS) and ten cases of atypical ductal hyperplasia (ADH). p53 mutations were detected, using single stranded conformational polymorphism (SSCP) and sequencing, in cell samples of three cases with DCIS and five cases with ADH. p53 mutations are therefore present in DCIS and ADH of the breast, considered as pre-malignant precursors to breast cancer, and some of them may represent early events in breast cancer development.


http://www.sciencedirect.com/science/article/B6T54-4423P0J-6/2/a4595c9563d4686fa0bf0694f346a51e

The minor variant frequency of a HER2 polymorphism (HER2V655) has been determined for 471 United States women enrolled in a multiracial case-control study. Allelic frequencies varied significantly by race. Genotypic distributions showed no excess breast cancer risk associated with
inheritance of HER2V655 either as carriers (OR=1.2, 95% CI=0.8-1.9), heterozygotes (OR=1.2, 95% CI=0.8-1.9), or homozygotes (OR=1.4, 95% CI=0.4-4.2). Nor was there a significant association when each racial group was considered separately. The current study suggests the HER2V655 allele is not a breast cancer risk factor for Caucasians, African-Americans, or Latinas.


Genomic deletions of the short arm of chromosome 8 are common in many human cancers and are frequently associated with a more aggressive tumour phenotype. One of the regions of loss of heterozygosity (LOH) on 8p22 identified in bladder cancer contains two genes, LZTS1 (FEZ1) and DBC2 (RHOBTB2) that have been shown to be mutated at low frequency in other cancers. We screened a panel of bladder tumours and bladder tumour-derived cell lines for mutations in these genes. Forty two percent of the tumours were found to have LOH in the 8p22 region and many of the cell lines have known loss of 8p. Several known polymorphisms and novel polymorphisms were detected. One possible mutation of LZTS1 (G374S) was found in a cell line. The functional significance of this is unknown but the novel serine residue created may represent a novel phosphorylation site. In DBC2, we found a single somatic mutation in a tumour (E349D) that lies in a highly conserved region of the protein. mRNA levels for both genes were reduced in the majority of bladder cancer cell lines. We conclude that neither LZTS1 nor DBC2 is commonly mutated in bladder cancer. However, neither can yet be excluded as the target of 8p22 LOH. The finding of a somatic mutation of DBC2 in a tumour sample and the down-regulation of both gene transcripts in bladder tumour cell lines may indicate that an alternative mechanism of inactivation of the second allele, for example promoter hypermethylation, is more common than mutation and this must now be examined.


http://www.sciencedirect.com/science/article/B6T54-3WBWV3F-7/2/ff49234a76b409594cd0ba9504329fa8

Sulfatide is a major acidic glycolipid in human gastric mucosa, and its sulfation is catalyzed by cerebrosyl sulfotransferase (CST). To investigate the expression of the CST gene in human gastric cancer, a reverse transcription PCR method was developed with the use of endoscopic biopic specimens. By this method, we examined the CST mRNA expression in 11 cases of gastric cancer, and in all the cases we detected various levels of the expression both in cancer tissues and in uninvolved adjacent tissues. The present assay method was suggested to be useful in the detection of CST mRNA from a limited amount of biopic samples.


http://www.sciencedirect.com/science/article/B6T54-40MT23J-B/2/4de11ac19e1b94c8bab99904ea543ef3

A two-step strategy was developed consisting of differential display reverse transcriptase
polymerase chain reaction (DDRT-PCR) with cultured normal human fetal astrocytes and U-373MG glioma cells followed by reverse Northern analysis of normal brain and primary tumor tissues. hu-dek, [alpha]-NAC, ribosomal proteins L7a and L35a, and five novel genes were identified. Since none of these genes has been previously shown to be associated with malignant brain tumor formation, this approach may be useful to identify novel targets for the diagnosis and treatment of brain tumors.


http://www.sciencedirect.com/science/article/B6T54-47T2SM3-6/2/420d91945b2c0f343802a32da89dd66

Genetic alterations in 28 non-small cell lung carcinoma patients were detected on chromosomes 13q and 14q with microsatellite markers by polymerase chain reaction techniques. Loss of heterozygosity of up to 50% was detected with chromosome 13 markers and of up to 37% for chromosome 14. Microsatellite instability was as high as 30% on chromosome 13 and up to 19% on chromosome 14. Accumulated mutation frequencies of up to 94 and 93% were observed for chromosomes 13 and 14, respectively. Of eight tumors displaying high mutation frequencies, 1 also carried a K-ras mutation and 4 had p53 mutations. A significant association was observed between p53 mutations and genetic instability.


http://www.sciencedirect.com/science/article/B6T54-490MTMB-9/2/0bb97467a2b57215ecc99c6d5a89dd42

To evaluate the potential association between NAT1/NAT2 polymorphisms and breast cancer, a case-control study was conducted in Korean women (254 cases, 301 controls). NAT1 *4/*10 genotype (42%) was the most common NAT1 genotype in this Korean population. The frequencies of slow, intermediate and rapid NAT2 acetylator genotype were 16, 39 and 44% in cases and 16, 42 and 42% in controls. Neither NAT1 rapid ( homozygous or heterozygous NAT1 *10) (OR=1.2, 95% CI=0.8-1.9) nor NAT2 rapid acetylator genotype (OR=1.2, 95% CI=0.8-1.7) showed significant association with breast cancer risk. Although the risk of NAT2 rapid acetylator genotype in postmenopausal women (OR=1.4, 95% CI=0.7-2.8) was higher than that in premenopausal women (OR=1.1, 95% CI=0.7-1.7), those were not statistically significant. However, combinations of NAT1, GSTM1 and GSTT1 genotypes showed a significant linear gene-dosage relationship with breast cancer (p for TREND=0.04) and those women with NAT2 rapid acetylator and both GSTM1 and GSTT1 null genotypes were at the elevated risk (OR=3.1, 95% CI=1.0-9.1). These results suggest that genetic polymorphisms of NAT1 and NAT2 have no independent effect on breast cancer risk, but they modulate breast cancer risk in the presence of GSTM1 and GSTT1 null genotypes.


http://www.sciencedirect.com/science/article/B6T54-4BYR971-1/2/45f9b4017b459c942d592ff01cbad525
Loss of heterozygosity (LOH) in the 10q21 region that harbors the tumor suppressor gene ANX7-GTPase gene have been found in 35% of prostate tumors. Therefore, the rationale for this study is that this gene could also be implicated in breast pathogenesis as well. We investigated allelic losses in microsatellites of the 10q21 region, and their correlations with ANX7 status, estrogen receptor (ER) status, progesterone receptor (PR) status, Ki-67 status and pathological phenotype in 30 breast carcinomas with matched control specimens. The LOH analysis was performed by amplifying DNA by PCR, using four markers of the 10q21 region (AFMa299ya5, AFM220xe5, AFM 063xc5, AFM200wf4). LOH in at least one marker of the 10q21 region (AFM220xe5 marker close to ANX7) was found in 66% of the first set of informative tumors containing 10 pairs of specimens. Subsequent comparison between 20 carcinomas using AFM220xe5, with and without LOH in terms of pathological parameters showed significant associations with differences in age (P=0.04), ER (P=0.05), Ki-67 (P=0.04) and PR (P=0.01); a trend toward significance was found for tumor size (P=0.06) and histological grade III (P=0.14). These results suggest that the ANX7 gene, or other genes of the 10q21 region, could be functionally related to breast cancer, probably influencing the hormone receptor expression associated with poor prognosis during development.


http://www.sciencedirect.com/science/article/B6T54-4B4S3VT-1/2/8c2c8ecc537f67a06f3741ab17af270fe

Multiple and extensive alterations in chromosome 9 were detected in thirty-four esophageal squamous cell carcinoma patients, using seventeen polymorphic markers localized to chromosome 9 to detect the loss of heterozygosity (LOH) by polymerase chain reaction techniques. The LOH rates detected in this study range from 42.9 to 80.0%. Three commonly deleted regions mapping to 9p23-p22, 9q13-q22.3, and 9q34 were observed. D9S1812 LOH at 9q22.1 was significantly associated with well- and moderately-differentiated tumors; LOH at D9S768, mapping to 9q13-21.3, indicated that drinking habits are not a significant risk factor for Chinese esophagus cancer. Interestingly, no case of microsatellite instability was observed.


http://www.sciencedirect.com/science/article/B6T54-41MJ006-4/2/fe1f87b2524a2040f3428817c4d29e6

Comparison of LCC2, the E2-independent, tamoxifen-resistant subline of the MCF-7 human breast cancer cell line with its parent line, disclosed that it is more resistant to growth inhibition and apoptosis induction by a variety of agents acting by diverse mechanisms. Thus, LCC2 cells can serve as a useful in-vitro model for the study of the molecular mechanisms of this resistance. It was found that bcl-2 protein and mRNA were elevated and that bax protein and mRNA were reduced in LCC2 compared with MCF-7 cells. Incubation of both lines in the presence of bcl-2 antisense caused growth inhibition and reduced bcl-2 protein levels only in MCF-7 cells, suggesting the involvement of bcl-2 in the regulation of normal growth of breast cancer cells. Increased bcl-2 expression in breast cancer cells may correlate with their resistance to growth inhibitory agents. Bcl-2 is a useful target for enhancing the effects of growth inhibitory agents.

Esophageal squamous cell carcinoma (ESCC), with a 5 year survival below 15%, is one of the most common fatal cancers worldwide. Significant reduction in mortality may be achieved by detecting and treating asymptomatic precursor lesions and curable early cancers. To explore this possibility and look for potential early detection markers, we examined alterations in 16 microsatellite markers in laser capture microdissected (LCM) endoscopic biopsies from the esophagus, including 15 dysplasias and 22 ESCCs, in patients from Shanxi Province, a region in north-central China. We found a significant increase in the total frequency of allelic loss with increasing disease severity. Allelic loss was seen in 2% of the markers in patients with low grade dysplasia (LGD), 15% of the markers in patients with high grade dysplasia (HGD), and 35% of the markers in patients with ESCC. Ten different markers (D3S4513, D5S2501, D8S1106, D9S118, D9S910, D13S1493, D13S894, D13S796, D15S655, and D17S1303) showed allelic loss in one or more of the premalignant lesions tested. The frequency of microsatellite instability (MSI) also increased with histological severity, from 22% in LGD to 33% in HGD and 59% in ESCC. These results indicate that the development of ESCC is associated with genetic instability, that this instability can be detected in endoscopic biopsies of recognized precursor lesions in patients without invasive cancer, and that these markers may be useful as predictive markers in the early detection of ESCC. Finally, we also report methodologic/technical modifications that enhance the use of LCM for screening endoscopic biopsies.
instability with malignant potential of head and neck cancer.


http://www.sciencedirect.com/science/article/B6T54-48YW1WX-1/2/ca27cb1e58a7e83324d8d30837a27398

Mutation of the class I [beta]-tubulin gene has been reported to be one of the mechanisms that cause resistance to paclitaxel. To assess the relationship between paclitaxel-resistance and class I [beta]-tubulin gene mutation in breast cancer, Japanese patients with breast cancer were screened for the class I [beta]-tubulin gene mutation. Total RNA was isolated from 82 breast cancer specimens and the corresponding normal tissues. Twenty-four of the 82 patients were treated with paclitaxel preoperatively and 12 of them did not respond to the treatment. Of the 82 breast cancer patients, 15 (18.3%) had silent polymorphism in exon 4, Leu217Leu (CTG/CTA). However, no mutations showing amino acid substitution of the [beta]-tubulin gene were detected in any of the patients, including 12 patients who did not respond to paclitaxel. Class I [beta]-tubulin gene mutation with amino acid substitution was not detected in 82 breast cancer specimens. Our results suggest that mutation of the class I [beta]-tubulin gene is unlikely to play an important role in the mechanism of resistance to paclitaxel in breast cancer.


http://www.sciencedirect.com/science/article/B6T54-4BY9WGH-36B/2/1c8f458cea0bac327bf5215c55a1e950

Enhanced c-erbB-2/neu expression has been linked with a poor prognosis in human bladder cancer. Previous reports have shown that a point mutation at nucleotide T2012 in the coding region of the transmembrane domain of the rat gene is sufficient to confer transformation potential on this gene. We examined the comparative levels of p185neu as well as the sequence around the hotspot (T2012) of the neu gene of rat bladder cells transformed by 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT) or established in culture from N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamidine (FANFT)-induced rat bladder tumors. We concluded that increased p185neu expression did not correlate significantly with tumorigenicity. No alterations in nucleotide sequences of the neu gene were observed in either in vitro model.


http://www.sciencedirect.com/science/article/B6T54-4BY9WF7-35T/2/6f01f26de23279f15d675d3513515e9f

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http://www.sciencedirect.com/science/article/B6T54-3T87G2T-9/2/260a5914c1d0ba8e8e5f90332311da3d

Male and female transgenic mice carrying the human prototype c-Ha-ras gene (rasH2 mice) and their wild littermates (non-Tg mice) received three subcutaneous injections of 0.3 mg N-methyl-N-nitrosourea (MNUR) once every 2 weeks for the first 4 weeks followed by a single intraperitoneal injection of 1000 or 0 mg/kg urethane (UR) 2 weeks later. They were then maintained without any other treatment for a further 13 weeks and sacrificed for assessment of pulmonary pathology. Inflammatory lesions, such as macrophage infiltration, alveolar bronchiolization and/or fibrosis, were induced in both rasH2 and non-Tg mice treated with MNUR or MNUR+UR. Lung proliferative lesions were induced in 100% of the UR-treated rasH2 mice but to a significantly lesser extent in the MNUR+UR case. The incidences of lung tumors in non-Tg mice treated with UR or MNUR+UR were relatively low. Point mutations of the transgene were detected in approximately 80% of lung tumors in rasH2 mice treated with UR and MNUR+UR, but murine Ki-ras mutations were rare. No marked difference in the mutation pattern was found between the UR-treated and the MNUR+UR-treated rasH2 mice. In non-Tg mice treated with UR or MNUR+UR, point mutations of the murine c-Ki-ras gene were observed in about 50% of the lung tumors examined. The present study confirmed that rasH2 mice are very sensitive to lung tumor induction by UR and suggested that alveolar epithelial cells in the reparative stage during pulmonary fibrosis are resistant to DNA damage by this carcinogen.


http://www.sciencedirect.com/science/article/B6T54-44XJ8WS-D/2/b8ffdfbee03f4286042324eb419f6101

Actinic keratoses (AKs) are pre-neoplastic lesions that can develop into squamous cell carcinomas (SCCs) of the skin. Often AK and SCC have commonly altered p53. A status of another tumor suppressor, the p16INK4a, was reported for SCC but not for AK. A comparative study of SCC and AK human samples by loss of heterozygosity (LOH) analysis determined that the p16INK4a/ARF locus is less frequently altered in AKs than in SCCs. These LOH data highly correlated with immunohistochemical findings demonstrating the presence of p16INK4a in the AK skin samples but its absence in SCC lesions. Our results imply that progression of AK into SCC may involve inactivation of p16INK4a.

We examined expression and function of osteopontin (OPN) in oral cancer cell lines using antisense oligonucleotide (AS). Quantitative real-time RT-PCR showed that expression in BSC-OF cells was significantly higher (10-fold) than that in KB cell. AS-study showed that foci of AS-treated BSC-OF cells possessed thin processes and radiated morphologically, although BSC-OF cells showed round foci. Cell growth in AS-group was lower (P<0.01). These results suggest that BSC-OF cell is useful for over-expression of OPN, and that OPN contributes to morphology, growth and invasion.


Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the incidence of colorectal carcinoma. We now report that the potent cyclooxygenase-1 inhibitor indomethacin had no effect on the growth of human colorectal carcinoma cell lines in vitro at concentrations up to 30 [μM]. The selective cyclooxygenase-2 inhibitors L-745337 and NS-398 reduced cyclooxygenase activity, but had no effect on cell growth at concentrations as high as 100 [μM]. Our results provide direct evidence that inhibition of cyclooxygenase activity does not necessarily inhibit the growth of colorectal carcinoma cell lines and imply that the growth-inhibitory effects of NSAIDs in vitro are not mediated by inhibition of cyclooxygenases.


In human cancer, alterations in the p53 tumor suppressor gene are the most common genetic alterations. The aim of the present study was to detect sensitivity of the p53 (+/-) mice and their littermates p53 (+/+ ) mice to N, N-dibutylnitrosamine (DBN) carcinogenicity. In experiment 1, 6-7-week-old p53 (+/-) and p53 (+/+ ) mice were treated with 0, 0.025 and 0.05% DBN, respectively, in drinking water for 20 weeks. Esophageal squamous cell and urinary bladder transitional cell carcinomas (TCCs) and fibrosarcomas were found to be significantly increased in p53 (+/-) mice treated with doses of DBN compared to p53 (+/+ ) mice administered similar doses. In experiment 2, 6-7-week-old p53 (+/-) and p53 (+/+ ) mice were administered 0 or 0.05 % DBN in drinking water for 8 weeks. Immunohistochemical staining revealed a significant increase in numbers of p53 and bromodeoxyuridine (BrdU) positive cells in the esophageal and urinary bladder epithelia of DBN-treated p53 (+/-) mice compared to p53 (+/+ ) mice administered DBN. Molecular analysis revealed point mutations in the residual p53 allele in four of eight (50%) esophageal mucosa of DBN-treated p53 (+/-) mice, and in three of eight (38%) of treated p53 (+/+ ) mice. The results show that p53 (+/-) mice were sensitive to DBN treatment with respect to esophageal and bladder tumor development, with a mechanism that could be confined to early mutations of the residual p53 allele and increased cellular proliferation in the target organs.

To examine the possible association between cervical cancer and p73 G4C14-to-A4T14 in exon 2 and p53 Arg72Pro polymorphisms, an incident case-control study was conducted in Japanese. The cases were 112 cervical cancer patients. Controls were 320 healthy women and 122 non-cancer female outpatients. Risk estimation for each genotype by an unconditional logistic model demonstrated a possible association between the p73 AA genotype and the risk of cervical cancer in our Japanese population (OR=1.57; 95% CI, 0.99-2.48, P=0.053). There was no significant difference in the p53 Arg72Pro genotype frequency between the controls and cases.


To test the association of endometrial cancer with the p73 G4C14-to-A4T14 polymorphism in exon 2 and the p53 Arg72Pro polymorphism, an incident case-control study was performed in Japanese subjects. The cases comprised 114 endometrial cancer patients, and the controls were 320 healthy females and 122 noncancer female outpatients. An unconditional logistic regression model demonstrated a significant association between the p73 AA genotype and an increased risk of endometrial cancer (OR=2.82, 95% CI=1.36-5.82), especially of type-I tumors (OR=3.24, 95% CI=1.53-6.87). In contrast, there was no significant difference in the p53 Arg72Pro genotype frequency between the controls and cases.


To examine the possible association between cervical cancer and Lymphotoxin-[alpha] (LT[alpha]) polymorphisms, C804A and A252G, an incident case-control study was conducted in Japanese. The cases were 131 cervical cancer patients. Controls were 320 healthy women. Risk estimation was conducted by an unconditional logistic model. Complete linkage disequilibrium was seen between LT[alpha] C804A and LT[alpha] A252G. We found that, compared with the 804CC genotype, 804CA and 804AA were associated with a decreased risk of cervical cancer (OR=0.64, 95% CI=0.40-1.02; and OR=0.45, 95% CI=0.21-0.95, respectively), especially of SCC (OR=0.54, 95% CI=0.32-0.91; and OR=0.39, 95% CI=0.16-0.92, respectively).


http://www.sciencedirect.com/science/article/B6T54-4066SCT-
We attempted to suppress glucose transporter 1 (GLUT1) expression by transfecting MKN45 cells with cDNA for antisense GLUT1. Glucose transport was significantly decreased in cells with antisense GLUT1 compared with wild-type cells or cells with vector alone. Suppression of GLUT1 mRNA resulted in a decreased number of cells in the S phase. This was accompanied by overexpression of p21 protein. Tumorigenicity in the nude mice injected with antisense GLUT1 expressing cells was significantly slower than in those with wild-type MKN45 cells. These results suggest that antisense GLUT1 mRNA inhibits tumor growth through a G1 arrest and that expression of antisense GLUT1 mRNA via gene therapy can be used as a tool in the treatment of cancer.


http://www.sciencedirect.com/science/article/B6T54-3WMJT68-9/2/5f27acb8f6de408eb1ead7904a7775b

The expression of the insulin-responsive glucose transporter (GLUT) 4 was studied in three histologically different human gastric cancer cell lines, MKN28, MKN45, and STSA. RT-PCR demonstrated GLUT1 and GLUT4 mRNA in all three cell lines. MKN28 cells expressed GLUT4 protein more than MKN45 and STSA cells by immunohistochemistry. Insulin stimulation of MKN28 cells resulted in a 22% increase in glucose uptake over that found under basal conditions (0.60+/-.05 fmol/cell per min after insulin stimulation versus 0.53+/-.07 fmol/cell per 3 min at basal). No increase in glucose uptake occurred with insulin stimulation in MKN45 or STSA cells. We conclude that the insulin responsive GLUT4 is expressed in MKN28, MKN45, and STKM1 human gastric cancer cell lines, albeit in different amounts. The greater expression of this transporter in MKN28 cells is likely responsible for the cell's ability to increase glucose uptake with insulin stimulation. However, the role played by GLUT4 in regulating the amount of glucose uptake would not be large in those human gastric cancer cell lines.


http://www.sciencedirect.com/science/article/B6T54-4BY9SD8-1YJ/2/007d8128501e8791c85beb7f2dd99582

Since muramyl dipeptide (MDP) was recognized as a potent monocyte/macrophage activating agent, many MDP analogues were synthesized and tested for their ability to augment the host immune defence system against neoplasms. This study was performed to determine whether the newly synthesized desmuramyl N-acyl dipeptides LK 409 and LK 410 were also capable of affecting the immune system. For this purpose, the peritoneal macrophages were incubated in vitro with these two agents and TNF-[alpha] production was measured. In addition, the effect of LK 409 and LK 410 on TNF-[alpha] and IL-1 RNA levels in in vivo stimulated macrophages was determined by quantitative polymerase chain reaction (RT-PCR). None of the LK 409 and LK 410 concentrations tested were able to render macrophages in vitro to excrete a detectable amount of TNF-[alpha] in the supernatant fluid. However, the TNF-[alpha] and IL-1 RNA levels in macrophages of in vivo treated mice (C57Bl/6) were increased in comparison to mock-treated mice. The results indicate that LK 409 and LK 410 are capable of inducing an increase in TNF-[alpha] and IL-1 RNA levels, yet in vitro TNF-[alpha] production remains under detectable levels.

Incidence of polymorphisms on exon 4 (CGC vs. CCC, p53Arg vs. p53Pro, A2 allele vs. A1 allele at codon 72, respectively) of the p53 gene was compared in 75 cases with hepatitis C virus (HCV) infection and 232 noninfected control subjects in a defined geographical area in Japan. Polymorphism was analyzed by the polymerase chain reaction-single strand conformation polymorphism method using DNA from peripheral blood leukocytes. When all cases and controls were compared, there was no significant correlation between hepatitis C virus (HCV) infection and the p53 polymorphism in question. However, when male cases infected with HCV type 1b, the most common viral genotype, were compared with controls matched by sex and age, significantly higher homozygotes for p53Pro were found in cases compared with controls (P=0.039). Significantly higher allelic frequency of this polymorphism was also observed with cases (P=0.010). We found no significant statistical difference between the p53 polymorphism and other genotypes of HCV (2a, 2b and others). On the basis of our study we believe there exists a significant correlation between male homozygotes for p53Pro with HCV type 1b infection.


Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in the folate metabolism, which affects DNA synthesis and methylation. Low enzyme activity may reduce the capacity of DNA methylation, and possibly reduce uracil misincorporation into DNA, which can result in double strand breaks. Both processes may be critical for the oncogenic transformation of human cells. Two common amino acid-changing and enzyme activity-reducing nucleotide polymorphisms (677C->T/Ala222Val and 1298A->C/Glu428Ala) have been described in MTHFR. We performed estimations of the relative risk associated with these two polymorphisms in samples from 287 colorectal cancer patients, compared to 346 healthy controls. Relative risk were further determined for subpopulations of cancer patients having sporadic (n=227) or suspected/verified hereditary disease (n=60) and tumours exhibiting high-level microsatellite instability (n=41) or not (n=246). No significant differences for the relative risk of colorectal cancer were observed for the MTHFR genotypes either alone or in combination in the analysed cohorts, although the frequency of the 1298AA+AC genotypes was increased among the 60 cases with hereditary disease. Whereas our results do not support an association of high enzyme activity and increased risk of colorectal cancer in general, we can not exclude an association of patients with hereditary disease and the MTHFR 1298A->C variant.

Previous studies have established that chronic dietary insufficiency of the lipotropic nutrients choline and methionine with or without chemical initiation is hepatocarcinogenic in the rat and certain mouse strains. In the present study, the folate/methyl-deficient model of multistage hepatocarcinogenesis was used to evaluate progressive in vivo changes in p16 promoter methylation in both preneoplastic and tumor tissues. Previous studies using this model have demonstrated stage-dependent alterations in genome-wide and p53 gene-specific methylation. In the present study, we used highly sensitive methylation specific PCR (MSP) to determine time of appearance of methylated sequences within p16 promoter. In addition, methylation-sensitive single nucleotide primer extension methodology was applied to determine methylation status of the remaining CpG sites within amplified methylated alleles. Using this approach, extensive methylation in p16 promoter was found in 100% of tumors, but the pattern of methylation varied depending on tumor type. The incidence and extent of de novo methylation in the CpG island of the p16 promoter increased with tumor progression. To further explore the evolution of p16 gene hypermethylation, we examined the appearance and progression of site-specific de novo methylation during early preneoplasia. Our data show that site-specific de novo methylation of 5′ CpG island of p16 gene precedes tumor development and undergoes dynamic expansion during tumor progression.


Functional inactivation of tumor suppressor genes during tumor progression has been shown to occur by either coding region mutation or promoter region methylation. Because of the functional equivalence of these two mechanisms, loss of tumor suppressor function generally occurs by one or the other mechanism, but rarely by both. Aberrant de novo methylation in most tumor suppressor promoter regions is found within CpG islands that occur near the transcription start site. The p53 promoter region is unique in that it does not contain a CpG island and therefore it is possible that methylation at critical CpG sites may be more important in gene silencing than total CpG methylation density. Other than site-specific aflatoxin B1-induced mutations, p53 coding region mutations are not frequently observed in most human primary hepatocellular carcinomas. In the present study, paired samples of human primary liver carcinoma and uninvolved tissue obtained from the same individual were evaluated for site-specific p53 promoter methylation status by methylation sensitive single nucleotide primer extension (Ms-SNuPE) and also for coding region mutations using polymerase chain reaction (PCR)- single strand conformation polymorphism (SSCP). The methylation pattern in the uninvolved tissue was variable at specific CpG sites, whereas the same sites had become highly methylated in tumor tissue from the same individual. Associated with de novo methylation, the level of p53 mRNA was significantly reduced in the tumor DNA relative to the uninvolved tissue DNA. None of the samples exhibited coding region mutations. Given that p53 mutations are rare in primary human liver tumors, these data suggest that transcriptional repression by p53 promoter methylation may contribute to tumor progression.

Chronic dietary methyl deficiency in F344 rats was used as an in vivo mammalian model in which to evaluate the genespecific alterations in DNA methylation patterns during multistage hepatocarcinogenesis. Using bisulfite mapping, the sitespecific ethylation profile within exons 6-7 of the 53 gene was determined in control liver, preneoplastic nodules (after 36 weeks of folate/methyl deficiency) and in hepatocellular carcinoma (after 54 weeks of deficiency). A progressive loss of methyl groups was observed at most CpG sites on both coding and non-coding strands during the first 36 weeks of folate/methyl deficiency, with the greatest loss occurring on the coding strand. When the same sequence was evaluated in tumor DNA after 54 weeks of deficiency, the majority of cytosines were unexpectedly found to have become remethylated. CpG sites that had previously lost methyl groups on both strands during preneoplasia as well as CpG sites that had been constitutively non-methylated, had undergone de novo methylation in tumor DNA. Maintenance methyltransferase and de novo methyltransferase activity in nuclear extracts were assessed using hemimethylated and non-methylated DNA substrates, respectively. In tumor, de novo methyltransferase capacity was increased ~4-fold relative to control or preneoplastic liver and associated with a relative increase in both p53 and genome-wide methylation density. In the preneoplastic nodules, the level p53 mRNA was increased and associated with hypomethylation in the coding region of the gene, whereas in tumor tissue, p53 mRNA was decreased and associated with relative hypermethylation. Taken together, these results provide additional insights into the dysregulation and instability in DNA methylation that accompanies the transition to tumor.


Inter-individual variation in metabolism of environmental toxicants, which is attributed to genetic polymorphism, may be a major risk factor in determining who will develop adverse health effects. This priority research area is the focus of many laboratories, and new techniques need to be developed to enhance the efficiency in generating data. We have developed and validated a new multiplex-polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) procedure for simultaneous genotyping of cytochrome P450 II E1 (CYP2E1), microsomal epoxide hydrolase (mEH), and glutathione S-transferase mu (GSTM1). Enzymes from these three polymorphic genes are involved with the phase I and II metabolism of a variety of environmental toxicants. Therefore, simultaneous characterization of these genes will not only reduce costs but will increase the efficiency of data collection, thereby contributing to health risk assessment efforts.


Ovarian carcinomas are known to rapidly develop drug resistance against chemotherapeutic agents. This phenomenon is often associated with the expression of p170-glycoprotein. A high rate of transcription of the corresponding mdr1-gene in resistant tumors is reported. Amplification
of the mdr1-gene has been observed in tumor cell lines exposed to cytotoxic drugs; however, significant information is lacking as to whether this holds true in clinical carcinomas. To fill this gap, we investigated the rate of gene amplification of the mdr1-gene in 63 recurrent ovarian carcinomas and we determined the resistance pattern of these cells using an ex vivo assay. The tumors showed varying ex vivo resistance patterns which did not correlate to clinical parameters. Amplification of the mdr1-gene was not observed in any of the cancer specimens. Therefore, we conclude that mdr1-gene amplification is not a common pathway for the development of chemoresistance in clinical ovarian carcinomas.


http://www.sciencedirect.com/science/article/B6T54-4CX72D5-4/2/69888c9bcfddde87679dbdd7597df478

Genetic or epigenetic inactivation of the DNA mismatch repair genes in tumor precursor cells results in a strong mutator phenotype, known as the microsatellite mutator phenotype (MMP), or microsatellite instability (MSI). This mutator phenotype causes mutations in genes responsible for the regulation of cell growth and survival/death and thus promotes the development and progression of tumors. In the present study, we examined the DNA topoisomerase II genes (topII[alpha] and topII[beta]) as mutational targets for MMP. We screened 10 MSI-positive human tumor cell lines and 30 MSI-positive colorectal tumors for mutations within the entire coding region of the topII[alpha] gene and two coding poly(A)7 sequences of topII[beta]. Mutations in either the topII[alpha] or topII[beta] gene were found with an overall frequency of 18% (in 10% of the primary tumors and in 44% of the cell lines). This indicates that modulation of the DNA topoisomerase II (TOPII) activity may be important for the development of MSI-positive cancer.


http://www.sciencedirect.com/science/article/B6T54-4BY9VGD-2TB/2/c4bb03aefa612b09c76732c5e6407a2f

We have investigated a series of ovarian tumours for evidence of mutations in the p53 tumour suppressor gene. In this study we have made use of the chemical mismatch cleavage technique which, from analyses of other genes, has been shown to consistently identify all point mutations present within a region of DNA. This approach revealed mutations of p53 in 11/20 tumours studied, mainly in exons 5 or 7. After sequencing the relevant regions of the gene it was shown that ten of these mutations would have resulted in an amino acid substitution in the protein and only one represented a polymorphism. The observed incidence of p53 missense mutations in our series (50%) was the highest recorded in ovarian tumours and demonstrated the potential of the mismatch cleavage technique as a reliable method for the detection of p53 mutations in human tumours.


http://www.sciencedirect.com/science/article/B6T54-46VBBNX-10/2/e8d3df0ee0d0a865009ff7df2523e56
Potassium bromate (KBrO3) is a rat renal carcinogen and a major drinking water disinfection by-product in water disinfected with ozone. Clear cell renal tumors, the most common form of human renal epithelial neoplasm, are rare in animals but are inducible by KBrO3 in F344 rats. Detection of cytoplasmic periodic acid-Schiff-positive granules in clear cell tumors, indicative of glycogen accumulation, provides evidence of their biochemical similarity to human counterparts. Mutation in the coding region of the von Hippel-Lindau (VHL) gene is frequently detected in human clear cell renal carcinomas. Detection of VHL mutations in KBrO3-induced rat renal tumors could enhance the relevancy of these rat renal tumors for human health risk assessment. Formalin-fixed paraffin-embedded control tissues and renal tumors from male F344 rats exposed to KBrO3 in the drinking water for 2 years were examined microscopically and were microdissected for DNA extraction. The coding sequence and a promoter region of the VHL gene were examined by polymerase chain reaction-single strand conformation polymorphism and/or DNA sequencing. Two of nine clear cell renal tumors carried the same C to T mutation at the core region of the Sp1 transcription factor binding motif in the VHL promoter and one of four untreated animals had C to T mutation outside the highly conserved core region. Mutation in the VHL coding sequence was only detected in one tumor. No VHL mutations were observed in three chromophilic tumors. KBrO3-induced rat renal tumors are morphologically similar to their human counterpart but the genetic basis of tumorigenesis is different.


http://www.sciencedirect.com/science/article/B6T54-4BY9PKN-14B/2/b0ce8cd3f10d35d4fbf9f16c520d1d8f

The steady state transcript levels of two hexokinase isozymes and type 1 glucose transporter in human tumor cell lines were analyzed. In HepG2 cells, both type II hexokinase and type 1 glucose transporter were highly expressed. However, in cell lines A431 and HeLa, in which the expression level of type 1 glucose transporter was lower than that in HepG2 cells, the amount of type II hexokinase transcript was almost negligible.


http://www.sciencedirect.com/science/article/B6T54-4CMJG37-1/2/933aad2fcbcd7589d143af0df1d2d0

Deoxycytidine kinase (dCK) is essential for the phosphorylation of gemcitabine and can predict response to gemcitabine in vivo. Conventional Competitive Template-Reverse Transcriptase-Polymerase Chain Reaction (CT-RT-PCR) was correlated with real time PCR using a Light Cycler (LC) with SYBR-Green detection to enable rapid and sensitive detection of dCK mRNA expression. We used cDNA from human xenografts to establish a relation between dCK activity and gemcitabine sensitivity. A significant correlation of LC-PCR was found with CT-RT-PCR (Pearson: r=0.956; Pr=0.972; P=0.003) and gemcitabine sensitivity (Pearson: r=0.695; P=0.048). The LC-PCR was also applied to needle biopsy specimens. In bladder tumors a similar correlation was found, while esophageal tumors with a high dCK expression responded to gemcitabine treatment. The LC is a rapid and reliable method for quantitation of dCK mRNA levels in tumors to predict clinical gemcitabine sensitivity.

http://www.sciencedirect.com/science/article/B6T54-411PGN3-9/2/55715104b7acb96a9c9f791e9e98c0c8

Expression of human mammaglobin (hMAM) was published to be exclusively expressed in mammary tissue, in solid tumors, axillary lymph nodes and disseminated cancer cells in blood of breast cancer patients. A quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) test was applied to investigate hMAM expression in blood of breast cancer patients. Mammaglobin mRNA expression was found not only in breast cancer cell lines but also in cell lines of other cancer origin. In our patient cohort hMAM expression in 11/98 (11%) samples of breast cancer and 3/12 (25%) ovarian cancer patients could be detected. hMAM mRNA expression as a candidate marker for the detection of disseminated cancer cells in blood of breast cancer patients showed low sensitivity and reduced tissue specificity. A prognostic significance of hMAM expression could not be demonstrated.


http://www.sciencedirect.com/science/article/B6T54-48BM4J8-2/2/6fa984346c825679803720a11b053c1f

Association between genetic polymorphisms of CYP1A1 and familial prostate cancer risk was examined by a case-control study of 185 individuals. Although the individual analysis of m1 or m2 genotype of CYP1A1 showed no significant association with prostate cancer risk, the presence of any mutated alleles significantly increased prostate cancer risk in comparison with wild-type genotypes by combination analysis (odds ratio [OR]=2.38; 95% confidence interval [CI]=1.72-3.29; P=0.0069). Furthermore, metastatic cancer had a significant association with mutated alleles of m1 and m2. These finding suggested that CYP1A1 polymorphisms has an association with prostate cancer risk, especially with progression of prostate cancer.


http://www.sciencedirect.com/science/article/B6T54-44KTJ57-82/2/e9254bd78908a87145f2083a34350e9b

We report three sporadic parathyroid tumors with biallelic inactivation of the multiple endocrine neoplasia type 1 (MEN1) gene. Three parathyroid tumors had two somatic mutations (K119del and 864del8, 363insT and 1767delT, and 508del33 and W341X, respectively). The mutations in both alleles detected by long-range polymerase chain reaction and subcloning in three tumors would likely result in a nonfunctional menin protein in parathyroid glands. These results show that the MEN1 gene is inactivated not only by a combination of somatic mutations and loss of heterozygosity, but also by somatic double mutations located on different alleles. The results directly confirmed the participation of MEN1 in the tumorigenesis of sporadic parathyroid tumors.

Tanakamaru, Z.-y., I. Mori, et al. (2001). "Essential similarities between spontaneous and MeIQx-
Aberrant crypt foci (ACFs) in the Fischer 344 (F344) rat colon, of control or 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx)-treated groups, were compared morphologically, immunohistochemically, and at the molecular biological level in order to elucidate their biological characteristics. Male 3-week-old rats were fed a diet supplemented with or without MeIQx at doses of 100 ppm or less for 16 weeks. The incidence of ACFs was the highest (90%) in animals given 100 ppm MeIQx but that in untreated rats was also surprisingly high (57%). Nine ACFs from nine MeIQx-treated rats and ten ACFs from ten untreated control rats were selected for detailed examination for their large size. There were no morphological differences in macroscopic and microscopic features between MeIQx-promoted and spontaneous ACFs. There were also no differences in immunohistochemical labeling for proliferating cell nuclear antigen (PCNA) and p53 protein between these ACFs although in both cases labeling was higher than in normal crypts. Dot blot hybridization revealed no c-K-ras mutations in codon 12 except in one ACF (11.1%) developing in a rat treated with 100 ppm MeIQx, in which a GGT->GAT single base substitution was detected. Our results thus suggest that in terms of morphology, cell proliferation, P53 expression and c-K-ras mutation, most ACFs found in rats given 100 ppm MeIQx are essentially identical to their spontaneous counterparts.


Dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are major metabolites of tetrachloroethylene (PCE) and trichloroethylene (TCE) and are found in chlorinated drinking water. All four chlorinated compounds are liver carcinogens in B6C3F1 mice. It has previously been reported that approximately 20% of hepatic tumors induced by PCE exhibited loss of heterozygosity (LOH) on chromosome 6, suggesting the presence of a tumor suppressor gene. In the current investigation, we determined whether TCA or DCA also induced LOH on chromosome 6. Liver tumors were initiated in 15 day old female B6C3F1 mice with N-methyl-N-nitrosourea (MNU) and promoted with 20 mmol/l DCA or TCA in their drinking water. Twenty-four and thirty-seven liver tumors promoted by DCA and TCA, respectively, were examined for LOH using 4 polymorphic loci on chromosome 6. Ten of 37 (27%) tumors (7 of 27 carcinomas and 3 of 10 adenomas) promoted by TCA exhibited LOH at least for two loci on chromosome 6. All 10 tumors that exhibited LOH, lost the C57BL/6J allele at both the D6mit9 and D6mit323 loci, while two also lost at least one of the C3H/HeJ alleles. No LOH on chromosome 6 was observed in the 24 liver tumors promoted by DCA. The LOH on chromosome 6 in TCA but not in DCA-promoted tumors supports it as an active metabolite of PCE and demonstrates different pathogenesis at least for some of the DCA and TCA-promoted liver cancer.

Human microsomal epoxide hydrolase (mEH) catalyzes a key step in the biotransformation of benzo[a]pyrene that yields the highly mutagenic (+)-anti-7,8-diol-9,10 epoxide (BPDE). Two polymorphisms have been described in the coding region of the mEH gene (EPHX1) that produce two protein variants: 113Tyr->113His (exon 3) and 139His->139Arg (exon 4). We performed a case-control study among Northwestern Mediterranean Caucasians to investigate a possible association between these EPHX1 variants and lung cancer risk. Both EPHX1 polymorphisms were analyzed in a group of lung cancer patients (n=176) and in a control group of healthy smokers (n=187). The results showed a significantly decreased risk for the rare homozygous 113His/113His (adjusted odds ratio (OR): 0.44, 95% confidence interval (CI): 0.27-0.71) and 139Arg/139Arg (adjusted OR: 0.55, 95% CI: 0.33-0.91) compared with the major wild-types 113Tyr/113Tyr and 139His/139His, respectively, as the references. Thereafter, we analyzed the EPHX1 variants in combination with three glutathione S-transferase polymorphic genes (GSTM1, GSTT1, and GSTP1) and we found a significant overrepresentation of cancer patients with a combination of exon 3 113Tyr/113Tyr EPHX1 and exon 5 105Ile/105Ile GSTP1 (adjusted OR: 2.34, 95% CI: 1.21-4.52). The polymorphic site within the exon 5 of GSTP1 results in a Ile->Val substitution, and the isoleucine GSTpi isofrom has been found in vitro to be less active than the valine isoform towards the conjugation of BPDE. The 113 Tyr/Tyr EPHX1 encodes for a high-activity mEH. Our results agree with these observations in vitro and suggest that a genetically determined combination of a high-activity mEH and a low-activity GSTpi may increase lung cancer risk among smokers.


http://www.sciencedirect.com/science/article/B6T54-4BNW2R7-2/2/4f868083aebdbf85e92609922efad9833

The expression pattern of erbB2 and its transmembrane polymorphisms (Ile654Val and Ile655Val) were investigated in a panel of human normal and neoplastic breast cell lines to evaluate whether the expression pattern was affected by changes in the gene structure. At least two peptides of lower molecular mass forms (95 and 68 kDa) than the holoreceptor (185 kDa), comprehensive of the tyrosine kinase domain, were detected in all cells. Both peptides were also phosphorylated, suggesting a functional role in signal transduction. The presence of the polymorphisms found in two cell lines was unrelated to the expression of the lower molecular mass proteins.


http://www.sciencedirect.com/science/article/B6T54-4239836-J/2/fd0587eae66b97cc8f742a65800a7942

We screened 90 cases of gastric carcinoma (GCA) samples for [beta]-catenin exon 3 mutation and assessed its possible relationship with microsatellite instability (MSI). Three mutations were detected in two samples, including a single mutation in an intestinal type and double mutations in a diffuse type GCA. One of the mutations found in the diffuse type GCA sample was a non-sense mutation at codon 68 (CAG->TAG). This novel mutation was predicted to disrupt the binding of [beta]-catenin to [alpha]-catenin and may be related to the diffuse type morphology. The other two mutations were missense mutations involved or related to the GSK-3[beta] phosphorylation site, which have been reported previously. No MSI can be demonstrated in the two cases with [beta]-catenin mutation. Our results suggested that [beta]-catenin mutation was infrequent in GCA and appeared not specific for MSI.

http://www.sciencedirect.com/science/article/B6T54-448Y28MB2/2/435c39873db92a670b751a0794574c4c

In this report, we describe the cloning of the coding region of human WIG-1 cDNA. The human 8 and 6 kb WIG-1 transcripts are both upregulated following ionizing irradiation of the human colon cancer cell lines HCT116 and LoVo which have wild type TP53 but not in DLD1 cells that lack wild type TP53. Basal levels of both WIG-1 transcripts were detected in human adult brain, kidney, and testis, but not in fetal brain, heart, pancreas, adrenal gland, fetal liver, and small intestine. FISH analysis mapped the human WIG-1 gene to 3q26.3. Investigation of squamous cell carcinomas of the lung by Southern blot and semiquantitative RT-PCR analysis showed amplification in combination with increased expression of WIG-1 in 1/7 tumors and increased expression in a further two cases.


http://www.sciencedirect.com/science/article/B6T54-42R0SBN7/2/1dc9f51edf4f6752e224a513e3ae846d

Tumor induction in rats by 7,12-dimethylbenz[a]anthracene (DMBA) will generate malignancies that display reproducible chromosomal abnormalities involving rat chromosome (RNO) 2. Thus, it has been reported that rat DMBA erythroleukemias display RNO2 abnormalities, which in this case were closely correlated to mutations in the Nras oncogene located in RNO2q34. Our cytogenetic analysis in a series of 17 DMBA-induced rat sarcomas showed that 11 (65%) tumors had a significant increase in RNO2 copy number. Furthermore, the incidence of point mutations in codons 12, 13 and 61 of Hras, Kras, and Nras was examined in the same set of sarcomas, and mutations were detected in three (18%) tumors, in codon 61 of Kras (CAA->CAT) (1 of 17) and Nras (CAA->CTA) (2 of 17). We conclude that the high frequency of RNO2 gain was in accordance with previous studies of DMBA-induced rat neoplasms, supporting the idea of a significant role of RNO2 in DMBA carcinogenesis. However, there was no clear-cut relationship between activated Nras and gain of RNO2 material, implying that mutational activation of Nras is not the causative factor underlying the gain of RNO2 copy number in rat DMBA sarcomas, in contrast to what has been suggested for DMBA-induced erythroleukemias.


http://www.sciencedirect.com/science/article/B6T54-44J722T7/2/d48d3fbed3b3d9b06f6509d3d136d701

A PCR-based subtractive hybridisation technique was used to identify genes involved in stromal-epithelial interactions in prostate cancer. Eight genes were identified as being differentially expressed in benign prostatic fibroblast cells after stimulation with tumourigenic LNCaP conditioned media. One of these genes, protein tyrosine phosphatase CAAX2 (PTPCAAX2; also described as PTP4A and OV-1), has recently been shown to be oncogenic in hamster pancreatic
epithelial cells. We show that PTPCAAX2 expression is up-regulated 4-fold in benign prostatic fibroblast cells 24 h after stimulation with LNCaP conditioned media and up-regulated 9-fold in prostatic tumour fibroblast cells. PTPCAAX2 overexpression was also detected in both androgen-dependent and androgen-independent prostate cancer cell lines and prostate tumour tissue, as determined by RT-PCR analysis and in situ hybridisation. These observations of PTPCAAX2 overexpression in prostate tumour cells and tissue suggest that PTPCAAX2 may potentially function as an oncogene in prostate cancer.


The expression and activity of CYP1A1 were examined in fresh, small-sized lung biopsy specimens from nine human subjects. CYP1A1 transcripts were detected by reverse transcription-polymerase chain reaction (RT-PCR) analysis of total lung RNA. CYP1A2 transcripts were detected in the RNA samples as well, and bioactivation of 2-aminofluorene (2-AF) or 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), a CYP1A2-preferential activity, was catalyzed by the lung S9 fractions also. Two major bands were detected in the whole homogenate by western blot analysis using CD3, a mouse anti rat CYP1A1 monoclonal that cross-reacts with rat CYP1A2 as well as with human CYP1A1 and CYP1A2. S9 fractions from the tissues catalyzed the bioactivation of benzo[a]pyrene (B[a]P), a CYP1A1-preferential activity, to mutagens in the Ames assay. Our findings are in agreement with the known presence of CYP1A1 in the human lung, and provide strong evidence for the expression of catalytically functional CYP1A2 in the tissue.

http://www.sciencedirect.com/science/article/B6T54-44HWVV1-2/2/17033bc37affb114359697656b9c47c0b

The inducibility of cytochrome P4501A1 gene (CYP1A1) expression was examined in human lung samples from 27 subjects, using an explant culture system and semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. CYP1A1 transcripts were present in all of the lung specimens and were induced by the prototypic inducers 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene (B[a]P), and by the atypical inducers pyridine, nicotine, and omeprazole. 2-Hydroxy[a]pyrene was a better inducer than pyridine, implicating metabolites in CYP1A1 induction by the parent compound. The prototypical inducers were the most effective inducers in many samples but were ineffective in some samples in which the atypical compounds were effective inducers. Cytochrome P4501A2 (CYP1A2) transcripts were also detected in most of the lung specimens and were inducible in some specimens. The results show the suitability of the explant culture system for examining the inducibility of human pulmonary CYP1A1 and CYP1A2, indicate the heterogeneity in individual sensitivity to the induction, and underscore the need to include atypical inducers in studies of CYP1A inducibility in humans.

Human PRL-1, PRL-2, and PRL-3 tyrosine phosphatases induce the malignant transformation of epithelial cells. We tested the hypothesis that the oncogenic effects of PRL occur by increasing cellular proliferation. Cells stably transfected with PRL-1 or PRL-2 exhibited 2.7-3.3-fold increases over control cells in the rate of DNA synthesis and the proportion of cells in S-phase, and they progressed more rapidly from G1 into S. In addition, cells overexpressing either PRL-1 or PRL-2 exhibited enhanced cyclin-dependent kinase 2 (CDK2) activity and significantly lower p21Cip1/Waf1 protein levels, and PRL-1 overexpressing cells had higher cyclin A protein levels than control cells. We conclude that PRL phosphatases increase cell proliferation by stimulating progression from G1 into S phase, and this process may be dependent on the down regulation of the cyclin dependent kinase inhibitor p21Cip1/Waf1.


We previously examined the tumorigenicity of 7-chlorobenz[a]anthracene (7-Cl-BA) and 7-bromobenz[a]anthracene (7-Br-BA) in the neonatal mouse bioassay and found that 7-Cl-BA and 7-Br-BA induced hepatocellular adenoma in 92 and 96% of the mice and hepatocellular carcinoma in 100 and 83% of the mice, respectively. In the present study, mRNA was isolated from each of the liver tumors induced by the two compounds and reverse-transcribed to cDNA. Portions of the K- and H-ras oncogene coding sequences were then amplified and analyzed for DNA sequence alterations. Eighty-three percent (20/24) of 7-Cl-BA-induced and 91% (20/22) of 7-Br-BA-induced liver tumors had activated ras protooncogenes. In contrast to the general finding of H-ras mutations in B6C3F1 mouse liver tumors, both compounds had 95% (19/20) of the mutations located at the first base of K-ras codon 13, resulting in a pattern of GC->GC. Thus, our results demonstrate that 7-Cl-BA and 7-Br-BA induce a unique type of ras (K-ras) oncogene activation in liver tumors of B6C3F1 mice.


A male patient synchronously or metachronously underwent six curative resections after diagnoses of cancers in the rectum, urinary bladder, stomach, colon, liver and lung. Five cancers, excluding early colon cancer, were analyzed for instability in seven microsatellite markers and in transforming growth factor [beta] type II receptor, insulin-like growth factor II receptor and BAX. All analyzed cancers had replication errors and instability in at least one target gene. These results suggest that abnormal DNA mismatch repair system plays a major role in the occurrence of multiple primary cancers in this case.

We have established a novel quantitative method based on the allele-specific PCR, which uses the linearly amplified fragment of the PCR products as the internal control. The improved characteristics of the procedure are the high sensitivity for quantitation of the mutant alleles at ratios of up to 1:10000 and the reduced necessity of the optimization of the PCR conditions for each mutation. Using this modified allele-specific PCR, we could quantify the tumor alleles in the urine sediments of three patients with urothelial cancers that harbored different p53 gene mutations. This method can be applied to other genetic targets that have other types of alterations, such as deletions or insertions.


Methylenetetrahydrofolate reductase (MTHFR) plays a pivotal role in folate metabolism by regulating the diversion of folate metabolites toward DNA methylation or toward DNA synthesis. Because aberrations in both of these pathways can be tumor promoting, the two common polymorphisms in the MTHFR gene, 677 C->T and 1298 A->C, have been implicated as risk factors for several cancers. Homozygosity for the 677 C->T polymorphism and compound heterozygosity for 677 C->T and 1298 A->C polymorphisms both reduce enzyme activity by more than 50% and can promote oncogenic alterations in DNA methylation especially when folate status is low. Thus, rapid identification of both polymorphisms in MTHFR gene would be of importance in understanding the genetics of abnormal folate metabolism as related to human cancer risk. Here we describe a multiplex polymerse chain reaction/restriction fragment length polymorphism procedure in which two sets of primers are used to amplify simultaneously the DNA regions spanning 677 and 1298 loci in one PCR reaction. The amplified products are digested by Hinfl or MboII followed by agarose gel electrophoresis for simultaneous detection of the 677 C->T and 1298 A->C polymorphisms in the same gel.


ASC/TMS1, a proapoptotic activator of procaspase-1, was reported to be aberrantly methylated in human breast cancer. We found that ASC was methylated in three of five human colon cancer cell lines lacking ASC protein expression. Demethylation treatment of these cell lines lacking ASC with 5-aza-2'-deoxycytidine partially restored ASC expression. Methylated ASC was also detected in six of ten colorectal cancer tissues. Although clear down-regulation of ASC in the whole region of a tumor tissue was hardly observed by immunostaining with anti-ASC mAb, complete suppression of ASC was identified in a minor population of the colorectal tumor cells. The biological significance of ASC methylation inducible ASC suppression in colorectal cancer will be discussed.
Previously, we reported the expression and function of system L amino acid transporter in KB human oral epidermoid carcinoma cells. In the present study, therefore, we investigated the expression and function of system L amino acid transporter in human normal oral keratinocytes (HNOK) and compared the expressions and functions of system L amino acid transporters in HNOK and KB cells. The HNOK expressed L-type amino acid transporter 1 (LAT1) and L-type amino acid transporter 2 (LAT2) with their subunit 4F2hc in the plasma membrane but the expression of LAT1 was very weak, which is in contrast to the KB cells expressing LAT1 but not LAT2 with the 4F2hc in the plasma membrane. The [14C] L-leucine uptake by HNOK, as well as KB cells, was inhibited by the system L selective inhibitor BCH. The majority of [14C] L-leucine uptake was, therefore, mainly mediated by LAT2 in the HNOK and by LAT1 in the KB cells. These results suggest that the transport of neutral amino acids including several essential amino acids into the HNOK and KB cells are mainly mediated by LAT2 and LAT1, respectively. The specific inhibition of LAT1 in oral cancer cells could be a new rationale for anti-cancer therapy.

We have examined the expression and function of system amino acid transporter in KB human oral epidermoid carcinoma cells. The KB cells express -type amino acid transporter 1 (LAT1) in plasma membrane, but not -type amino acid transporter 2 (LAT2). The [14C]-leucine uptake by KB cells is inhibited by system selective inhibitor BCH. The majority of [14C]-leucine uptake is, therefore, mediated by LAT1. These results suggest that the transport of neutral amino acids including several essential amino acids into the KB cells mediated by LAT1 and the specific inhibition of LAT1 in oral cancer cells will be a new rationale for anti-cancer therapy.

Inflammation has been considered to be related to carcinogenesis. Previously, we demonstrated that 1-hydroxyanthraquinone (1-HA), a naturally occurring carcinogen, induced severe inflammation such as ulcerative colitis in colonic mucosa. We also showed that indomethacin inhibited the tumorigenicity of 1-HA. In this study, we examined the expressions of major enzymes in arachidonic acid cascade related to inflammation in the colon mucosa of rats treated with 1-HA. After the treatment of 1% 1-HA diet, colon lesions were observed and RNA was extracted from mucosa and neoplasms. The mRNA expressions of group II phospholipase A2, cyclooxygenase-2 and 5-lipoxygenase, were examined by using a reverse transcriptase polymerase chain reaction. The expressions of phospholipase A2 and cyclooxygenase were
significantly increased in non-neoplastic mucosa in rats treated with 1-HA compared with those in control rats. The expressions in the neoplasms induced by 1-HA were also increased. Phospholipase A2, especially, was much higher in the neoplasms than in non-neoplastic mucosa. However, the expression of 5-lipoxygenase showed no change in the non-neoplastic mucosa and neoplasms of rats treated with 1-HA, compared with that in control rats. These findings suggest that the inflammation induced by 1-HA may be related to the metabolites through a cyclooxygenase pathway, which indicates a prostaglandin synthesis, but not through a lipoxygenase pathway, which indicates a leukotriene synthesis in arachidonic acid cascade.


http://www.sciencedirect.com/science/article/B6T54-3VXJ6SV-D/2/aad3f4baed7ec26e6101dce843fa1d46

FGF-like growth factors have been detected in the urine of patients with bladder or renal cell carcinoma. FGF-1-like and FGF-2-like proteins have been detected in the urine of patients with bladder carcinoma. However, the expression of FGFs and their receptor in bladder and renal cell carcinoma cells remains limited. We measured the mRNA levels of FGFs and their receptor in these carcinoma cell lines by means of RT-PCR. We detected FGF-8 mRNA expression in murine cell lines of bladder and renal cell carcinomas but not in those of the normal bladder and kidney. Furthermore, FGF-8 mRNA expression was detected in all human bladder and renal cell carcinoma cell lines tested. We also frequently detected FGF-1, FGF-2 and FGF-5 mRNA expression in human bladder and renal cell carcinoma cell lines. These results indicate that FGF-8 is also candidate for marker of these types of carcinoma as well as FGF-1 and FGF-2.


Peroxisome proliferator-activated receptor [delta] (PPAR[delta]) is ligand-activated transcription factor of the nuclear receptor superfamily which is recently implicated in carcinogenesis. We examined the expression profiles of PPAR[delta] in human gastric cancer, normal gastric mucosa and gastric cancer cell lines by RT-PCR, Western blot and immunohistochemistry. PPAR[delta] mRNA and protein was found to be ubiquitously expressed in all 5 gastric cancer cell lines, 40 gastric cancer samples and 10 normal gastric mucosa from non-cancer patients. Positive immunoreactivity was detected in the nuclei of normal and malignant gastric epithelium. Treatment of gastric cell line MKN45 that overexpressed cyclooxygenase-2 (COX-2) with specific COX-2 inhibitor NS398 resulted in a time- and dose-dependent suppression of PPAR[delta] expression. In contrast, there was no suppression of PPAR[delta] in MKN28 gastric cell line with low COX-2 expression. Our results demonstrated the ubiquitous expression of PPAR[delta] in normal and cancer gastric epithelium. Suppression of PPAR[delta] may be one of the mechanisms underlying the chemopreventive effects of COX-2 inhibitor.


http://www.sciencedirect.com/science/article/B6T54-44HWVV1-
Alteration in ganglioside composition in F-11 cells by suppression of GD3-synthase gene expression resulted in greatly reduced tumor growth and metastasis when the cells were injected into nude mice. To identify genes whose expression is correlated with the decreased level of ganglioside GD3, we analyzed gene expression profiles of the GD3-suppressed F-11 cells and the control F-11 cells using DNA microarrays. We identified a set of GD3-related genes, most of which are involved in tumor growth and development. The genes that define the proliferation-transformation signature are down-regulated, such as creatine kinase-B (CKB), upstream stimulation factor 1 (USF-1), type II cAMP-dependent protein kinase regulatory subunit (RII PKA), and tyrosine hydroxylase (TH). On the other hand, the genes that define the differentiation-reverse transformation signature are up-regulated, including p160 myb-binding protein (P160), brain factor-2, insulin-like growth factor-binding protein (IGFBP), and growth/differentiation factor 11. Transcriptional levels of the genes that showed the most distinct GD3-related expression change were validated by reverse transcription-polymerase chain reaction (RT-PCR). Defining GD3-related genes may lead to identification of clinically relevant therapeutics and to understanding of the mechanism(s) by which ganglioside GD3 affects tumor growth and metastasis.


We previously demonstrated that N-(4-hydroxyphenyl)retinamide (4-HPR) and gamma-irradiation, when used in combination, had a synergistic effect in inducing apoptosis in bladder cancer cells, suggesting that 4-HPR may increase radiosensitivity in bladder cancer cells. To unravel molecular correlates in this radiosensitizing effect of 4-HPR, we examined the baseline and 4-HPR-induced expression of GADD45 to elucidate possible mechanisms by which 4-HPR enhanced the effect of gamma-irradiation in three bladder cancer cell lines. To investigate the role of p53 in mediating the radiosensitizing effect of 4-HPR, we also examined mutations in exons 5-9 by using direct sequencing and the levels of p53 expression by using RT-PCR and Western blot, before and after treatment with 4-HPR in these bladder cancer cell lines. Two cell lines had low expression of GADD45, and a dose-dependent increase in GADD45 expression induced by 4-HPR was found in bladder cancer cell lines without p53 mutations in exons 5-9. A combination of gamma-irradiation and 4-HPR showed a significantly greater effect in enhancing GADD45 expression than either agent used alone. The results indicate that the combined treatment with 4-HPR and gamma-irradiation has a stronger effect on GADD45 expression than the treatment with either agent alone, which suggests that the two agents may have an additive/synergistic effect. However, a normal p53 function appears to be necessary for the dose-dependent induction of GADD45 by 4-HPR. Once our results are verified and replicated by other investigators, 4-HPR may have a potential clinical implication in effectively treating bladder cancer in combination with low-gamma-irradiation therapy.
Sp proteins play an important role in angiogenesis and growth of cancer cells, and specificity protein 1 (Sp1) has been linked to vascular endothelial growth factor (VEGF) expression in pancreatic cancer cells. RNA interference was used to investigate the role of Sp family proteins on regulation of VEGF expression and proliferation of Panc-1 pancreatic cancer cells. Using a series of constructs containing VEGF promoter inserts, it was initially shown that Sp1 and Sp3 were required for transactivation, and this was primarily dependent on proximal GC-rich motifs. We also showed that Sp4 was expressed in Panc-1 cells, and RNA interference assays suggested that Sp4 cooperatively interacted with Sp1 and Sp3 to activate VEGF promoter constructs in these cells. However, the relative contributions of Sp proteins to VEGF expression were variable among different pancreatic cancer cell lines. Small inhibitory RNAs for Sp3, but not Sp1 or Sp4, inhibited phosphorylation of retinoblastoma protein, blocked G0/G1 -> S-phase progression, and up-regulated p27 protein/promoter activity of Panc-1 cells; similar results were observed in other pancreatic cancer cells, suggesting that Sp3-dependent growth of pancreatic cancer cells is caused by inhibition of p27 expression.

Tenascin-C (TN) is an extracellular matrix protein that is expressed at low levels in normal adult tissue but is highly expressed around many tumors including breast carcinoma. TN exists as multiple isoforms generated through alternative splicing, and these isoforms have different effects on cell growth and migration. This study has analyzed in detail the pattern of TN isoform expression in benign, preinvasive, and invasive breast lesions using reverse transcription-PCR and Southern blotting. Significant differences in the profile of TN isoforms were identified. Although all tissues expressed the fully truncated TN, expression of two additional isoforms, one containing exon 16 (TN16) and one containing both exons 14 and 16 (TN14/16), were significantly associated with the invasive phenotype (P < 0.001). A subset of ductal carcinoma in situ (DCIS) cases were also found to express these isoforms, which may be indicative of a high risk of invasion in these lesions. Expression of these isoforms correlated with the presence of TN protein in the stroma in place of or in addition to basement membrane TN. Immunohistochemistry and in situ hybridization confirmed the production of exon 14-containing higher molecular weight isoforms by stromal fibroblasts in malignant tissue and both periductal fibroblasts and residual myoepithelial cells in DCIS. Although no evidence of tumor cell synthesis of TN was detected in the tissues, two highly invasive breast cancer cell lines (MDA-MB 231 and MDA-MB 468) were found to produce TN in contrast with tumor cells with a lower invasive capacity (MCF-7 and T47D). These results demonstrate for the first time that specific TN isoforms are expressed in invasive breast carcinomas and that these isoforms are identified in a subset of DCIS and suggest that detection of TN16 and/or TN14/16 may be used as a predictor for invasion. Functional studies are now essential to establish the effect of these isoforms on tumor behavior and evaluate whether they will provide appropriate targets for therapeutic intervention.
Overexpression of the epidermal growth factor (EGF) pathway has been implicated in melanoma pathogenesis, and a recent case-control study identified a single nucleotide polymorphism (G to A) in the EGF gene where the G allele was associated with increased EGF expression and an increased risk of melanoma. To further evaluate this association, we conducted a case-control analysis from the Genes, Environment, and Melanoma study at the University of Michigan site using two different study designs. Incident cases of histopathologically confirmed first primary melanoma that were diagnosed between January 1, 2000 and December 31, 2000 from the University of Michigan Melanoma Clinic (n = 330) were compared with the following two different sources of nonmelanoma controls: spouse/friend controls (n = 84) and healthy volunteer controls from a case-control study of psoriasis (n = 148). Using a second analytic design, comparisons between multiple primary melanoma cases (n = 62) and single primary melanoma cases (n = 330) were also evaluated to estimate odds ratios (ORs). Genotyping for the single nucleotide substitution (G to A) at position 61 in the 5′ untranslated region of the EGF gene was performed from genomic DNA, and epidemiological risk factors were assessed through a telephone interview. When EGF genotypes were compared between incident primary melanoma cases and the nonmelanoma controls, the risk associated with the homozygous G/G genotype was not statistically significantly associated with an increased risk for incident primary melanoma compared with the homozygous A/A genotype [OR, 1.09; 95% confidence interval (CI); 0.65-1.85]. No strong associations with EGF G/G genotype were observed in comparisons of multiple primary and single primary melanoma cases (OR, 0.66; 95% CI; 0.25-1.73). Case subjects with tumors \[IMG]\_3.5 mm compared with those <3.5 mm were not significantly associated with the G/G genotype (OR, 0.54; 95% CI; 0.12-2.35). Our data do not support a strong association between EGF 61*G and melanoma and demonstrate the potential utility of case-case designs for evaluating the role of single nucleotide polymorphisms and cancer. Additional independent studies will be required to elucidate relationships between genetic variation in the EGF gene and risk of melanoma.

Athar, M., C. Li, et al. (2004). "Inhibition of Smoothened Signaling Prevents Ultraviolet B-Induced Basal Cell Carcinomas through Regulation of Fas Expression and Apoptosis." Cancer Res. 64(20): 7545-7552.

Abnormal activation of the hedgehog-signaling pathway is the pivotal abnormality driving the growth of basal cell carcinomas (BCCs), the most common type of human cancer. Antagonists of this pathway such as cyclopamine may therefore be useful for treatment of basal cell carcinomas and other hedgehog-driven tumors. We report here that chronic oral administration of cyclopamine dramatically reduces (\[\sim\]66%) UVB-induced basal cell carcinoma formation in Ptc1+/- mice. Fas expression is low in human and murine basal cell carcinomas but is up-regulated in the presence of the smoothened (SMO) antagonist, cyclopamine, both in vitro in the mouse basal cell carcinoma cell line ASZ001 and in vivo after acute treatment of mice with basal cell carcinomas. This parallels an elevated rate of apoptosis. Conversely, expression of activated SMO in C3H10T1/2 cells inhibits Fas expression. Fas/Fas ligand interactions are necessary for cyclopamine-mediated apoptosis in these cells, a process involving caspase-8 activation. Our data provide strong evidence that cyclopamine and perhaps other SMO antagonists are potent in vivo inhibitors of UVB-induced basal cell carcinomas in Ptc1+/- mice and likely in humans because the majority of human basal cell carcinomas manifest mutations in PTCH1 and that a major mechanism of their inhibitory effect is through up-regulation of Fas, which augments apoptosis.

http://cancerres.aacrjournals.org/cgi/content/abstract/63/4/887

We expressed the full-length CD44v2-10 isoform in SKHep1 cells, a nonmetastatic human hepatocellular carcinoma cell line that does not express any endogenous CD44v isoforms. In SCID mice, expression of CD44v2-10 by SKHep1 cells had no effect on s.c. primary tumor development but caused pulmonary metastases in 41% (7 of 17) of animals compared with control SKHep1 cells (0 of 16; P < 0.01). CD44v2-10 expression by SKHep1 cells resulted in enhanced heparan sulfate (HS) attachment and an enhanced capacity to bind heparin-binding growth factors. Mutation of the v3 domain to prevent HS attachment and growth factor binding abolished the metastatic phenotype, demonstrating that HS modification of CD44v2-10 plays a critical role in the development of metastases in this model. However, in vitro proliferation, motility, and invasion were not altered by CD44v2-10 expression.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/7/2534

Tissue invasion by tumor cells involves their migration across basement membranes through activation of extracellular matrix degradation and cell motility mechanisms. Chemokines binding to their receptors provide chemotactic cues guiding cells to specific tissues and organs; they therefore could potentially participate in tumor cell dissemination. Melanoma cells express CXCR4, the receptor for the chemokine stromal cell-derived factor-1{alpha} (SDF-1{alpha}). Using Matrigel as a model, we show that SDF-1{alpha} promotes invasion of melanoma cells across basement membranes. Stimulation of membrane-type 1 matrix metalloproteinase (MT1-MMP) activity by SDF-1{alpha} was necessary for invasion, involving at least up-regulation in the expression of this metalloproteinase, as detected in the highly metastatic BLM melanoma cell line. Moreover, SDF-1{alpha} triggered the activation of the GTPases RhoA, Rac1, and Cdc42 on BLM cells, and expression of dominant-negative forms of RhoA and Rac1, but not Cdc42, substantially impaired the invasion of transfectants in response to SDF-1{alpha}, as well as the increase in MT1-MMP expression. Furthermore, CXCR4 expression on melanoma cells was notably augmented by transforming growth factor-(beta)1, a Matrigel component, whereas anti-transforming growth factor-(beta) antibodies inhibited increases in CXCR4 expression and melanoma cell invasion toward SDF-1{alpha}. The identification of SDF-1{alpha} as a potential stimulatory molecule for MT1-MMP as well as for RhoA and Rac1 activities during melanoma cell invasion, associated with an up-regulation in CXCR4 expression by interaction with basement membrane factors, could contribute to better knowledge of mechanisms stimulating melanoma cell dissemination.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/5/929
Enzymes that activate and detoxify benzene are likely genetic determinants of benzene-induced toxicity. NAD(P)H: quinone oxidoreductase-1 (NQO1) detoxifies benzoquinones, proposed toxic metabolites of benzene. NQO1 deficiency in humans is associated with an increased risk of leukemia, specifically acute myelogenous leukemia, and benzene poisoning. We examined the importance of NQO1 in benzene-induced toxicity by hypothesizing that NQO1-deficient (NQO1/-) mice are more sensitive to benzene than mice with wild-type NQO1 (NQO1+/+; 129/Sv background strain). Male and female NQO1/- and NQO1+/+ mice were exposed to inhaled benzene (0, 10, 50, or 100 ppm) for 2 weeks, 6 h/day, 5 days/week. Micronucleated peripheral blood cells were counted to assess genotoxicity. Peripheral blood counts and bone marrow histology were used to assess hematotoxicity and myelotoxicity. p21 mRNA levels in bone marrow cells were used as determinants of DNA damage response. Female NQO1/- mice were more sensitive (6-fold) to benzene-induced genotoxicity than the female NQO1+/+ mice. Female NQO1/- mice had a 9-fold increase (100 versus 0 ppm) in micronucleated reticulocytes compared with a 3-fold increase in the female NQO1+/+ mice. However, the induced genotoxic response in male mice was similar between the two genotypes ([>=10-fold increase at 100 ppm versus 0 ppm]). Male and female NQO1/- mice exhibited greater hematotoxicity than the female NQO1+/+ mice. p21 mRNA levels were induced significantly in male mice (>10-fold) from both strains and female NQO1/- mice (>8-fold), which indicates an activated DNA damage response. These results indicate that NQO1 deficiency results in substantially greater benzene-induced toxicity. However, the specific patterns of toxicity differed between the male and female mice.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/18/6820

RGS proteins negatively regulate heterotrimeric G protein signaling. Recent reports have shown that RGS proteins modulate neuronal, cardiovascular, and lymphocytic activity, yet their role in carcinogenesis has not been explored. In an epidemiologic study of 477 bladder cancer patients and 446 matched controls, three noncoding single-nucleotide polymorphisms (SNPs) in RGS2 and RGS6 were each associated with a statistically significant reduction in bladder cancer risk. The risk of bladder cancer was reduced by 74% in those individuals with the variant genotype at all three SNPs (odds ratio, 0.26; 95% confidence interval, 0.09-0.71). When the SNPs were analyzed separately, the RGS6-rs2074647 (C[-&gt;T]) polymorphism conferred the greatest overall reduction in risk of bladder cancer (odds ratio, 0.66; 95% confidence interval, 0.46-0.95). These reductions in risk were more pronounced in ever smokers, suggesting a gene-environment interaction. In transfection assays, the RGS6-rs2074647 (C[-&gt;T]) polymorphism increased the activity of a luciferase-RGS fusion protein by 2.9-fold, suggesting that this SNP is functionally significant. Finally, we demonstrate that RGS2 transcripts and several splice variants of RGS6 are expressed in bladder cancer cells. These data provide the first evidence that RGS proteins may be important modulators of cancer risk and validate RGS6 as a target for further study.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/14/4687

Deregulation of members of the transforming growth factor (TGF)-beta signaling pathway occurs often in colon cancers and is believed to affect the formation of primary colon cancer. Mutational inactivation of TGFBR2 is the most common genetic event affecting the TGF-beta signaling pathway and occurs in [-]20-30% of all colon cancers. By mating Fabp4xat-132 Cre mice with Tgfr2flx/flx mice, we have generated a mouse model that is null for Tgfr2 in the
colonic epithelium, and in this model system, we have assessed the effect of loss of TGF-{beta} signaling in vivo on colon cancer formation induced by azoxymethane (AOM). We have observed a significant increase in the number of AOM-induced adenomas and adenocarcinomas in the Fabpl4xat-132 Cre Tgfbr2flx/flx mice compared with Tgfbr2flx/flx mice, which have intact TGF-{beta} receptor type II (TGFBR2) in the colon epithelium, and we have found increased proliferation in the neoplasms occurring in the Fabpl4xat-132 Cre Tgfbr2flx/flx mice. These results implicate the loss of TGF-{beta}-mediated growth inhibition as one of the in vivo mechanisms through which TGFBR2 inactivation contributes to colon cancer formation. Thus, we have demonstrated that loss of TGFBR2 in colon epithelial cells promotes the establishment and progression of AOM-induced colon neoplasms, providing evidence from an in vivo model system that TGFBR2 is a tumor suppressor gene in the colon.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/1/7

The regulation of the epidermal growth factor receptor (egfr) gene in human cancer is not yet fully understood. Recent data on a polymorphic CA repeat located at the 5'-regulatory sequence in intron 1 of the egfr gene [egfr CA simple sequence repeat (SSR) I] point to a possible inheritance of cancer risk associated with the egfr gene. Furthermore, we have detected frequent allelic imbalances restricted to the egfr CA SSR I in breast cancer tissue and nontumorous breast tissue adjacent to invasive and in situ breast cancer representing amplifications. Therefore, we conducted a population-based case-control study to assess the relationship between the egfr polymorphism and breast cancer risk. Cases with a first primary breast cancer by age 50 years and age-matched population controls provided information on known and suspected risk factors. The allelic length of the egfr CA SSR was determined in 616 cases and 1072 population-sampled controls. Genotypes were categorized for analysis by allele length. Multivariate logistic regression was used to compare genotype distributions, accounting for other risk factors, and to investigate gene-environment interactions. We found a modifying effect, albeit no main effect, of the allelic length of the egfr polymorphism on breast cancer risk. The presence of two long alleles ([IMG]="BORDER="0">19 CA) was associated with a significantly elevated odds ratio (OR) of 10.4 [95% confidence interval (CI), 1.85-58.70] among women with a first-degree family history of breast cancer (P = 0.015 for interaction). The risk increase associated with high red meat consumption (OR, 10.68; 95% CI, 1.57-72.58) and the protective effect of high vegetable intake (OR, 0.07; 95% CI, 0.004-1.07) was also most pronounced among carriers of two long alleles ([IMG]="BORDER="0">19 CA). The length of the egfr CA SSR may increase the risk for familial breast cancers, and its effect could be modulated by dietary factors.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/18/5727

Recent studies suggest that genetic polymorphisms of the estrogen receptor-{alpha} (ER-{alpha}) gene may be associated with breast cancer risk. To evaluate the role of this gene in the risk of breast cancer, we genotyped a newly identified GT dinucleotide repeat [(GT)n] polymorphism located in the promoter region (6.6 kb upstream of the transcription start site) in 947 breast cancer cases and 993 age frequency-matched community controls from a population-based case-control study conducted among Chinese in urban Shanghai. Sixteen alleles were identified, the most common one having 16 GT repeats [(GT)16]. Compared with subjects homozygous for
this allele, subjects carrying the (GT)17 or (GT)18 allele had a decreased risk of breast cancer. The odds ratios (ORs) were 0.81 [95% confidence interval (CI), 0.62-1.06] and 0.58 (95% CI, 0.36-0.94), respectively, for one and two copies of the (GT)17 or (GT)18 allele. The inverse association with carrying either of these alleles was stronger among women with >30 years of menstrual cycles (OR 0.66; 95% CI 0.51-0.85) than those with a shorter duration of menstrual cycles (OR 0.97; 95% CI 0.73-1.27), and the test for an interaction was statistically significant (P = 0.04). Among breast cancer patients, the presence of either the (GT)17 or (GT)18 allele was associated with a reduced expression of progesterone receptor. Results of this study indicate that the GT dinucleotide repeat polymorphism in ER-\( \alpha \) gene promoter region may be a new biomarker for genetic susceptibility to breast cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/8/2406

Oxytocin receptors (OTRs) are expressed in numerous tissues, including human normal endothelium. Here we investigated the expression and biological significance of OTRs in Kaposi's sarcoma (KS), an intensely angioproliferative disease of possible vascular origin with a prominent inflammatory component. Immunohistochemistry and in situ hybridization studies showed OTR expression in tumor cells of cutaneous classic and AIDS-related KS lesions. OTR mRNA and protein were also detected on cultured KS-IMM spindle cells by reverse transcription-PCR and immunofluorescence procedures. In these cells, OTR expression was up-regulated by the supernatants of resting CD4+ and CD8+ lymphocytes through a still unidentified factor. Functionality of OTRs was demonstrated because OT treatment of KS-IMM cells led to a significant increase in cell proliferation, coupled to the increase of intracellular calcium, but did not effect cell migration in vitro or angiogenesis in vivo. In addition, we demonstrated that CD4+ and CD8+ cells produce OT themselves, thus constituting an intralesional source of peptide. These results indicate that: (a) functioning OTRs are expressed in KS cells and modulated by the inflammatory counterpart of KS lesions; (b) via OTRs, OT stimulates KS-IMM cell proliferation and could, therefore, be considered a new possible relevant growth factor involved in KS progression; and finally (c) the evidence of OT synthesis by CD4+ and CD8+ lymphocytes strongly suggests the existence of local endocrine-immunological cross-talk in Kaposi's sarcoma.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/9/2028

Despite the increasing clinical applications of circulating EBV DNA analysis as a tumor marker, the molecular nature of these EBV DNA molecules remains unclear. We subjected plasma/serum samples of nasopharyngeal carcinoma and lymphoma patients to DNase digestion and ultracentrifugation and showed that circulating EBV DNA molecules are "naked" DNA fragments instead of being contained inside virions. We further showed that these EBV DNA fragments were relatively short, and 87% of them were shorter than 181 bp. These results provide fundamental information that may improve our understanding of the release of tumor-derived nucleic acids into the blood of cancer patients.

Chang, J.-Y., J.-F. Liu, et al. (2002). "Novel Mutation of Topoisomerase I in Rendering Cells Resistant to
To identify mechanisms of camptothecin (CPT) resistance and the relationship between CPT-resistant cells and other anticancer agents, a CPT-resistant cell line (CPT30) and its partial revertant cell line (CPT30R) were established from a human nasopharyngeal carcinoma cell line (HONE-1). CPT30 and CPT30R cells displayed a 14- and 3.5-fold resistance to CPT compared with HONE-1 cells, respectively. The resistant and partial revertant cell lines showed cross-resistance to topotecan and increased sensitivity to cisplatin, carboplatin, and 1,3-bis(chloroethyl)-1-nitrosurea. The topoisomerase (Top) I catalytic activity of CPT30 and CPT30R cells was 30% and 200%, respectively, compared with that of HONE-1 cells. The expression of Top I protein and mRNA levels in CPT30 cells was 40% and 30% less than that in HONE-1 cells, respectively, whereas in CPT30R cells, the levels of Top I protein and mRNA were 50% and 20% higher, respectively, than that in HONE-1 cells. Both the resistant and revertant cell line whole-cell lysates demonstrated different levels of sensitivity to CPT in in vitro assays in comparison with that of HONE-1 cells. Furthermore, CPT exhibited 15- and 7-fold better binding affinity in stabilizing protein-linked DNA breaks in HONE-1 cells than in CPT30 and CPT30R cells, respectively. Direct DNA sequencing of the reverse transcription-PCR product and genomic DNA revealed a point mutation resulting in E418K mutation in the Top I of both CPT30 and CPT30R cells. Wild-type Top I RNA and genomic DNA were also detected in these two cell lines. A yeast system was used to examine whether this mutation could be responsible for CPT resistance. Our results showed that a single amino acid change (E418K) resulted in CPT resistance. Therefore, quantitative and qualitative changes in Top I were responsible for CPT resistance in CPT30 cells. CPT resistance in CPT30R cells was caused by mutation of Top I.


Glycine N-methyltransferase (GNMT) affects genetic stability by (a) regulating the ratio of S-adenosylmethionine to S-adenosylhomocysteine and (b) binding to folate. Based on the identification of GNMT as a 4 S polyaromatic hydrocarbon-binding protein, we used liver cancer cell lines that expressed GNMT either transiently or stably in cDNA transfections to analyze the role of GNMT in the benzo(a)pyrene (BaP) detoxification pathway. Results from an indirect immunofluorescent antibody assay showed that GNMT was expressed in cell cytoplasm before BaP treatment and translocated to cell nuclei after BaP treatment. Compared with cells transfected with the vector plasmid, the number of BaP-7,8-diol 9,10-epoxide-DNA adducts that formed in GNMT-expressing cells was significantly reduced. Furthermore, the dose-dependent inhibition of BaP-7,8-diol 9,10-epoxide-DNA adduct formation by GNMT was observed in HepG2 cells infected with different multiplicities of infection of recombinant adenoviruses carrying GNMT cDNA. According to an aryl hydrocarbon hydroxylase enzyme activity assay, GNMT inhibited BaP-induced cytochrome P450 1A1 enzyme activity. Automated BaP docking using a Lamarckian genetic algorithm with GNMT X-ray crystallography revealed a BaP preference for the S-adenosylmethionine-binding domain of the dimeric form of GNMT, a novel finding of a cellular defense against potentially damaging exposures. In addition to GNMT, results from docking experiments showed that BaP binds readily with other DNA methyltransferases, including HhaI, HaellI, PvuII methyltransferases and human DNA methyltransferase 2. We therefore hypothesized that BaP-DNA methyltransferase and BaP-GNMT interactions may contribute to carcinogenesis.
Clusterin is a widely expressed glycoprotein that has been paradoxically observed to have both pro- and antiapoptotic functions. Recent reports suggest this apparent dichotomy of function may be related to two different isoforms, one secreted and cytoplasmic, the other nuclear. To clarify the functional role of clusterin in regulating apoptosis, we examined its expression in human colon cancer tissues and in human colon cancer cell lines. We additionally explored its expression and activity using models of adenomatous polyposis coli (APC)- and chemotherapy-induced apoptosis. Clusterin RNA and protein levels were decreased in colon cancer tissues largely devoid of wild-type APC when compared with matched normal tissue controls, suggesting a means for invasive cancers to avoid apoptosis. Conversely, induction of apoptosis by expression of wild-type APC or by treatment with chemotherapy led to increased clusterin RNA and protein levels localizing to apoptotic nuclei. We found that transient transfection of clusterin to colon cancer cell lines directly enhanced basal and chemotherapy-induced apoptosis. Clusterin-induced apoptosis was inhibited by antisense clusterin and was found to be highly dependent on p21 but not p53 expression, yet a deficit in p21 can be subverted by clusterin transfection. Collectively, these data support the hypothesis that nuclear clusterin function is proapoptotic when induced by APC or chemotherapy in the context of p21 expression. Absent of p21, clusterin is not induced, and apoptosis is significantly inhibited. These data support a potential therapeutic role for clusterin in enhancing chemotherapy-induced apoptosis and in promoting apoptosis in cells deficient in p21.


Breast cancer metastasis suppressor 1 (BRMS1) functions as a metastasis suppressor gene in breast cancer and melanoma cell lines, but the mechanism of BRMS1 suppression remains unclear. We determined that BRMS1 expression was inversely correlated with that of urokinase-type plasminogen activator (uPA), a prometastatic gene that is regulated at least in part by nuclear factor-{kappa}B (NF-{kappa}B). To further investigate the role of NF-{kappa}B in BRMS1-regulated gene expression, we examined NF-{kappa}B binding activity and found an inverse correlation between BRMS1 expression and NF-{kappa}B binding activity in MDA-MB-231 breast cancer and C8161.9 melanoma cells stably expressing BRMS1. In contrast, BRMS1 expression had no effect on activation of the activator protein-1 transcription factor. Further, we showed that suppression of both constitutive and tumor necrosis factor-{alpha}-induced NF-{kappa}B activation by BRMS1 may be due to inhibition of I{kappa}B{alpha} phosphorylation and degradation. To examine the relationship between BRMS1 and uPA expression in primary breast tumors, we screened a breast cancer dot blot array of normalized cDNA from 50 breast tumors and corresponding normal breast tissues. There was a significant reduction in BRMS1 mRNA expression in breast tumors compared with matched normal breast tissues (paired t test, P < 0.0001) and a general inverse correlation with uPA gene expression (P < 0.01). These results suggest that at least one of the underlying mechanisms of BRMS1-dependent suppression of tumor metastasis includes inhibition of NF-{kappa}B activity and subsequent suppression of uPA expression in breast cancer and melanoma cells.
Studies of Wnt activation in gastric cancer have yielded conflicting results. The goals of this study were to determine the frequency of Wnt pathway activation and β-catenin mutation in these tumors. Three hundred eleven gastric cancers were examined for β-catenin expression by immunostaining and dissected using laser capture microscopy to obtain DNA from those tumors with nuclear β-catenin. Exon 3 of β-catenin was amplified using PCR and sequenced. Ninety gastric cancers (29%) displayed nuclear β-catenin. DNAs from 73 tumors were amplified and sequenced; 19 (26%) contained mutations in exon 3 of β-catenin, whereas no mutations were detected in 19 tumors negative for β-catenin nuclear staining (P < 0.05). Most mutations were adjacent to or abolished known regulatory phosphorylation sites. Mutations in exon 3 of β-catenin are common in gastric cancer that display nuclear β-catenin. These results suggest that Wnt pathway activation contributes to carcinogenesis in a subset of gastric adenocarcinomas.


Biallelic mutations in Fanconi anemia complementation group genes disrupt DNA repair and result in the complex Fanconi anemia phenotype. In addition, germ line mutations in the BRCA2/FANCD1 Fanconi anemia complementation group gene have also been implicated in predisposition to a number of cancers including pancreatic cancer. The recent identification of FANCC and FANCG mutations in resected pancreatic tumors selected for loss of heterozygosity on chromosome 9, some of which were present in the germ line DNA, suggests that inactivation of these and other Fanconi complementation group genes may contribute to pancreatic cancer. To further assess the relevance of FANCC and FANCG mutations to pancreatic cancer we conducted a mutation screen of these genes in DNA from blood of 421 sequentially collected pancreatic cancer cases diagnosed at the Mayo Clinic. Two truncating FANCC mutations but no truncating FANCG mutations were identified in young onset (<55 years) pancreatic cancer cases with no family history of pancreatic cancer. Both mutations were associated with loss of heterozygosity of the wild-type allele in corresponding pancreatic tumors. In addition, no truncating mutations were identified in germ line DNA from blood of 658 control individuals undergoing routine colonoscopy. Taken together these data support the assertion that inherited mutations in FANCC can predispose to pancreatic cancer.

cell invasiveness through Matrigel-coated porous membranes in an EC human cell line Hec1A, which expresses the LH/hCG receptor. This effect turned out to depend on hrLH binding to its specific receptors and to the subsequent activation of protein kinase A (PKA). Moreover the hrLH-induced increase in Hec1A invasiveness relied upon a PKA-dependent functional activation of β1 integrin receptors, as well as the subsequent induction of matrix metalloproteinase-2 secretion in its active form. The same mechanisms were also found to be operative in primary EC cells. In fact, a significant percentage of primary ECs expressed the LH/hCG receptor, and hrLH addition to primary EC cells, which expressed the specific receptors produced an increase in cell invasiveness only in those tumor cells possessing the specific receptors. This effect was also dependent on PKA activity. We conclude that LH/hCG can regulate EC cells invasiveness, and this result provides a rationale for the use of inhibitors of LH secretion such as GnRH analogues in the treatment of EC.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/18/6032

This study assessed the immunological and clinical responses of women with human papillomavirus (HPV) 16-associated high-grade vulval intraepithelial neoplasia (VIN) vaccinated with TA-HPV, a recombinant vaccinia virus encoding modified HPV 16 and 18 E6 and E7. Eighteen women with HPV 16-positive high-grade VIN were vaccinated with TA-HPV. The extent of their baseline disease was compared after 24 weeks by lesion measurements and histological analysis. Viral load was assessed pre- and postvaccination by real time PCR. Cell-mediated immunity to HPV 16 E6 and/or E7 peptides (HLA-A2 epitopes) or vaccinia-infected cell lysates was determined by IFN-(gamma) enzyme-linked immunospot (ELISPOT) and T cell proliferation using an HPV 16 L2E6E7 fusion protein. Antibodies were measured by ELISA using vaccinia-infected cell lysates or HPV 16 and 18 E6 and E7 glutathione S-transferase-fusion proteins. Lesion-infiltrating CD4+, CD8+, CD1a+, and CD68+ immune cells were assessed by immunohistochemistry. The single vaccination with TA-HPV was well tolerated, and all patients showed an increased ELISPOT and/or antibody response to vaccinia. There were significant differences in HPV-16 E7-specific ELISPOT and L2E6E7 proliferative responses in the patients at one or more time points postvaccination as compared with the prevaccination status; two patients showed transient increased antibody responses. Overall, 13 women showed an increased HPV 16-specific immune response by one or more methodologies after immunization. Eight patients demonstrated a reduction in lesion diameter of at least 50% and a further four patients showed significant symptom relief. Viral load was reduced or cleared in six of eight lesion responders but also in six of ten nonresponders. Before vaccination, clinical responders had significantly higher levels of lesion-associated CD4+, CD8+, and CD1a+-immune cells than nonresponders. There were no differences in CD68 (macrophages) between responders and nonresponders before or after vaccination. Nonresponders did show a significant increase in CD4+ and CD8+ but not CD1a+-immune cells postvaccination but at lower levels overall than responder patients. Local immune infiltration may be a critical factor in potential responsiveness to vaccine therapy in HPV-associated neoplasia and should be carefully monitored in future placebo-controlled trials of immunotherapy for VIN.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/14/3891
The cyclooxygenase (COX) pathway is important in colorectal carcinogenesis with the majority of cancers overexpressing COX-2; however, the role of COX-2 in the development of colorectal adenomas is less well defined. Accordingly, we analyzed 108 colorectal adenomas for COX-1 and COX-2 transcription in archival formalin-fixed, paraffin-embedded tissue using real-time PCR and normalized to {beta}-actin. Neither COX-1 nor COX-2 mRNA expression differed with regard to age or gender of the subject. COX-2 mRNA expression was significantly higher in distal adenomas (2.2 \ (+/-) 1.9) compared with proximal (0.7 \ (+/-) 0.5) adenomas (P < 0.0001) and in larger (>7 mm) compared with smaller (<7 mm) adenomas (2.3 \ (+/-) 2.2 and 1.7 \ (+/-) 1.3, respectively, P = 0.04). COX-2 expression did not differ significantly in tubular compared with tubulovillous adenomas, although there appeared to be a trend toward higher COX-2 expression in tubulovillous adenomas with increasing villous content. Additionally, there was not a significant difference in either COX-1 or COX-2 based on the degree of dysplasia Therefore, if COX-2 inhibitors work through a COX-2 mechanism, these agents may have differential effects on colorectal adenomas that are distal and larger.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/23/7093

Infection of the gastrointestinal tract by the human polyomavirus, JCV, which has been frequently detected in raw urban sewage, can occur via intake of contaminated water and food. In light of earlier reports on the tumorigenecity of JCV, we investigated the presence of the JCV genome and the expression of viral proteins in a collection of 27 well-characterized epithelial malignant tumors of the large intestine. Results from gene amplification revealed the presence of the viral early genome in 22 of 27 samples. Expression of the viral oncogenic early protein, T-antigen, and the late auxiliary protein, Agnoprotein, was observed in >50% of the samples. The absence of the viral capsid protein in the tumor cells excludes productive replication of the virus in neoplastic cells. Laser capture microdissection confirmed the presence of the JCV genome and expression of T-antigen in precancerous villous adenomas and regions of invasive adenocarcinoma. The ability of JCV T-antigen to interact with {beta}-catenin and the nuclear detection of {beta}-catenin in T-antigen-positive cells suggests dysregulation of the Wnt pathway in the tumor cells. The coinfection of T-antigen and {beta}-catenin in colon cancer cells enhanced transcription of the c-myc promoter, the downstream target of {beta}-catenin. These observations provide evidence for a possible association of JCV with colon cancer and suggest a novel regulatory role for T-antigen in the deregulation of the Wnt signaling pathway through {beta}-catenin in tumors of the gastrointestinal tract.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/18/5745

Induced chemoresistance leads to the reduction of apoptotic responses. Although several drugs are in development that circumvent or decrease existing chemoresistance, none has the potential to prevent or reduce its induction. Here, we present data from a drug that could perhaps fill this gap. Cotreatment of chemotherapy with (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU, RP101) prevented the decrease of apoptotic effects during the course of chemotherapy and reduced nonspecific toxicity. Amplification of chemoresistance genes (Mdr1 and Dhfr) and overexpression of gene products involved in proliferation (DDX1) or DNA repair (UBE2N and APEX) were inhibited, whereas activity of NAD(P)H: quinone oxidoreductase 1 (NQO1) was enhanced. During recovery, when treatment was with BVDU only, microfilamental proteins were up-regulated, and
proteins involved in ATP generation or cell survival (STAT3 and JUN-D) were down-regulated. That way, in three different rat tumor models, the antitumor efficiency of chemotherapy was optimized, and toxic side effects were reduced. Because of these beneficial properties of BVDU, a clinical pilot Phase I/II study with five human tumor entities has been started at the University of Dresden (Dresden, Germany). So far, no unwanted side effects have been observed.

http://cancerres.aacrjournals.org/cgi/content/abstract/63/17/5438

Multiple myeloma (MM) is an incurable B-cell malignancy able to mediate massive destruction of the axial skeleton. The aim of this study was to examine the involvement of the tumor necrosis factor-ligand family member, receptor activator of nuclear factor-(kappa)B ligand (RANKL), and its naturally occurring antagonist, osteoprotegerin (OPG), in MM biology. Using flow cytometry and two independent anti-RANKL antibodies, we demonstrate RANKL expression in CD38+++CD45+ and CD38+++CD45- myeloma plasma cell (MPC) subpopulations derived from patients with osteolytic MM. In addition, highly purified subpopulations of MPC express mRNA for both transmembrane and soluble RANKL isoforms but lack expression of OPG mRNA and protein. We also show that RANKL expressed by MPC is functional as in vitro coculture of CD38+++CD45+ and CD38+++CD45- MPC subpopulations with peripheral blood mononuclear cells resulted in the formation of multinucleate, tartrate-resistant acid phosphatase-positive osteoclasts-like cells capable of forming typical resorption pits. Furthermore, high expression of membrane-associated RANKL by CD38+++ MPC correlated with the presence of multiple radiological bone lesions in individuals with MM. Together, our data strongly suggest that RANKL expression by MPC confers on them the ability to participate directly in the formation of osteoclast in vivo and extends our knowledge of the involvement of RANKL and OPG in the osteolysis characteristic of this disease.

http://cancerres.aacrjournals.org/cgi/content/abstract/65/6/2147

Clinical staging of cervical lymph nodes from patients with squamous cell carcinoma of the head and neck (SCCHN) has only 50% accuracy compared with definitive pathologic assessment. Consequently, both clinically positive and clinically negative patients frequently undergo neck dissections that may not be necessary. To address this potential overtreatment, sentinel lymph node (SLN) biopsy is currently being evaluated to provide better staging of the neck. However, to fully realize the potential improvement in patient care afforded by the SLN procedure, a rapid and accurate SLN analysis is necessary. We used quantitative reverse transcription-PCR (QRT-PCR) to screen 40 potential markers for their ability to detect SCCHN metastases to cervical lymph nodes. Seven markers were identified with good characteristics for identifying metastatic disease, and these were validated using a set of 26 primary tumors, 19 histologically positive lymph nodes, and 21 benign nodes from patients without cancer. Four markers discriminated between positive and benign nodes with accuracy >97% but only one marker, pemphigus vulgaris antigen (PVA), discriminated with 100% accuracy in both the observed data and a statistical bootstrap analysis. A rapid QRT-PCR assay for PVA was then developed and incorporated into a prototype instrument capable of performing fully automated RNA isolation and QRT-PCR. The automated analysis with PVA provided perfect discrimination between histologically positive and benign lymph nodes and correctly identified two lymph nodes with micrometastatic tumor deposits. These assays were completed (from tissue to result) in ~30 minutes, thus demonstrating the
feasibility of intraoperative staging of SCCHN SLNs by QRT-PCR.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/1/186

The p53 tumor suppressor protein plays a pivotal role in preventing uncontrolled cellular proliferation. By contrast, zinc deprivation enhances esophageal cell proliferation and the induction of esophageal tumors in rodents by N-nitrosomethylbenzylamine (NMBA). We investigated whether p53 deficiency rendered zinc-deficient (ZD) mice more susceptible to NMBA-induced esophageal/forestomach carcinogenesis. At 6-7 weeks of age, p53 null (-/-), heterozygous (+/-), and wild-type (+/+) mice were placed on ZD or zinc-sufficient (ZS) diets to form six experimental groups: ZD:p53-/-; ZD:p53+/-; ZD:p53+/-; ZS:p53-/-; ZS:p53+/-; and ZS:p53+/. After 3 weeks, 15-23 mice in each group were treated once with NMBA (2 mg/kg body weight). Control animals were untreated. Zinc deficiency alone induced unrestrained cellular proliferation in the esophagus and forestomach of p53-/- mice. Forestomach tumors were first detected in a ZD:p53-/- mouse at 13 days. By 30 days, 100% (21 of 21) of ZD:p53-/- mice developed forestomach tumors and 38% showed esophageal tumors versus 42 and 0% in ZS:p53-/- mice (P < 0.004, esophagus; P < 0.001, forestomach). ZD:p53-/- mice showed an accelerated progression to malignancy, with 10% of esophageal tumors and 38% of forestomach tumors presenting as carcinomas. Nearly 20% of ZD:p53-/- mice developed esophageal Barrett's metaplasia, a lesion not previously seen in NMBA-induced neoplasia. ZD:p53+/+ mice had significantly higher tumor incidence than ZS:p53-/- mice. The order of tumor incidence in forestomach was as follows: naught incidence in ZS:p53+/+ mice; ZD:p53-/- > ZD:p53+/+ > ZS:p53-/- > ZS:p53+/+ > [&gt;=] ZS:p53+/+ > ZS:p53+/+. The rapid rate of tumor induction/progression in ZD:p53-/- mice was accompanied by an increase in the rate of cell proliferation and a decrease in apoptosis. cDNA array expression analysis of known genes identified a 5-fold up-regulation of cytokeratin 14 mRNA expression in ZD:p53-/- versus ZS:p53+/+ forestomach, a result showing gene-modulating effects of zinc deficiency. Cytokeratin 14 is a biomarker in human esophageal carcinogenesis. Our findings provide in vivo evidence for the collaboration of a deficiency of both p53 and zinc in esophageal carcinogenesis and reveal molecular targets of this collaboration.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/24/8674

To identify methylation-mediated silencing of genes in hepatocellular carcinoma (HCC), we surveyed genes induced by treatment with 5-aza-2'-deoxycytidine (5-Aza-CdR) in six human hepatoma cell lines using cDNA microarray analysis and determined the methylation status of 5'CpG islands by bisulfite DNA sequencing or methylation-specific PCR. Fifty genes exhibited a >5-fold induction in response to treatment with 5-Aza-CdR in at least one of the hepatoma cell lines examined. Among these genes, the hepatocyte growth factor activator inhibitor 2/placental bikunin (HAI-2/PB) gene was maximally induced by 5-Aza-CdR in three of six cell lines studied (HLE, HuH7, and Hep3B). Bisulfite sequencing revealed that the 5' CpG island of this gene was densely methylated in HLE, HuH7, and Hep3B cells. After treatment with 5-Aza-CdR, re-expression and demethylation of HAI-2/PB gene were detected in these cells. These findings suggest that HAI-2/PB expression may be inappropriately repressed by promoter hypermethylation in HCC. Methylation-specific PCR analysis demonstrated that HAI-2/PB
hypermethylation occurred in 21 of 26 HCC tumors (80.8%), whereas in the corresponding nontumorous liver tissues, it was found in 7 of 26 samples (26.9%). In addition, HAI-2/PB hypermethylation was not detected in any of the seven normal liver samples from individuals without HCC. Reverse transcription-PCR analysis demonstrated that promoter hypermethylation was associated with the reduced expression of the HAI-2/PB gene in HCC tumors. In conclusion, we have found that the HAI-2/PB gene is silenced by promoter hypermethylation in human hepatoma cells by means of cDNA microarray analysis after 5-Aza-CdR treatment, and that HAI-2/PB hypermethylation occurs frequently in primary HCC tumors.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/7/2617

Peritoneal dissemination is one of the main causes of death in cancer patients. Pathophysiology of metastasis has been well investigated, but the mechanism of diffuse spread of tumor colonies in the peritoneal cavity is not fully understood. CD9 is a member of tetraspanin and its down-regulation is known to be involved in poor prognosis. To investigate the significance of the down-regulation of CD9, HTOA, an ovarian carcinoma cell line that highly expressed CD9, was transiently transfected with small interfering RNA (siRNA) against CD9, and CD9-negative cells (HTOACD9-) were purified. HTOACD9- showed altered adhesion patterns on Matrigel, collagen, fibronectin, and laminin compared with those of control siRNA-transfected HTOA (control-HTOA). Flow cytometry and fluorescence cytostainings revealed that the expression levels of integrins \{beta\}1, \{alpha\}2, \{alpha\}3\{beta\}1, \{alpha\}5, and \{alpha\}6 were lower in HTOACD9- than those of control-HTOA. HTOACD9- showed altered expression of junctional and cytoskeletal molecules. By time-lapse video microscopy, control-HTOA showed solid adhesion to extracellular matrix and formed cobblestone pattern, whereas HTOACD9- showed weaker adhesion and were distributed as diffuse spots. To examine whether the expression level of CD9 change during tumor dissemination, HTOA-P, a highly disseminative subclone of HTOA, was established. HTOA-P showed distinctive down-regulation of CD9 at mRNA and protein levels, and showed similar morphologic alteration as HTOACD9- did. These findings indicate that the down-regulation of CD9 may be an acquired event in the process of tumor dissemination. Down-regulated CD9 may attenuate the expression of several integrins and rearrange junctional and cytoskeletal molecules that might contribute to dissemination of ovarian carcinomas.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/11/3058

Because some studies have linked polymorphic variants of the estrogen-metabolizing genes CYP17 and catechol-O-methyltransferase (COMT) with risk for hormonally related cancers, we sought to determine whether selected polymorphisms of these genes differed between women with and without ovarian cancer. From a population-based study of ovarian cancer, we analyzed DNA from a total of 480 cases and controls. PCR amplification was performed using primers that amplify restriction sites for MspAI (A2 polymorphism-CYP17) and NlaIII (Val/Met polymorphism-COMT). Digestion of the PCR products was performed. Genotypes identified by gel electrophoresis were assigned as homozygous wild type (WW), heterozygous variant (Wv), and homozygous variant (vv). Frequencies were compared using \{chi\}2 or Fisher's exact tests. Logistic regression was used to calculate crude and adjusted relative risks (RRs) for ovarian
cancer associated with possession of any variant allele overall, and within demographic, weight, and smoking history categories, and by histological subtype of ovarian cancer. A portion (68.9%) of cases either carried or was homozygous for the A2 variant of CYP17 compared with 53.9% of controls, for a RR (and 95% confidence interval) of 1.86 (1.26, 2.75; P = 0.003), adjusted for age, parity, oral contraceptive use, site of study, and family history of breast or ovarian cancer. The increased risk was most apparent for women >50 and women with invasive serous cancers. A portion (71.9%) of cases either carried or was homozygous for the Val/Met variant of COMT, compared with 76.9% of controls (P = 0.27). Although the inverse association of ovarian cancer with possession of a Val/Met variant was not significant overall, it was for mucinous tumors of the ovary, with an adjusted RR of 0.28 (0.13, 0.61; P = 0.0012). Possession of the A2 variant of CYP17 appears to increase risk for ovarian cancer, whereas possession of the Val/Met variant of COMT decreases the risk for mucinous tumors. Confirmation in other populations and further exploration of potential pathogenetic mechanisms will be necessary.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/10/3572

The expression of the Mr 67,000 laminin receptor, a nonintegrin laminin receptor, was found to be up-regulated in neoplastic cells and to directly correlate with invasion and metastatic potential. In the present study, we investigated the role of laminin receptor in mediating laminin effects and the involvement of the mitogen-activated protein kinases (MAPK) cascades and dual-specificity phosphatases in laminin signaling in human melanoma cells. Using stable transfection of A375SM melanoma cells, we established lines expressing reduced or elevated laminin receptor. The antisense-transfected cells demonstrated reduced attachment to laminin and reduced invasion through Matrigel-coated filters. In addition, both matrix metalloproteinase-2 (MMP-2) mRNA expression and activity were significantly reduced in the antisense-transfected cells. Antisense-transfected cells showed a reduction in mRNA level of the (alpha)6B integrin subunit isoform, whereas no change in the mRNA level of the (alpha)6A isoform was observed. We found that exogenous laminin reduced the phosphorylated (active) form of extracellular signal-regulated kinase, c-Jun NH2-terminal protein kinase, and p38 in all of the cells, irrespective of the expression of the laminin receptor. Furthermore, the phosphorylation of extracellular signal-regulated kinase, c-Jun NH2-terminal protein kinase, and p38 was significantly higher in the cell lines expressing reduced laminin receptor, regardless of the exposure to exogenous laminin. This increase of MAPK phosphorylation was accompanied by a significant reduction in MKP-1 phosphatase mRNA level and a significant increase in PAC-1 phosphatase mRNA level. In conclusion, our results confirm the involvement of the laminin receptor in different mechanisms related to tumor dissemination and provide first evidence of the involvement of MAPK and dual-specificity phosphatases in its signal transduction pathway.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/9/3014

Loss of PTEN tumor suppressor function is observed in tumors of breast, prostate, thyroid, and endometrial origin. Allelic losses in the proximity of the PTEN locus (10q23) also occur in sporadic colorectal cancers (CRCs), but biallelic inactivation of this site has not been frequently demonstrated. We hypothesized that alternative mechanisms of PTEN allelic inactivation, such as promoter hypermethylation, might be operative in CRC and that PTEN inactivation may be related to recognized forms of genomic instability. We characterized a cohort of 273 sporadic CRCs by
determining their microsatellite instability (MSI) status. Of these, 146 cancers were examined for PTEN promoter methylation by methylation-specific PCR. Mutations at the poly(A)6 repeat sequences in PTEN exons 7 and 8 and deletions at the 10q23 locus were also identified using microsatellite analysis. The presence of PTEN protein was determined by immunostaining, and the results were correlated with the promoter methylation status. We observed that PTEN promoter hypermethylation was a frequent occurrence in MSI-high (MSI-H) tumors (19.1% of MSI-H versus 2.2% of MSI-low/microsatellite stable tumors; \( P = 0.002 \)). A PTEN mutation or a deletion event was present in 60% of the tumors with promoter region hypermethylation. Hypermethylation of the PTEN promoter correlated significantly with either decreased or complete loss of PTEN protein expression (\( P = 0.004 \)). This is the first demonstration of PTEN inactivation as a result of promoter hypermethylation in MSI-H sporadic CRCs. These data suggest that this silencing mechanism plays a major role in PTEN inactivation and, in colon cancer, may be more important than either allelic losses or inactivating mutations. The significant correlation of PTEN hypermethylation with MSI-H tumors further suggests that PTEN is an additional important "target" of methylation along with the hMLH1 gene in the evolution of MSI-H CRCs and also confers the "second hit" in the biallelic inactivation mechanism for some proportion of tumors.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/2/432

Loss of genetic material on chromosome 6 has been associated with progression of human melanomas. We showed previously that introducing chromosome 6 into metastatic human melanoma cell lines suppresses metastasis without affecting the ability of the hybrids to form progressively growing tumors. By subtractive hybridization comparing nonmetastatic chromosome 6-containing (neo6/C8161) versus parental (C8161) metastatic cells, the KISS1 metastasis suppressor gene was isolated. However, KISS1 mapped to chromosome 1q32. To identify upstream regulator(s) of (and downstream effectors of) KISS1, microarray hybridization comparing C8161 and neo6/C8161 variants was performed. TXNIP/V Dup1, a thioredoxin-binding protein, was expressed more highly in neo6/C8161 and in nonmetastatic melanomas. Increased TXNIP expression inhibited metastasis and up-regulated KISS1. Surprisingly, TXNIP also mapped to chromosome 1q. PCR karyotyping that refined the region on chromosome 6 identified CRSP3/DRIP130, a transcriptional coactivator, as a metastasis suppressor. CRSP3 transfectant cells had up-regulated KISS1 and TXNIP expression and were suppressed for metastasis. Quantitative real-time reverse-transcription PCR of clinical melanoma samples showed that loss of CRSP3 expression correlated with decreased KISS1 expression and increased metastasis. Thus, we implicated a specific gene on chromosome 6 in the etiology of melanoma metastasis and identified potential up-stream regulators of KISS1 and TXNIP.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/9/3334

Epidemiological studies and clinical observations suggest that nonsteroidal anti-inflammatory drugs and certain selective cyclooxygenase (COX)-2 inhibitors may reduce the relative risk of clinically evident prostate cancer. This prompted us to investigate the chemopreventive potential of celecoxib, a selective COX-2 inhibitor, against prostate carcinogenesis in a transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Similar to prostate cancer in humans,
prostate malignancies in TRAMP mice progress from precursor intraepithelial lesions, to invasive carcinoma that metastasizes to lymph nodes, liver, lungs, and occasionally to bone. The basal enzyme activity and protein expression of COX-2 is significantly higher (>4-fold) in the dorsolateral prostate of TRAMP mice up to 24 weeks of age compared with their nontransgenic littermates. Eight-week-old TRAMP mice were randomly divided and fed either control diet (AIN 76A) or a custom prepared AIN 76A diet containing 1500-ppm celecoxib ad libitum for 24 weeks, a dosage that would compare with the normal recommended dose for the treatment of human disease. Studies from two independent experiments, each consisting of 10 mice on test, showed that the cumulative incidence of prostate cancer development at 32 weeks of age in animals fed with AIN 76A diet was 100% (20 of 20) as observed by tumor palpation, whereas 65% (13 of 20), 35% (7 of 20), and 20% (4 of 20) of the animals exhibited distant site metastases to lymph nodes, lungs, and liver. Celecoxib supplementation to TRAMP mice from 8-32 weeks of age exhibited significant reduction in tumor development (5 of 20) with no signs of metastasis. Celecoxib feeding resulted in a significant decrease in prostate (56%; P < 0.0003) and genitourinary weight (48%; P < 0.008). Sequential magnetic resonance imaging analysis of celecoxib-fed mice documented lower prostate volume compared with the AIN 76A-fed group. Histopathological examination of celecoxib-fed animals showed reduced proliferation, and down-modulation of COX-2 and prostaglandin E2 levels in the dorsolateral prostate and plasma, respectively. These results correlated with retention of antimetastasis markers, viz E-cadherin, and (alpha)- and (beta)-catenin, along with a significant decrease in vascular endothelial growth factor protein expression. Celecoxib supplementation also resulted in enhanced in vivo apoptosis in the prostate as monitored by several techniques including a recently perfected technique of 99mTc-labeled annexin V in live animals followed by phosphor imaging. One striking observation in an additional study was that celecoxib feeding to mice with established tumors (16 weeks of age) significantly improved their overall survival (P = 0.014), compared with AIN 76A-fed group. Our findings suggest that celecoxib may be useful in chemoprevention of prostate cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/18/5808

It has previously been shown that the Copenhagen (COP) rat contains several genetic loci that contribute to its mammary tumor-resistant phenotype after 7,12-dimethylbenz(a)anthracene (DMBA) administration. One of these loci, mammary carcinoma susceptibility 1 (Mcs1), is located on the centromeric end of chromosome 2 and appears to act in a semidominant fashion. To confirm the existence and independent action of this locus and also aid in the identification of the physical location of the Mcs1 gene, congenic lines were generated by transferring the Mcs1 COP allele onto a Wistar Furth (WF) genetic background. Male carriers were genotyped using microsatellite markers spanning 20-30 cm of the Mcs1 locus. One of the congenic lines minimally retained the COP allele at D2Mit29 on the centromeric end of chromosome 2 and extended distally to D2Rat201. Heterozygous Mcs1 carrier rats were interbred, and the female offspring were treated with DMBA. The female rats from the Mcs1 congenic line that carried one or two COP alleles of the Mcs1 region had a significantly reduced (65 and 85%, respectively) tumor development (P < 0.001) compared with rats carrying zero COP alleles at this locus. A WF.COP-D2Mit29/D2Rat201 homozygous congenic strain derived at the N10 generation was treated with DMBA, and the COP homozygous rats developed 1.5 {+/-} 0.3 carcinomas/rat versus 6.3 {+/-} 0.5 in WF control rats (P < 0.0001). Fine mapping of this congenic interval using several recombinant lines identified three genetic loci within the Mcs1 congenic region that independently supported a tumor resistance phenotype. These genetic loci have been termed Mcs1a, Mcs1b, and Mcs1c. In rats for which each locus was homozygous for the COP allele, tumor development was reduced by ~60% compared with littermate controls. The identification of these independent loci within the Mcs1 COP allele provide a model of the genetic complexity of cancer.
Cyclooxygenase-2 (COX-2) is an inducible enzyme that converts arachidonic acid to prostaglandins. Overexpression of the COX-2 gene in mammary glands of transgenic mice was sufficient to induce tumorigenesis. We analyzed COX-2 expression in human breast cancers (and breast cancer cell lines) and adjacent ductal carcinoma in situ (DCIS) as well as its association with HER2/neu and clinicopathological variables. Archival primary breast carcinomas (n = 57), adjacent DCIS (n = 14) and DCIS alone (n = 2) were analyzed for COX-2 and HER2 expression by immunohistochemistry using specific monoclonal antibodies. An immunohistochemical scoring system was used. HER2 gene amplification had been analyzed previously by fluorescence in situ hybridization (n = 20). Histology of carcinomas included infiltrating ductal (n = 44), lobular (n = 2), and other (n = 7). Frozen breast cancers and adjacent normal tissue pairs (n = 9) were analyzed for COX-2 mRNA by reverse transcription-PCR. COX-2 and HER2 expression were also analyzed in human breast cancer cell lines (MCF-7, MCF-7/HER2, SK-BR-3, and MDA-MB-231) by immunoblotting. Cytoplasmic COX-2 expression was detected at an intermediate or high level in epithelial cells in 18 of 42 (43%) invasive breast cancers and in 10 of 16 (63%) cases of DCIS. Normal-appearing breast epithelia adjacent to cancer expressed COX-2 in 81% of cases and was generally focal and of similar or decreased intensity relative to adjacent neoplastic epithelia. COX-2 mRNA was detected in all samples analyzed by reverse transcription-PCR and was increased in eight of nine breast cancers relative to paired normal tissue. In archival tumors, no significant correlation was found between COX-2 and HER2 expression/amplification and clinicopathological variables. COX-2 expression was induced in MCF-7 cells stably transfected with HER2, in contrast to parental MCF-7 cells, and was detected in MDA-MB-231, but not SK-BR-3 cells. COX-2 is frequently overexpressed in invasive breast cancers and in adjacent DCIS and, thus, may be an early event in mammary tumorigenesis. Forced HER2 expression in MCF-7 cells was shown to up-regulate COX-2, although no association was found in human tumors. Our results suggest that nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors may be useful in the chemoprevention and therapy of human breast cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/10/2506

Little is known concerning promoters or gene therapy specific for ovarian cancer. To explore the potential use of IAI.3B isolated from ovarian cancer cells in gene therapy for ovarian cancer, we identified the promoter region of the IAI.3B gene and created a replication-selective adenovirus, AdE3-IAI.3B, driven by the promoter. Transient transfection experiments showed that the DNA segment located between -1816 and -1 bp resulted in preferential expression in ovarian cancer cells with negligible expression in squamous cell carcinoma and normal cells. The promoter activity of IAI.3B was almost the same as that of cytomegalovirus and an order of magnitude higher than those of midkine and cyclooxygenase-2 in ovarian cancer cells. AdE3-IAI.3B replicated as efficiently as the wild-type adenovirus and caused extensive cell killing in a panel of ovarian cancer cells in vitro. In contrast, squamous cell carcinoma and normal cells were not able to support AdE3-IAI.3B replication. In animal studies, AdE3-IAI.3B administered to flank and i.p. xenografts of ovarian cancer cells led to a significant therapeutic effect. These results demonstrate the usefulness of the IAI.3B promoter for generation of ovarian cancer-specific adenoviral vectors and provide a potential for the development of ovarian cancer-specific
oncolytic viral therapies.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/23/6796

Urothelial carcinoma of the renal pelvis and ureter may develop sporadically or as a manifestation of hereditary nonpolyposis colorectal cancer. The majority of hereditary nonpolyposis colorectal cancer is caused by mutation of the human DNA mismatch repair (MMR) genes and is detected by associated microsatellite instability (MSI). Seventy-three unselected urothelial carcinomas of the ureter and/or renal pelvis were screened for MSI using the National Cancer Institute-designated reference panel (plus BAT40). Instability of at least two microsatellite markers (MSI-high) was detected in 15 samples (21%). Immunohistochemical staining of the MMR proteins (hMSH2, hMLH1, or hMSH6) was absent in 13 of 15 (87%) MSI tumors, and alteration of coding sequence microsatellites (TGF(\beta)RII, Bax, hMSH3, and hMSH6) was found at frequencies of 7-33% in these samples. Tumors with MSI had significantly different clinical and histopathological features including higher prevalence in female patients, low tumor stage and grade, and a papillary and frequently inverted growth pattern. Our results suggest a molecular pathway of tumorigenesis that is similar to MMR-deficient colorectal cancers and consistent with the notion that the site distributions of hereditary or sporadic MSI-high tumors may reflect tissue-specific susceptibility to lesions processed by the MMR machinery.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/20/7361

Flotillin 2 (flot-2) is a highly conserved protein isolated from caveolae/lipid raft domains that tether growth factor receptors linked to signal transduction pathways. Flot-2 protein and mRNA were increased in tumorigenic and metastatic melanoma cell lines in vitro, and the immunostaining intensity increased substantially across a tissue array of melanocytic lesions. Flot-2 transfection transformed SB2 melanoma cells from nontumorigenic, nonmetastatic to highly tumorigenic and metastatic in a nude mouse xenograft model. SB2 cells stably transfected with the flot-2 cDNA (SB2-flot)-2 cells proliferated faster in the absence of serum, and their migration through Matrigel was additionally enhanced by thrombin. When SB2-flot-2 cells were compared with SB2-vector-control cells on a cancer gene pathway array, SB2-flot-2 cells had increased expression of protease activated receptor 1 (PAR-1) mRNA, a transmembrane, G-protein-coupled receptor involved in melanoma progression. PAR-1 and flot-2 were coimmunoprecipitated from SB2-flot-2 cells. Up-regulation of PAR-1 was additionally confirmed in SB2-flot-2 cells and melanoma cell lines. SB2-flot-2 cells transfected with flot-2-specific small-interfering RNAs made substantially less flot-2 and PAR-1 mRNA. In conclusion, flot-2 overexpression is associated with melanoma progression, with increased PAR-1 expression, and with transformation of SB2 melanoma cells to a highly metastatic line. Flot-2 binds to PAR-1, a known upstream mediator of major signal transduction pathways implicated in cell growth and metastasis, and may thereby influence tumor progression.

Hogarty, M. D., C. L. Winter, et al. (2002). "No Evidence for the Presence of an Imprinted Neuroblastoma
Deletion of the distal short arm of chromosome 1 occurs in 35% of primary neuroblastomas (NBs). These deletions tend to be large and extend to the telomere, but a common region within sub-band 1p36.3 is consistently lost. Despite intensive investigation, no candidate tumor suppressor gene within this region has been shown to undergo tumor-specific mutation consistent with biallelic inactivation. In addition, initial studies demonstrated preferential loss of the maternally inherited 1p homologue in NBs with 1p loss of heterozygosity (LOH) without MYCN amplification. This has led to the widely accepted hypothesis that a genomically imprinted NB suppressor gene is the target of 1p deletion in this subset. To test this hypothesis we have studied 293 primary NBs for LOH within 1p36.3 and determined the parental origin of the deleted 1p homologue. LOH within 1p36.3 was demonstrated in 55 NBs (19%). Of these, 29 occurred in tumors without MYCN amplification: 13 had deletion of the maternally inherited 1p, whereas 16 had deletion of the paternally inherited 1p (P = 0.58). These data strongly refute a parent-of-origin effect for 1p deletions in NB and exclude the existence of an imprinted NB suppressor locus in this region.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/16/5728

High mobility group A (HMGA) proteins are chromatinic proteins that do not have transcriptional activity per se, however, by interacting with the transcription machinery, they regulate, negatively or positively, the expression of several genes. We searched for genes regulated by HMGA1 proteins using microarray analysis in embryonic stem (ES) cells bearing one or two disrupted hmgal alleles. We identified 87 transcripts increased and 163 transcripts decreased of at least 4-fold in hmgal-/- ES cells. For some of them, a HMGA1-dose dependency was observed, because an intermediate level was observed in the heterozygous ES cells. When the expression analysis of these genes was extended to embryonic fibroblasts and adult tissues such as heart, spleen, and liver from hmgal-knockout mice, contrasting results were obtained. In fact, aside some genes showing the same HMGA1 regulation observed in ES cells, there were some genes that did not modify their expression, and others showing a HMGA1-mediated regulation but in an opposite direction. These results clearly indicate that HMGA1-mediated gene regulation depends on the cellular context. Finally for a couple of analyzed HMGA1-regulated genes, electrophoretic mobility shift assay and chromatin immunoprecipitation revealed a direct binding of HMGA1 proteins to their promoters, suggesting a HMGA1-direct regulation of their expression.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/17/4955

Allelic variants of the MDR-1 gene have been shown recently to influence protein expression and P-glycoprotein (P-gp) function in healthy volunteers. Therefore, 405 acute myeloid leukemia patients were investigated for somatic genotypes of the three most frequent single nucleotide polymorphisms (SNPs) in exons 12, 21, and 26. In all three loci, homozygous wild-type alleles were classified as genotype A, heterozygous as B, and homozygous mutant (alternative) allele as
C. Patients with the C genotype in exons 12 and 26 showed a lower median age (both \( P < 0.05 \)). Additionally, the C genotype in exons 12 and 26 was associated with cytogenetic poor risk aberrations (both \( P < 0.05 \)). A possible regulatory impact of the SNPs on MDR1 mRNA expression was investigated by a Real time-PCR assay. MDR1 expression was strongly correlated with a decreased complete remission rate (\( P = 0.01 \)) but failed to predict decreased overall survival (OS). There was a significant association of the A genotype in exons 21 (\( P = 0.05 \)) and 26 (\( P < 0.05 \)) with lower MDR1 expression, whereas the B variants showed highest MDR1 values at all three investigated gene loci. The A genotype in exon 26 was associated with lower OS (\( P < 0.01 \)). In these patients, worse OS is likely attributable to an increased risk of relapse (\( P < 0.001 \)). We were able to detect a linkage disequilibrium of the investigated SNPs, indicating combined polymorphisms that could affect the regulation of MDR1 expression. The A genotype of all SNPs demonstrated both lowest MDR1 values and significantly decreased OS (\( P < 0.05 \)) with a high probability of relapses (\( P < 0.01 \)). These observations indicate that allelic variants of the MDR1 gene may influence therapy outcome by additional mechanisms, different from P-gp expression on acute myeloid leukemia blasts, possibly involving pharmacokinetic effects of P-gp.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/13/3708

Thymomas are thymic epithelial tumors. Because most of them are rich in nonneoplastic T-cells, recurrent genetic aberrations have been reported only in the rare, lymphocyte-poor WHO types A, B3, and C. We have now investigated virtually the whole spectrum of thymomas, including the commoner types AB and B2, microdissecting or culturing neoplastic cells from these lymphocyte-rich thymomas and applying 41 microsatellite markers covering 17 loci on 10 chromosomes. In 28 cases, comparative genomic hybridization data were available. Apart from type A, there was striking heterogeneity between thymomas. Allelic imbalances were seen in 87.3% of the 55 cases, and MSI in 9.9%. Losses of heterozygosity (LOHs) were much the commonest aberration. Overall, they were most prevalent at four regions on chromosome 6. Aberrations elsewhere, affecting mainly 8p11.21 and 7p15.3, suggested a cortical footprint because they recurred only in the thymopoietically active type AB and B thymomas. LOHs were also seen at the adenomatous polyposis coli (APC) locus (5q21-22) in subsets of these thymomas, whereas combined LOHs at the APC, retinoblastoma (13q14.3), and p53 (17p13.1) loci were confined to a subset of B3 thymomas that had possibly evolved from APC-hemizygous B2 thymomas by tumor progression; indeed, thymomas combing B2 plus B3 features are common. Notably, some AB and B thymomas shared LOHs despite their nonoverlapping morphology and different clinical behavior. Finally, allelic imbalances at 8p11.21 and 16q22.1 (CDH1) were significantly more frequent in stage IV metastatic thymomas. We conclude that the WHO-defined histological thymoma types generally segregate with characteristic genetic features, type A thymomas being the most homogeneous. Many findings support the view that B2 and B3 thymomas form a continuum, with evidence of tumor progression. However, other findings imply that types A and AB are biologically distinct from the others, any potential invasiveness being severely restricted by a medullary commitment in the precursor cell undergoing neoplastic transformation.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/1/154
Mutations in the currently known mismatch repair genes cannot explain all cases of hereditary nonpolyposis colorectal cancer (HNPCC), and novel predisposing genes are actively sought. Recently, mutations in the DNA repair gene EXO1 have been implicated in HNPCC. One truncating and several missense changes were observed in familial colorectal cancer (CRC) cases but not in controls. We evaluated a series of European CRC patients and population controls to clarify whether EXO1 variants may indeed predispose to familial CRC. Several variants observed in patients were also observed in controls with similar frequencies, including the truncating variant proposed previously to be a disease-causing mutation. Thus, little evidence was obtained to support a major causative role of EXO1 in HNPCC, although we cannot exclude a role for EXO1 as a low penetrance cancer susceptibility or modifying gene.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/7/2619

Nonsteroidal signaling via the androgen receptor (AR) plays an important role in hormone-refractory prostate cancer. Previously, we have reported that the pleiotropic cytokine, interleukin (IL)-6, inhibited dihydrotestosterone-mediated expression of prostate-specific antigen in LNCaP cells (Jia et al., Mol Can Res 2003;1:385-92). In the present study, we explored the mechanisms involved in this inhibition and considered possible effects on AR nuclear translocation, recruitment of transcription cofactors, and the signaling pathways that may mediate this inhibitory effect. IL-6 neither induced nuclear localization of the AR nor inhibited dihydrotestosterone-induced nuclear translocation of the receptor. IL-6 did not affect AR or p160 coactivator recruitment to the transcription initiation complex on the prostate-specific antigen enhancer and promoter. Moreover, it did not lead to the recruitment of the coactivator silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) or histone deacetylase 1 (HDAC1) at the same sites. IL-6 did, however, prevent the recruitment of the secondary coactivator, p300, to the complex and partially abrogated by signal transducers and activators of transcription-3 knock-down using small interfering RNA. Our results show that IL-6 modulates androgen action through the differential recruitment of cofactors to target genes. These findings may account for the pleiotropic actions of IL-6 in malignant prostate cells.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/24/9185

Androgen receptor (AR) signals play a decisive role in regulating the growth and differentiation of both normal and cancerous prostate cells by triggering the regulation of target genes, in a process in which AR cofactors have critical functions. Because of the highly prostate-specific expression pattern of HOXB13, we studied the role of this homeodomain protein in prostate cells. Expression of HOXB13 was limited to AR-expressing prostate cells. Reporter transcription assay demonstrated that HOXB13 significantly suppressed hormone-mediated AR activity in a dose-responsive manner, and suppression was specific to AR with which HOXB13 physically interacts. Overexpression of HOXB13 further down-regulated the androgen-stimulated expression of prostate-specific antigen, and suppression of endogenous HOXB13 stimulated transactivation of AR. Functionally, HOXB13 suppressed growth of LNCaP prostate cancer cells, which could be counteracted by additional hormone-activated AR. On the other hand, the growth-suppressive function of HOXB13 in AR-negative CV-1 cells was not affected by AR. These results suggest
that HOXB13 functions as an AR repressor to modulate the complex AR signaling and subsequent growth regulation of prostate cancer cells. In addition to the loss of HOXB13 expression, maintaining AR may be an important step for prostate cancer cells to tolerate the suppressor function of HOXB13. Altogether, our data present a novel mechanism for the HOXB13-mediated repression of AR signaling, which can be interpreted to a growth-suppressive event.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/5/1567

Cyclooxygenase-2 (COX-2) was recently reported (M. Tsujii and R. N. DuBois, Cell, 83: 493-501, 1995) to affect the metastatic potential of cells. Previous studies (M. Fukuda, Cancer Res., 56: 2237-2244, 1996) indicated that sialyl Lewis antigen expression is correlated with hematogenous metastasis of colon cancer. In the present study, we investigated the interaction between COX-2 activity, expression of sialyl Lewis antigens, in vitro cancer cell adhesion to endothelial cells, and in vivo metastatic potential. Effects of COX-2 activity and prostaglandin E2 on cell adhesion, expression of sialyl Lewis antigens, and glycosyltransferase genes were determined in Caco-2-m (COX-2 low level), Caco-2-COX-2 (programmed to overexpress COX-2), and HT-29 (COX-2 high level) cells. Metastatic spread of these cells to the liver was also investigated. Caco-2-COX-2 cells had increased SPan-1 levels and increased adherence to endothelial cells via SPan-1 compared with Caco-2-m cells. HT-29 cells expressed sialyl Lewis a and adhered to endothelial cells via sialyl Lewis a. Treatment with a COX-2 inhibitor, celecoxib, decreased SPan-1 and sialyl Lewis a expression and adherence to endothelial cells. (beta)3Gal-T5 and ST3Gal III and IV expression was inhibited by celecoxib and was enhanced by prostaglandin E2 treatment. Caco-2-COX-2 and HT-29 cells metastasized to the liver, whereas Caco-2-m cells did not. Pretreatment with celecoxib reduced the metastatic potential as well as anti-sialyl Lewis antibodies. Our results indicate a direct link between COX-2 and enhanced adhesion of carcinoma cells to endothelial cells, and enhanced liver metastatic potential via accelerated production of sialyl Lewis antigens. COX-2 inhibitors may suppress metastasis.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/15/4583

Studies of families with Birt-Hogg-Dube syndrome (BHD) have recently revealed protein-truncating mutations in the BHD gene, leading to tumorigenesis of the skin and of different cell types of kidney. To additionally evaluate the role of BHD in kidney tumorigenesis, we studied 39 sporadic renal tumors of different cell types: 7 renal oncocytomas, 9 chromophobe renal cell carcinomas (RCCs), 11 papillary RCCs, and 12 clear cell RCCs. We screened for BHD mutations and identified a novel somatic mutation in exon 13: c.1939_1966delinsT in a papillary RCC. We performed loss of heterozygosity (LOH) analysis on 28 matched normal/tumor sets, of which 10 of 28 (36%) demonstrated LOH: 2 of 6 (33%) chromophobe RCCs, 5 of 6 (83%) papillary RCCs, 3 of 12 (25%) clear cell RCCs, but 0 of 4 renal oncocytomas. BHD promoter methylation status was examined by a methylation-specific PCR assay of all of the tumors. Methylation was detected in 11 of 39 (28%) sporadic renal tumors: 2 of 7 (29%) renal oncocytomas, 1 of 9 (11%) chromophobe RCCs, 4 of 11 (36%) papillary RCCs, and 4 of 12 (33%) clear cell RCCs. Five tumors with methylation also exhibited LOH. Mutation and methylation were absent in 9 kidney cancer cell lines. Our results showed that somatic BHD mutations are rare in sporadic renal tumors. The alternatives, LOH and BHD promoter methylation, are the two possible inactivating
mechanisms involved. In conclusion, unlike other hereditary kidney cancer-related genes (i.e., VHL and MET), which are cell type-specific, BHD is involved in the entire spectrum of histological types of renal tumors, suggesting its major role in kidney cancer tumorigenesis.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/8/3146

We did expressional profiling on 24 paired samples of normal esophageal epithelium, Barrett's metaplasia, and esophageal adenocarcinomas. Matching tissue samples representing the three different histologic types were obtained from each patient undergoing esophagectomy for adenocarcinoma. Our analysis compared the molecular changes accompanying the transformation of normal squamous epithelium with Barrett's esophagus and adenocarcinoma in individual patients rather than in a random cohort. We tested the hypothesis that expressional profiling may reveal gene sets that can be used as molecular markers of progression from normal esophageal epithelium to Barrett's esophagus and adenocarcinoma. Expressional profiling was done using U133A GeneChip (Affymetrix), which represent approximately two thirds of the human genome. The final selection of 214 genes permitted the discrimination of differential gene expression of normal esophageal squamous epithelium, Barrett's esophagus, and adenocarcinoma using two-dimensional hierarchical clustering of selected genes. These data indicate that transformation of Barrett's esophagus to adenocarcinoma is associated with suppression of the genes involved in epidermal differentiation, including genes in 1q21 loci and corresponding to the epidermal differentiation complex. Correlation analysis of genes concordantly expressed in Barrett's esophagus and adenocarcinoma revealed 21 genes that represent potential genetic markers of disease progression and pharmacologic targets for treatment intervention. PCR analysis of genes selected based on DNA array experiments revealed that estimation of the ratios of GATA6 to SPRR3 allows discrimination among normal esophageal epithelium, Barrett's dysplasia, and adenocarcinoma.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/13/3546

Aromatase plays a critical role in breast cancer development by converting androgen to estrogen. In this report, results are presented to demonstrate that estrogen, the product of aromatase, can up-regulate its expression. Estrogen receptor (ER) transient transfection experiments were performed using the SK-BR-3 breast cancer cell line, which is ER negative and expresses aromatase. When SK-BR-3 cells were transfected with the expression plasmid pCI-ER(alpha), but not pCI-ER(beta), aromatase activity was elevated by 17(beta)-estradiol (E2) in a dose-dependent manner. The E2 induction could be enhanced by cotransfection with the coactivator GRIP1 and suppressed by antiestrogens such as tamoxifen and ICI 182,780. The aromatase activity in the ER(alpha)-transfected SK-BR-3 cells could also be induced by environmental chemicals that were known to have an estrogen-like activity. Using aromatase gene exon 1-specific reverse transcription-PCR, the level of promoter I.1-driven transcripts was found to be elevated in E2-treated ER(alpha)-transfected cells. This suggested that E2 induced aromatase expression through the up-regulation of promoter I.1. Using DNA deletion analysis of the 5'-flanking region of promoter I.1, the section between -300 and -280 bp upstream from exon I.1 was identified to be important for mediating E2 induction. However, a direct binding of ER(alpha) to this 20-bp region could not be demonstrated. It was found that E2 induction could be
suppressed by the mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor, PD98059, and the epidermal growth factor receptor tyrosine kinase inhibitor, PD153035 hydrochloride. A significant induction of aromatase expression was also detected in ER-positive MCF-7 breast cancer cells after transfection with pCI-ER{alpha} and E2 treatment. Furthermore, after ER{alpha} transfection and E2 treatment, the aromatase activity in Her-2-overexpressing MCF-7 cells was drastically higher than that of the wild-type MCF-7 cells. In addition, aromatase induction in MCF-7 cells could also be suppressed by PD153035 hydrochloride. These results suggest that E2 up-regulates aromatase expression by a nongenomic action of ER{alpha} via cross-talk with growth factor-mediated pathways.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/16/4554

Loss of function mutations in the fumarate hydratase (fumarase, FH) gene were recently identified as the cause for dominantly inherited uterine and cutaneous leiomyomas and renal cell cancer. To further evaluate the role of FH in tumorigenesis, we screened FH mutations from tumor types seen in hereditary leiomyomatosis and renal cell cancer mutation carriers—41 uterine and 10 cutaneous leiomyomas, 52 renal cell carcinomas, 53 sarcomas, 29 prostate carcinomas, and 15 lobular breast carcinomas. Few mutations were detected. Biallelic inactivation of FH was found in one uterine leiomyosarcoma, one cutaneous leiomyoma, and one soft tissue sarcoma. Whereas the two former lesions were shown to originate from a germ-line mutation, the soft tissue sarcoma is to our knowledge the first example of purely somatic inactivation of FH in tumors.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/7/2092

Patients with longstanding extensive ulcerative colitis have an increased risk of developing colorectal cancer (CRC). There are significant differences in the early pathogenesis of colitis-associated tumors compared with common CRC, whereas the frequency, degree, and significance of microsatellite instability (MSI) as a marker of mismatch repair deficiency in colitis tumors remain unclear. Here we describe the application of the DSS model of chronic colitis to mice with a defect in the Msh2 mismatch repair gene to discern these early events. These mice do not develop CRC spontaneously without an external trigger. The aim of this study was to determine the effect of the Msh2 defect on the frequency and grade of colitis-associated colorectal dysplasia and adenocarcinoma in Msh2-/-, Msh2+/-, and wild-type (Msh2+/+) mice and on the MSI status of the tumors. We show that in mice with chronic colitis, 60% of the Msh2-/- and 29% of the wild-type mice developed high-grade dysplasia or adenocarcinoma, but heterozygosity for the Msh2 defect did not increase tumor susceptibility over wild-type genotype. The largest difference between genotypes was in the frequency of high-grade dysplasia, with 46.7, 8, and 12.5% in Msh2-/-, Msh2+/-, and Msh2+/+ mice, respectively. The Msh2-/- mice developed MSI-high tumors, whereas the majority of the Msh2+/- and wild-type tumors had no MSI. In the Msh2-/- mice, MSI appeared early in non-neoplastic colon tissue, presumably as a result of markedly increased epithelial cell proliferation associated with inflammation. These observations suggest that a homozygous mismatch repair defect predisposes to tumors triggered by chronic inflammation but is not the only factor involved because tumors also developed in the wild-type mice. This model of colitis offers opportunities to characterize the different molecular
Numerous investigations have shown that in primary breast adenocarcinomas DNA aneuploidy in contrast to DNA diploidy indicates high malignancy potential. On the basis of the study of 104 breast carcinomas, we describe a subtype of aneuploidy, which demonstrates a low degree of malignancy. In image cytometric DNA histograms, this subtype possessed a low percentage (≤8.8%) of nonmodal DNA values as measured by the stemline scatter index (SSI), which is defined as sum of the percentage of cells in the S-phase region, the G2 exceeding rate and the coefficient of variation of the tumor stemline. The cut point of SSI = 8.8% (P = 0.03) enabled us to also subdivide diploid and tetraploid tumors into clinically low and high malignant variants. One possible reason for aneuploidy is impaired distribution of chromosomes at mitosis caused by numerical or structural centrosome aberrations. Cyclins A and E seem to be involved in centrosome duplication. Real-time quantitative PCR measurements of cyclin A and E transcript levels and immunohistochemical determination of cyclin A protein expression showed statistically significantly increased values in the tumors with a high SSI (>8.8%), compared with those with a low SSI. A pilot study demonstrated centrosomal aberrations in an average of 9.6% of the measured cells in four aneuploid carcinomas with high SSI values and in an average of 2.5% of the cells in three aneuploid and three diploid tumors with low SSI. Our data indicate that the SSI, most likely reflecting the degree of genomic instability, allows additional classifying of the known aneuploid, diploid, and tetraploid categories of primary breast adenocarcinomas into low and high malignant subtypes.

Immortal cell lines and tumors maintain their telomeres via the telomerase pathway or via a telomerase-independent pathway, referred to as alternative lengthening of telomeres (ALT). Here, we show the reversible conversion of the human papillomavirus type 16 E6-induced immortal human fibroblasts E6 Cl 6 from telomerase-positive (Tel+) to telomerase-negative (Tel-) cells. Tel+ cells converted spontaneously to Tel- cells that reverted to Tel+ cells following treatment with trichostatin A (TSA) and/or 5-aza-2'-deoxycytidine (5-AZC), which induced the reversion from complete to partial methylation of the CpG islands of the human telomerase reverse transcriptase (hTERT) promoter in Tel- E6 Cl 6 cells. Tel- E6 Cl 6 cells lacked the phenotypes characteristic of ALT cell lines such as very long and heterogenous telomeres and ALT-associated promyelocytic leukemia nuclear bodies (APB) but grew for >240 population doublings (PD) after they became telomerase negative. The ratios of histone H3 (H3) lysine (K) 9 methylation to each of H3-K4 methylation, H3-K9 acetylation, and H3-K14 acetylation of the chromatin containing the hTERT promoter in Tel- E6 Cl 6 cells and ALT cell lines were greater than those in Tel+ cells and decreased following treatment with TSA and/or 5-AZC, inversely corresponding to telomerase activity. Our findings suggest the possibility that human tumors may be able to reversibly interconvert their telomere maintenance phenotypes by chromatin structure-mediated regulation of hTERT expression.
The WWOX (WW domain containing oxidoreductase) gene was recently identified as a candidate tumor suppressor gene at 16q23.3-24.1, a chromosome region that spans the common fragile site FRA16D. To evaluate the potential role of the WWOX gene in esophageal squamous cell carcinomas, we examined 36 tumors for genetic alterations of the WWOX gene. Loss of heterozygosity (LOH) at the WWOX locus was observed in 14 (39%) tumors. A tumor-specific missense mutation was found in one tumor, and LOH analysis had shown that the other allele was missing. Furthermore, we detected aberrant WWOX gene transcripts with absence of exons 6-8 in two tumors, and complete absence of transcript in one tumor. These results indicate that alteration and inactivation of the WWOX gene may play a role in esophageal squamous cell carcinogenesis.

Promoter hypermethylation is an alternative way to inactivate tumor suppressor genes in cancer. Alterations of chromosome 3p are frequently involved in many types of cancer, including esophageal squamous cell carcinoma. Here, we investigated the methylation status and loss of heterozygosity (LOH) of 3p tumor suppressor genes. We examined the promoter methylation status of von Hippel-Lindau disease (VHL), retinoic acid receptor {beta} (RAR-{beta}), RAS association domain family 1A (RASSF1A), and fragile histidine triad (FHIT) genes in 22 esophageal squamous cell carcinoma cell lines and 47 primary tumors and corresponding noncancerous tissues by a methylation-specific PCR. In addition, we analyzed 47 paired samples for LOH at eight loci on chromosome 3p. Hypermethylation in VHL, RAR-{beta}, RASSF1A, and FHIT was detected in 36, 73, 73, and 50% of tumor cell lines, respectively. In primary tumors, hypermethylation in VHL, RAR-{beta}, RASSF1A, and FHIT was detected in 13, 55, 51, and 45%, respectively. In corresponding noncancerous tissues, hypermethylation in RAR-{beta} and FHIT was frequently detected in 38 and 30%, respectively, whereas no VHL hypermethylation and only 4% of RASSF1A hypermethylation were detected. Furthermore, in clinical stages I and II, hypermethylation in RAR-{beta} (67%) and FHIT (78%) was frequently detected, whereas no VHL hypermethylation and 11% of RASSF1A hypermethylation were detected. On the other hand, the correlation between FHIT hypermethylation and LOH at FHIT region was statistically significant (P = 0.008). Our findings suggest that hypermethylation of the RAR-{beta} and FHIT may play an important role in the early stage of esophageal squamous cell carcinogenesis. In addition, FHIT may be inactivated in accordance with the two-hit inactivation model, involving deletion of one allele and hypermethylation of the other.
(LOH) of the SEMA3B in non-small cell lung cancers (NSCLCs). We analyzed the methylation status of semaphorin 3B (SEMA3B) promoter and LOH at 3p21.3 in eight NSCLC cell lines and 27 primary tumors. Hypermethylation of SEMA3B was found in 50% of the cell lines and 41% of the primary tumors studied. The presence of hypermethylation was statistically associated with loss of SEMA3B expression in both cell lines (P = 0.02) and primary tumors (P < 0.01). There was no correlation between SEMA3B and tumor stage. On the other hand, the correlation between SEMA3B methylation status and LOH at 3p21.3 was significant (P = 0.02). Notably, 7 of 8 tumors with both hypermethylation and LOH of SEMA3B showed the absence of the expression. Treatment with 5-AZAC restored SEMA3B expression in NSCLC cell line. These results indicate that SEMA3B gene alterations may play an important role in the malignant transformation of NSCLC via a two-hit mechanism, including epigenetic changes and allelic loss, for tumor suppressor gene inactivation.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/24/9193

The two regulatory subunits (R1 and R2) of protein kinase A (PKA) are differentially expressed in cancer cell lines and exert diverse roles in growth control. Recently, mutations of the PKA regulatory subunit 1A gene (PRKAR1A) have been identified in patients with Carney complex. The aim of this study was to evaluate the expression of the PKA regulatory subunits R1A, R2A, and R2B in a series of 30 pituitary adenomas and the effects of subunit activation on cell proliferation. In these tumors, neither mutation of PRKAR1A nor loss of heterozygosity was identified. By real-time PCR, mRNA of the three subunits was detected in all of the tumors, R1A being the most represented in the majority of samples. By contrast, immunohistochemistry documented low or absent R1A levels in all tumors, whereas R2A and R2B were highly expressed, thus resulting in an unbalanced R1/R2 ratio. The low levels of R1A were, at least in part, due to proteasome-mediated degradation. The effect of the R1/R2 ratio on proliferation was assessed in GH3 cells, which showed a similar unbalanced pattern of R subunits expression, and in growth hormone-secreting adenomas. The R2-selective cAMP analog 8-Cl cAMP and R1A RNA silencing, stimulated cell proliferation and increased Cyclin D1 expression, respectively, in human and rat adenomatous somatotrophs. These data show that a low R1/R2 ratio promoted proliferation of transformed somatotrophs and are consistent with the Carney complex model in which R1A inactivating mutations further unbalance this ratio in favor of R2 subunits. These results suggest that low expression of R1A protein may favor cAMP-dependent proliferation of transformed somatotrophs.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/22/8184

Alterations in histones, chromatin-related proteins, and DNA methylation contribute to transcriptional silencing in cancer, but the sequence of these molecular events is not well understood. Here we demonstrate that on disruption of estrogen receptor (ER) (alpha) signaling by small interfering RNA, polycomb repressors and histone deacetylases are recruited to initiate stable repression of the progesterone receptor (PR) gene, a known ER[alpha] target, in breast cancer cells. The event is accompanied by acquired DNA methylation of the PR promoter, leaving a stable mark that can be inherited by cancer cell progeny. Reestablishing ER[alpha] signaling alone was not sufficient to reactivate the PR gene; reactivation of the PR gene also requires DNA
demethylation. Methylation microarray analysis further showed that progressive DNA methylation occurs in multiple ER(α) targets in breast cancer genomes. The results imply, for the first time, the significance of epigenetic regulation on ER(α) target genes, providing new direction for research in this classical signaling pathway.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/9/3726

Epithelial ovarian cancer derived from the human ovarian surface epithelium (HOSE) is the leading cause of death from gynecologic malignancies among American women. Metabolic activation of endogenous and exogenous chemicals by cytochrome P450 (CYP) class I enzymes has been implicated in its etiology. In this study, we showed overexpression of CYP1A1 mRNA, but not CYP1B1 transcripts, in ovarian cancer cell lines when compared with primary cultures or immortalized HOSE cell lines. Importantly, we identified a novel, enzymatically active, spliced variant of CYP1A1 (CYP1A1v) formed by excision of an 84-bp cryptic intron in exon 2. CYP1A1v is overexpressed in ovarian cancer cell lines and exhibits a unique subcellular distribution restricted to the nucleus and mitochondria, contrary to the endoplasmic reticulum localization of the wild-type enzyme. In concordance, total CYP1A1 activity, as measured by the ethoxyresorufin O-deethylase assay, was detected in mitochondrial, nuclear, and microsomal fractions of ovarian cancer cells but was notably absent in all subcellular fractions of HOSE cells. Immunocytochemistry studies in 30 clinical specimens revealed overexpression of CYP1A1 in various types of ovarian cancers compared with benign epithelia and frequent localization of the enzyme to cancer cell nuclei. Forced expression of CYP1A1wt or CYP1A1v in HOSE cells resulted in nuclear localization of the enzyme and acquisition of anchorage-independent growth, which was further exacerbated following exposure to benzo(a)pyrene or 17(β)-estradiol. Collectively, these data provided the first evidence that CYP1A1 overexpression and alternative splicing could contribute to ovarian cancer initiation and progression.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/16/4781

Oligonucleotide arrays that detect single nucleotide polymorphisms were used to generate genome-wide loss of heterozygosity (LOH) maps from laser capture microdissected paraffin-embedded samples using as little as 5 ng of DNA. The allele detection rate from such samples was comparable with that obtained with standard amounts of DNA prepared from frozen tissues. A novel informatics platform, dChipSNP, was used to automate the definition of statistically valid regions of LOH, assign LOH genotypes to prostate cancer samples, and organize by hierarchical clustering prostate cancers based on the pattern of LOH. This organizational strategy revealed apparently distinct genetic subsets of prostate cancer.

Discovery and functional evaluation of biologically significant regulatory single nucleotide polymorphisms (SNP) in carcinogen metabolism genes is a difficult challenge because the phenotypic consequences may be both transient and subtle. We have used a gene expression screening approach to identify a functional regulatory SNP in glutathione S-transferase M3 (GSTM3). Anttila et al. proposed that variation in GSTM3 expression was affected by exposure to cigarette smoke and inheritance of the GSTM1-null genotype. To investigate the mechanism of GSTM3 expression was affected by exposure to cigarette smoke and inheritance of the GSTM1-null genotype. To investigate the mechanism of GSTM3 expression variation, we measured GSTM3 expression in lymphoblast cells from a human Centre d'Etude du Polymorphisme Humain family and observed a low expression phenotype. Promoter sequencing revealed two novel GSTM3 promoter SNPs: A/C and A/G SNPs, 63 and 783 bp upstream of the codon 1 start site, respectively. In this pedigree, the two children homozygous for the -63C/C genotype had 8-fold lower GSTM3 expression relative to the two children with the -63A/A genotype, with no association between A-783G SNP and GSTM3 expression. Further evaluation using genotyped glioma cell lines and with luciferase reporter constructs showed that the -63C allele was associated with lower GSTM3 expression (P < 0.0001 and P < 0.003). RNA pol II chromatin immunoprecipitation was combined with quantitative probed-based allelic discrimination genotyping to provide direct evidence of a 9-fold reduced RNA pol II binding capacity for the -63C allele. These results show that the GSTM3 -63C allele strongly affects gene expression in human cell lines and suggests that individuals who carry the low expression allele may be deficient in glutathione transferase catalyzed biological functions.


Germ-line mutation of the von Hippel-Lindau (VHL) gene predisposes to the development of multifocal, benign lesions, including retinal and central nervous system hemangioblastomas, pheochromocytomas, and renal and pancreatic cysts. Progression to malignancy in VHL disease is associated primarily with the development of renal cell carcinoma (RCC) and pancreatic islet cell tumors (PICT). Although many reports have documented the multiple functions of the VHL protein, few have investigated the intriguing question related to the tissue-specificity of malignant conversion in VHL disease, a problem not easily explained by strict genotype-phenotype correlations. We investigated a novel VHL kindred with a preponderance of PICTs to determine whether loss of additional genetic loci associated with the sporadic forms of RCC and PICTs might play a role in malignant conversion in this disease. We report the high frequency loss of heterozygosity (LOH) of genetic loci distinct from and mapping proximal to VHL within human chromosome 3p in the VHL kindred under study. Furthermore, chromosome 3p LOH occurs subsequent to VHL mutation and cyst formation, and correlates with malignant progression in VHL-associated PICTs. High frequency LOH was also observed in sporadic PICTs in regions of 3p associated with LOH in sporadic clear cell RCC as well as homozygous deletion in lung cancer. A stepwise model for malignant conversion in VHL disease is herein proposed.

Expression of survivin is elevated in most malignancies, especially in radiation-resistant cell lines. In this study, we investigated how radiation affects survivin expression in primary endothelial cells as well as in malignant cell lines. We found that 3 Gy significantly reduced survivin protein level in human umbilical vein endothelial cells (HUVECs) but not in tumor cell lines. Flow cytometry studies suggest that the down-regulation of survivin is independent of cell cycle. In addition, survivin mRNA level was also down-regulatable by irradiation. However, it was abrogated by actinomycin D-mediated inhibition of gene transcription. Luciferase reporter gene assays suggest that irradiation suppressed the survivin promoter. p53 overexpression reduced survivin expression, but overexpression of a p53 mutant failed to abolish the radiation-induced down-regulation in HUVECs. Alteration of p53 status in Val138 lung cancer cell line also failed to restore the radiation-inducible down-regulation. Overexpression of survivin in 293 cells prevented apoptosis induced by irradiation and increased cell viability after irradiation. The inhibition of survivin using antisense oligonucleotides caused a significant decrease in cell viability of irradiated H460 lung cancer cells. These data suggest that radiation transcriptionally down-regulates survivin in HUVECs. This regulatory mechanism is defective in malignancies and is not mediated by p53. Survivin overexpression may lead to resistance to radiotherapy by inhibiting apoptosis and enhancing cell viability. The inhibition of survivin results in sensitization of H460 lung cancer cells to radiation. These studies suggest that survivin may be a target for cancer therapy.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/16/4997

Visinin-like protein-1 (VILIP-1) is a member of the neuronal EF-hand Ca2+-sensor protein family. VILIP-1 is expressed in the central nervous system where it plays a crucial role in regulating cAMP levels, cell signaling, and differentiation. Screening of mouse skin tumor cell lines for differentially expressed genes showed high-level VILIP-1 expression in less aggressive squamous cell carcinoma (SCC) and papilloma cell lines. Conversely, expression was markedly decreased or lost in invasive SCC and spindle cell carcinoma cell lines. In addition, immunohistochemistry of normal skin and primary tumors showed that VILIP-1 is expressed in basal cells of the normal intrafollicular epidermis as well as in basal cells of papillomas. The expression was decreased in low-grade SCCs and disappeared in most high-grade SCCs. When two high-grade carcinoma cell lines were transfected with VILIP1-cDNA, the VILIP-1 transfectants had significantly higher cAMP levels than the respective vector alone-transfected lines. VILIP-1-transfected cells were less invasive (both in vivo and in vitro) than the control transfectedants. Reduced invasiveness and elevation of cAMP levels were accompanied by decreased MMP-9, as well as decreased RhoA activity. These results indicate that VILIP-1 plays an important role in regulating tumor cell invasiveness and that its loss could aid in enhancing the advanced malignant phenotype.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/22/8420

In this study, we have evaluated 11 pancreatic tumor cell lines and tumor cells from surgical samples of patients with pancreatic adenocarcinoma for expression of the chemokine receptor CXCR4. Six of 11 cell lines expressed detectable mRNA of CXCR4, with three cell lines (AsPC1, Capan1, and Hs766T) having substantial amounts of transcripts. Expression was higher in lines
derived from metastatic lesions compared with those derived from primary tumors. Different inflammatory cytokines did not modify expression, whereas IFN-\(\gamma\) down-regulated and hypoxia up-regulated CXCR4 transcripts. Transcript expression was associated with surface expression in pancreatic carcinoma cell lines. All surgical carcinoma samples tested expressed higher levels of CXCR4 than normal pancreatic ducts, which were used as reference tissue. The chemokine CXCL12 induced chemotaxis in CXCR4-positive pancreatic carcinoma cell lines, which was inhibited by anti-CXCR4 monoclonal antibody and by the antagonist AMD3100. Transendothelial migration, Matrigel invasion, and activation of matrix metalloproteases were also enhanced by CXCL12. In CXCR4-positive cell lines, CXCL12 stimulated cell proliferation. The cell line Hs766T produces high levels of CXCL12, and addition of the CXCR4 antagonist AMD3100 partially inhibited proliferation, indicating an autocrine loop. Moreover, the addition of exogenous CXCL12 inhibited apoptosis induced by serum starvation. These results indicate that the CXCR4 receptor is frequently expressed in metastatic pancreatic tumor cells. CXCR4 not only stimulates cell motility and invasion but also promotes survival and proliferation. Strategies to target CXCR4 expressed on tumor cells may be of benefit in patients with pancreatic cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/24/8791

5-Fluorouracil (5-FU) is the most common chemotherapeutic agent used in the treatment of colorectal cancer, yet objective response rates are low. Recently, camptothecin (CPT) has emerged as an effective alternative therapy. Decisive means to determine treatment, based on the likelihood of response to each of these agents, could greatly enhance the management of this disease. Here, the ability of cDNA microarray-generated basal gene expression profiles to predict apoptotic response to 5-FU and CPT was determined in a panel of 30 colon carcinoma cell lines. Genes whose basal level of expression correlated significantly with 5-FU- and CPT-induced apoptosis were selected, and their predictive power was assessed using a "leave one out" jackknife cross-validation strategy. Selection of the 50 genes best correlated with 5-FU-induced apoptosis, but not 50 randomly selected genes, significantly predicted response to this agent. Importantly, this gene expression profiling approach predicted response more effectively than four previously established determinants of 5-FU response: thymidylate synthase and thymidine phosphorylase activity; and p53 and mismatch repair status. Furthermore, reanalysis of the database demonstrated that selection of the 149 genes best correlated with CPT-induced apoptosis maximally and significantly predicted response to this agent. These studies demonstrate that the basal gene expression profile of colon cancer cells can be used to predict and distinguish response to multiple chemotherapeutic agents and establish the potential of this methodology as a means by which rational decisions regarding choice of therapy can be approached.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/23/8702

Allelic loss of chromosome region 3p21.3 occurs early and frequently in non-small-cell lung cancer, and numerous tumor suppressor genes at this locus may be targets of inactivation. Using an incident case series study of non-small-cell lung cancer, we sought to determine the prevalence of loss of heterozygosity (LOH) in the 3p21.3 region and to examine the associations between this alteration and patient outcome, exposure to tobacco smoke, occupational asbestos...
exposure, and additional molecular alterations in these tumors. We examined LOH at 7 microsatellite markers in the chromosome 3p21.3 region, and LOH was present in at least one of the loci examined in 60% (156 of 258) of the tumors, with the prevalence of LOH at individual loci ranging from 15 to 56%. Occupational asbestos exposure and TP53 mutation were significantly associated with more extensive 3p21 LOH. In squamous cell carcinomas, measures of cumulative smoking dose were significantly lower in patients with LOH at 3p21, particularly in TP53 mutant tumors. Examining patient outcome, we found that in squamous cell carcinomas, having any LOH in this region was associated with a better overall survival (log-rank test, P < 0.04). Together, these results indicate that allelic loss at 3p21 can affect patient outcome, and that this loss may initially be related to carcinogen exposure, but that extension of this loss is related to TP53 mutation status and occupational asbestos exposure.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/21/7113

The fight against lung cancer is greatly compromised by the lack of effective early detection strategies. Genomic abnormalities and specifically the amplification of chromosomal region 3q26-3qter in lung cancer represent a major signature of neoplastic transformation. Here, we address the significance of p53 homologue p63 mapping to 3q27 in lung tumorigenesis. We analyzed p63 gene copy number (CN) by fluorescence in situ hybridization and expression by immunohistochemistry in tissue microarrays of 217 non-small cell lung cancers (NSCLCs) and correlated them with survival. We additionally characterized our findings in a subset of 24 NSCLCs by reverse transcription-PCR and Western blotting. We analyzed p63 CN and protein expression in 41 preinvasive squamous lesions. The p63 genomic sequence was amplified in 88% of squamous carcinomas, in 42% of large cell carcinomas, and in 11% of adenocarcinomas of the lung. The predominant splice variant of p63 expressed was {Delta}Np63{alpha}. Western analyses revealed {Delta}Np63{alpha} expression in normal bronchus and squamous carcinomas but not in normal lung or in adenocarcinomas. Furthermore, p63genomic amplification and protein staining intensity associated with better survival. We found a significant increase in CN in preinvasive lesions graded severe dysplasia or higher. Our data demonstrate that there is early and frequent genomic amplification of p63 in the development of squamous carcinoma of the lung and that patients with NSCLC showing amplification and overexpression of p63 have prolonged survival. These observations suggest that p63 genomic amplification has an early role in lung tumorigenesis and deserves additional evaluation as a biomarker for lung cancer progression.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/15/4594

The genetic basis of disease susceptibility can be studied by several means, including research on animal models and epidemiological investigations in humans. The two methods are infrequently used simultaneously, but their joint use may overcome the disadvantages of either method alone. We used both approaches in an attempt to understand the genetic basis of aflatoxin B1 (AFB1)-related susceptibility to hepatocellular carcinoma (HCC). Ingestion of AFB1 is a major risk factor for HCC in many areas of the world where HCC is common. Whether humans vary in their ability to detoxify the active intermediate metabolite of AFB1, AFB1-exo-8,9-epoxide, is not certain but may explain why all exposed individuals do not develop HCC. To determine whether human variability in detoxification may exist, in a study of 231 HCC cases and 256 controls, we genotyped eleven loci in two families of AFB1 detoxification genes; the glutathione
S-transferases (GSTs) and the epoxide hydrolases (EPHX). After adjustment for multiple comparisons, only one polymorphism in the epoxide hydrolase family 2 locus remained significantly associated with HCC (odds ratio = 2.06, 95% confidence interval = 1.13-3.12). To determine whether additional susceptibility loci exist, we developed a mouse model system to examine AFB1-induced HCC. Susceptibility of 7-day-old mice from two common inbred strains (C57BL/6J, DBA/2J) was assessed. DBA/2J animals were 3-fold more sensitive to AFB1-induced HCC and significantly more sensitive to AFB1 acute toxicity than were C57BL/6J animals. Analysis of the xenobiotic metabolizing genes in the two strains revealed single nucleotide polymorphisms in three genes, Gsta4, Gstt1, and Ephx1. Although the GSTT1 and EPHX1 loci did not appear to be related to HCC in the total population of the human study, a polymorphism in GSTA4 was significantly related to risk in the male subset. The mouse model also demonstrated that absent or compromised p53 was not necessary for the development of carcinogenesis. These results indicate that the comparison of results from human studies and the AFB1-susceptible mouse model may provide new insights into hepatocarcinogenesis.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/13/3855

Colorectal cancers from the mutator phenotype pathway display distinctive pathological features and confer a lesser aggressiveness than colorectal adenocarcinomas originated by the suppressor pathway. The goal of this work was to test whether tumors developed through the mutator pathway could show a decrease in matrix metalloproteinase (MMP) activity. We evaluated levels and activity of gelatinase A (MMP-2) and gelatinase B (MMP-9), as well as stromelysin-1 (MMP-3) expression in 101 sporadic colorectal tumors in consideration of the microsatellite instability (MSI) status of the groups. Gelatinases were analyzed by ELISA and zymography. The MMP-3 study was performed by real-time quantitative PCR. MMP-9 total levels were significantly higher in MSI-H tumors. However, levels of the active MMP-9 form were significantly much lower in this group of tumors. Data from real-time quantitative PCR indicated that levels of MMP-3 for MSI-L/MSS tumors were much higher as compared with those observed in MSI-H cancers (P = 0.033). Moreover, all MSI-H tumors showed nucleotide insertions and/or deletions in MMP-3 promoter. These mutations were not observed in the group of MSI-L/MSS tumors. Our data indicate that the MMP-3 promoter constitutes a novel target of the defective mismatch repair machinery in sporadic colorectal tumors, resulting in a dramatic decrease in the levels of the active MMP-9 form, which may result in a lessened capacity for invasion.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/5/1800

Histone deacetylation and DNA methylation establish epigenetic modifications, which through chromatin remodeling may result in gene silencing. We hypothesized that chemokine receptors C-C chemokine receptor 7 (CCR7) and C-X-C chemokine receptor 4 (CXCR4) on melanoma cells undergo epigenetic regulation. We investigated whether a histone deacetylase inhibitor and a demethylating agent influence CCR7 and CXCR4 expression on melanoma cells. Initially, microarray analysis was done to screen changes in chemokine receptor expression on melanoma cells after treatment with trichostatin A (TSA) and 5-Aza-2-deoxycytidine (5-Aza). CCR7 and CXCR4 mRNA expression were uniformly altered and selected for further investigation. Quantitative real-time reverse transcription-PCR assay, immunohistochemistry, and Western blot analysis were used to assess changes in mRNA and protein expression induced by TSA and 5-
Aza in melanoma lines. Cell migration assays were conducted to assess the effects of altered CCR7 and CXCR4 expression on cell function. Treatment with TSA or 5-Aza increased gene expression of both CCR7 and CXCR4 in melanoma lines. TSA was the strongest enhancer. With combined treatment, CCR7 and CXCR4 mRNA expression was also up-regulated. Immunohistochemistry after combined treatment showed enhanced staining of both CCR7 and CXCR4 compared with control cells. Melanoma cell migration in TSA- and 5-Aza-treated cells was 7- and 2-fold higher than control cells for CCR7 and CXCR4, respectively. In summary, a histone deacetylase inhibitor and a demethylating agent up-regulated CCR7 and CXCR4 expression on melanoma cells. This increase in chemokine receptor expression correlated with functional activity. Most importantly, we have identified an epigenetic mechanism that may endogenously regulate chemokine receptor expression on melanoma cells.


Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant genetic predisposition syndrome that accounts for 2-7% of all colorectal cancers. Diagnosis of HNPCC is based on family history (defined by Amsterdam or Bethesda Criteria), which often includes a history of multiple synchronous or metachronous cancers. The majority of HNPCC results from germ-line mutations in the DNA mismatch repair (MMR) genes hMSH2 and hMLH1 with rare alterations in hMSH6 and hPMS2 in atypical families. Both HNPCC and sporadic MMR-deficient tumors invariably display high microsatellite instability (MSI-H). Two types of HNPCC families can be distinguished: type I (Lynch I) with tumors exclusively located in the colon; and type II (Lynch II) with tumors found in the endometrium, stomach, ovary, and upper urinary tract in addition to the colon. A proposed association of breast cancer with type II HNPCC is controversial. To address this important clinical question, we examined MSI in a series of 27 female patients who presented with synchronous or metachronous breast plus colorectal cancer. Although MSI-H was found in 5 of 27 (18.5%) of the colon cancers, in all cases the matched breast cancer was microsatellite stable. We also examined the breast tumors from three women who were carriers of MMR gene mutations from HNPCC families. None of these three breast tumors displayed MSI nor was the expression of MMR proteins altered in these tumors. We conclude that breast cancer largely arises sporadically in HNPCC patients and is rarely associated with the HNPCC syndrome.


Previous studies have suggested that common breast cancers are associated with EBV. We used a highly sensitive quantitative real-time PCR method to screen whole tumor sections of breast cancers for the presence of the EBV genome. EBV DNA was detected in 19 of 92 (21%) tumors, but viral load was very low in positive samples (mean = 1.1 copy EBV/1000 cells, maximum = 7.1 copies EBV/1000 cells). Importantly, quantitative real-time PCR failed to detect the EBV genome in microdissected tumor cells from any case. Using a monoclonal antibody (2B4-1) reactive against the EBV nuclear antigen-1, we noted strong staining of tumor nuclei in a proportion of those breast cancers that had tested negative for the presence of the EBV genome. Because nuclear staining with the 2B4-1 antibody was previously observed more frequently in poor prognosis breast cancers, we examined a larger series of breast cancers with complete clinical
follow-up. Strong punctate staining of tumor cell nuclei was observed in 47 of 153 (31%) breast cancers; 2B4-1-positive tumors were significantly more likely to be ER-negative (P < 0.0001), to be of higher grade (P = 0.001) and larger (P = 0.03), to involve more regional lymph nodes (P = 0.01), and to have higher Nottingham Prognostic Index scores (P = 0.0003). Conclusions are: (a) EBV can be regularly detected in whole sections of breast cancers but viral copy number is very low; (b) in these cases, tumor cells do not harbor virus; and (c) reactivity with the monoclonal antibody 2B4-1 is detectable in the absence of the EBV genome and is strongly associated with ER-negative breast tumors and with prognostically unfavorable disease. Additional studies should be directed to the identification of this protein and to elucidation of its role in breast cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/22/7606

Recent studies showed that SV40 is detected in >40% of non-Hodgkin's lymphoma (NHL) in United States, suggesting SV40-contaminated poliovaccines widely used during the period 1955-1963 to be a major source of SV40 in NHL. We examined the presence of SV40 sequences in 122 cases with NHL and 3 with Hodgkin's lymphoma from Japan. The detection rate of SV40 sequences in diffuse large B-cell lymphoma (19%) was higher than that in peripheral blood cells of normal healthy volunteers in Japan (4.7%; P < 0.05) reported previously as controls for comparison with the study results from cancer patients, suggesting a role for SV40 in the development of diffuse large B-cell lymphoma. In contrast, the frequency of SV40 sequences in NHL cases born between 1951 and 1963 (12%), during which SV40-contaminated poliovaccines might have been inoculated, is not significantly different from that in cases born before 1950 (11%) or after 1964 (15%). SV40 is a new candidate etiologic factor for malignant lymphoma not only in the United States but also in Japan.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/22/7657

Deletions found in several types of human tumor, including carcinomas of the colorectum, breast, and lung, suggest the presence of a potential tumor suppressor gene(s) on chromosome 15. Common regions of deletion in these tumors are at 15q15 and 15q21. Here, we have analyzed loss of heterozygosity (LOH) on chromosome 15 to ascertain its potential involvement in the development and progression of transitional cell carcinoma (TCC) of the bladder. A panel of 26 polymorphic markers, spanning 15q12-15q22, were used to map regions of LOH in 51 TCCs. LOH was found for at least one marker in the region 15q14-15q15.3 in 20 of 51 (39%) tumors. Deletion mapping defined two minimum regions of deletion: a distal region between the markers D15S514 and D15S537 at 15q15.1-15q15.3 (estimated as 3 Mb) and a more proximal region between the markers D15S971 and D15S1042 at 15q14 (estimated as 1.1 Mb). Analysis of a panel of 33 bladder tumor cell lines revealed regions of contiguous homozygosity for markers in 15q15, indicating likely LOH. Fluorescence in situ hybridization analysis demonstrated that mitotic recombination is the predicted mechanism of LOH in two of these. These regions of LOH on 15q may contain tumor suppressor genes the loss or inactivation of which is associated with TCC development. The DNA repair gene RAD51 at 15q15.1 represents a candidate 15q tumor suppressor gene. Expression analysis of rad51 protein in tumor cell lines revealed variable levels of expression but no significant loss of expression in cell lines with likely 15q LOH.

http://cancerres.aacrjournals.org/cgi/content/abstract/65/5/2026

A major limitation of adoptive immunotherapy is the availability of T cells specific for both terminally differentiated tumor cells and their clonogenic precursors. We show here that marrow-infiltrating lymphocytes (MILs) recognize myeloma cells after activation with anti-CD3/CD28 beads with higher frequency than activated peripheral blood lymphocytes from the same patients. Furthermore, activated MILs target both the terminally differentiated CD138+ plasma cells and the myeloma precursor as shown by profound inhibition in a tumor clonogenic assay. The presence of antigen in the marrow microenvironment seems to be important for the maintenance of tumor specificity. Taken together, these results highlight the intrinsic tumor specificity of MILs and describe a novel approach for the generation of tumor-specific T-cell populations suitable for adoptive immunotherapy of multiple myeloma.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/21/7801

Tumor growth and progression are critically controlled by alterations in the microenvironment often caused by an aberrant expression of growth factors and receptors. We demonstrated previously that tumor progression in patients and in the experimental HaCaT tumor model for skin squamous cell carcinomas is associated with a constitutive neoexpression of the hematopoietic growth factors granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF), causing an autocrine stimulation of tumor cell proliferation and migration in vitro. To analyze the critical contribution of both factors to tumor progression, G-CSF or GM-CSF was stably transfected in factor-negative benign tumor cells. Forced expression of GM-CSF resulted in invasive growth and enhanced tumor cell proliferation in a three-dimensional culture model in vitro, yet tumor growth in vivo remained only transient. Constitutive expression of G-CSF, however, caused a shift from benign to malignant and strongly angiogenic tumors. Moreover, cells recultured from G-CSF-transfected tumors exhibited enhanced tumor aggressiveness upon reinjection, i.e., earlier onset and faster tumor expansion. Remarkably, this further step in tumor progression was again associated with the constitutive expression of GM-CSF strongly indicating a synergistic action of both factors. Additionally, expression of GM-CSF in the transfected tumors mediated an earlier recruitment of granulocytes and macrophages to the tumor site, and expression of G-CSF induced an enhanced and persistent angiogenesis and increased the number of granulocytes and macrophages in the tumor vicinity. Thus both factors directly stimulate tumor cell growth and, by modulating the tumor stroma, induce a microenvironment that promotes tumor progression.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/2/567

SEL1L, the human orthologue of the *Caenorhabditis elegans* sel-1 gene, is differentially
expressed in breast primary tumors and in normal breast tissues. Analysis of a series of human primary breast carcinomas, using a monoclonal antibody raised against a SEL1L recombinant protein, revealed down-modulation or absence of SEL1L expression in about two-thirds of the tumors as compared with normal breast epithelial cells. Overall survival analysis of breast carcinoma patients indicated a statistically significant correlation between SEL1L down-modulation and poor prognosis. MCF-7, human breast carcinoma cells, were transfected with a construct containing the entire SEL1L cDNA driven by an inducible promoter and showed a dramatic reduction in anchorage-dependent growth and colony formation in soft agar. Growth of the transfected cells in Matrigel, an extracellular matrix rich with laminin, restored colony-formation ability. These results point to the role for SEL1L in breast tumor growth and aggressiveness, possibly involving cell-matrix interactions.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/3/797

Activating mutations in ras oncogenes occur at high frequency in human malignancies and expression of activated ras in immortalized cells lines is generally transforming. However, somewhat paradoxically, ectopic expression of ras in some myeloid cell lines has been shown to induce growth suppression associated with up-regulation of the cyclin-dependent kinase inhibitor p21CIP1/WAF1 in a p16INK4a, p15INK4b, and p53 independent fashion. We have used cDNA array technology to compare the expression profile induced by activated N-ras (N-rasG13R) in growth-suppressed myeloid cells with that induced in myeloid cells, which are transformed by N-rasG13R. The expression profile induced in growth suppressed cells was consistent with differentiation and included the up-regulation of the transcription factor IFN regulatory factor-1 (IRF-1), a known transcriptional activator of p21CIP1/WAF1 expression and a target of oncogenic mutations associated with myeloid leukemia. Antisense suppression of IRF-1 prevented N-rasG13R-associated growth arrest and up-regulation of p21CIP1/WAF1. These results define a novel tumor suppressive response to oncogenic signaling and provide a mechanistic link between growth suppression and differentiation in myeloid cells.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/5/1670

Previous reports have suggested a connection between reduced levels of the catalytic subunit of DNA-dependent protein kinases (DNA-PKcs), a component of the nonhomologous DNA double-strand breaks end-joining system, and a reduction in ATM. We studied this possible connection in other DNA-PKcs-deficient cell types, and following knockdown of DNA-PKcs with small interfering RNA, Chinese hamster ovary V3 cells, lacking DNA-PKcs, had reduced levels of ATM and hSMG-1, but both were restored after transfection with PRKDC. Atm levels were also reduced in murine scid cells. Reduction of ATM in a human glioma cell line lacking DNA-PKcs was accompanied by defective signaling through downstream substrates, post-irradiation. A large reduction of DNA-PKcs was achieved in normal human fibroblasts after transfection with two DNA-PKcs small interfering RNA sequences. This was accompanied by a reduction in ATM. These data were confirmed using immunocytochemical detection of the proteins. Within hours after transfection, a decline in PRKDC mRNA was seen, followed by a more gradual decline in DNA-PKcs small interfering RNA sequences. This was accompanied by a reduction in ATM. These data were confirmed using immunocytochemical detection of the proteins. Within hours after transfection, a decline in PRKDC mRNA was seen, followed by a more gradual decline in DNA-PKcs small interfering RNA sequences. This was accompanied by a reduction in ATM. These data were confirmed using immunocytochemical detection of the proteins. Within hours after transfection, a decline in PRKDC mRNA was seen, followed by a more gradual decline in DNA-PKcs small interfering RNA sequences. This was accompanied by a reduction in ATM. These data were confirmed using immunocytochemical detection of the proteins. Within hours after transfection, a decline in PRKDC mRNA was seen, followed by a more gradual decline in DNA-PKcs mRNA observed, beginning 2 days post-transfection. The amount of ATM began to decline,
starting about 3 days post-treatment, then it declined to levels comparable to DNA-PKcs. Both proteins returned to normal levels at later times. These data illustrate a potentially important cross-regulation between the nonhomologous end-joining system for rejoining of DNA double-strand breaks and the ATM-dependent damage response network of pathways, both of which operate to maintain the integrity of the genome.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/3/977

Both the integrin and insulin-like growth factor binding protein (IGFBP) families independently play important roles in modulating tumor cell growth and progression. We present evidence for a specific cell surface localization and a bimolecular interaction between the \(\alpha_v\beta_3\) integrin and IGFBP-2. The interaction, which could be specifically perturbed using vitronectin and \(\alpha_v\beta_3\) blocking antibodies, was shown to modulate IGF-mediated cellular migration responses. Moreover, this interaction was observed in vivo and correlated with reduced tumor size of the human breast cancer cells, MCF-7(\(\beta_3\)), which overexpressed the \(\alpha_v\beta_3\) integrin. Collectively, these results indicate that \(\alpha_v\beta_3\) and IGFBP-2 act cooperatively in a negative regulatory manner to reduce tumor growth and the migratory potential of breast cancer cells.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/6/2353

The Notch family of proteins plays an integral role in determining cell fates, such as proliferation, differentiation, and apoptosis. We show that Notch-1 and its ligands, Delta-like-1 and Jagged-1, are overexpressed in many glioma cell lines and primary human gliomas. Immunohistochemistry of a primary human glioma tissue array shows the presence in the nucleus of the Notch-1 intracellular domain, indicating Notch-1 activation in situ. Down-regulation of Notch-1, Delta-like-1, or Jagged-1 by RNA interference induces apoptosis and inhibits proliferation in multiple glioma cell lines. In addition, pretreatment of glioma cells with Notch-1 or Delta-like-1 small interfering RNA significantly prolongs survival in a murine orthotopic brain tumor model. These results show, for the first time, the dependence of cancer cells on a single Notch ligand; they also suggest a potential Notch juxtacrine/autocrine loop in gliomas. Notch-1 and its ligands may present novel therapeutic targets in the treatment of glioma.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/19/6469

In a total of 38 typical T-cell lineage acute lymphocytic leukemia (T-ALL) and T-cell lineage chronic lymphocytic leukemia (T-CLL) cases investigated, we found that CC chemokine receptor CCR9 was selectively and frequently expressed on T-ALL CD4+ T cells, was moderately
expressed on T-CLL CD4+ T cells, and was rarely expressed on normal CD4+ T cells. These findings were demonstrated at protein and mRNA levels using flow cytometry and real-time quantitative reverse transcription-PCR technique and were verified by digital confocal microscopy and Northern blotting. Thymus-expressed chemokine, a ligand for CCR9, selectively induced T-ALL CD4+ T-cell chemotaxis and adhesion. Interleukin (IL)-2 and IL-4, together, down-regulated the expression and functions of CCR9 in T-ALL CD4+ T cells including chemotaxis and adhesion. It was also demonstrated that IL-2 and IL-4, together, internalized CCR9 on T-ALL CD4+ T cells and subsequently inhibited functions of CCR9 in these cells. Thymus-expressed chemokine mRNA was highly expressed in CD4+ T cells, involving lymph node and skin in T-ALL patients, and was expressed at moderate levels in lymph node and skin tissues in T-CLL patients. Our findings may provide new clues to understanding various aspects of T-ALL CD4+ T cells, such as functional expression of CCR9-thymus-expressed chemokine receptor-ligand pairs as well as the effects of IL-2 and IL-4, which may be especially important in cytokine/chemokine environment for the pathophysiological events of T-ALL CD4+ T-cell trafficking.


Malignant transformation of Barrett's esophagus is characterized by three distinct premalignant stages: intestinal metaplasia (MET), low- (LGD), and high-grade dysplasia (HGD). We reported recently an increase in the frequency of loss of 7q33-q35 between LGD and HGD as determined by comparative genomic hybridization (P. H. J. Riegman et al., Cancer Res., 61: 3164-3170, 2001). Now the 7q32.3-q36.1 region was additionally characterized by allelotype analysis with 11 polymorphic markers in 15 METs, 20 LGDs, 20 HGDs, and 20 Barrett’s adenocarcinomas from different patients. Low percentages of imbalance were determined in METs and LGDs, 7% and 10%, respectively, whereas HGDs and Barrett's adenocarcinomas revealed high percentages of loss, 75% and 65%, respectively. This difference in frequency between LGDs and HGDs appeared highly significant: P = 0.00007. The majority of imbalances were found at D7S2439 and D7S483, located on 7q36.1. These data suggest that markers from this area can be used as a diagnostic tool in Barrett’s esophagus, i.e., to distinguish between watchful waiting and active treatment.


Paired DNA samples of tumor and normal thyroid tissue from adult patients possibly exposed to radioactive Chernobyl fallout [11 cases of papillary thyroid carcinoma (PTC) and 6 follicular adenomas] and from control samples (9 PTC occurring in Japanese patients) were examined for the relative mitochondrial DNA (mtDNA) content, prevalence and level of common deletion (CD), and large-scale deletions in mtDNA. Elevated relative mtDNA content as estimated by real-time PCR was found in tumor tissue in most cases, but no significant correlation with the level of radiiodine contamination of patients' residency nor with clinicopathological data were found. CD was detected in every DNA specimen from all types of tissue regardless of the presence of oxyphillic cell changes. Elevated level of the CD was predominantly found in tumor tissue of the radiation-associated group but not in sporadic PTC. No correlation was noted with clinicopathological parameters, radiiodine contamination, and relative mtDNA content. The quantity of large-scale deletions in mtDNA was elevated in most tumor tissues, especially in the radiation-associated group and tended to correlate with the level of radiopollutant in PTC. In
contrast to sporadic PTC, highly significant-positive correlation between the presence of large scale mtDNA deletions and relative mtDNA content was found in radiation-associated tumors (P = 0.001 and P = 0.019 in PTC and follicular adenoma, respectively). Normal tissue displayed the inverse tendency. No association with level of the CD was found in either group of cases. Concordant increase of both relative mtDNA content and number of mtDNA deletions was detected more often in radiation-associated PTC than in sporadic PTC. Thus, simultaneous determination of the number of large-scale mtDNA deletions and relative mtDNA content may be useful to elucidate molecular distinctive features of radiation-associated thyroid tumors.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/13/3794

Medulloblastoma is the most common malignant brain tumor in children. Chromosome arm 17p13.3 is reduced to homozygosity in 35-50% of medulloblastomas, making it the most frequent genetic alteration in these tumors. HIC-1 (hypermethylated in cancer) is a putative tumor suppressor gene located in the area of common deletion. HIC-1 resides in a CpG island and is hypermethylated in many different tumor types. Therefore, we studied a series of tumor specimens for hypermethylation and deletion of the region containing the HIC-1 gene to determine whether these two mechanisms of gene inactivation play a complimentary role in medulloblastoma. Southern blotting was performed using the methylation-sensitive restriction endonuclease NotI. Methylation of NotI restriction sites located in HIC-1 was demonstrated in 26 (72%) of 36 tumors and 11 (92%) of 12 specimens of normal brain. Of these 26 tumors, 23 differed significantly from normal brain. A greater proportion of the cells from the tumors showed methylated alleles of the HIC-1 gene. A group of 15 (42%) of 36 tumors exhibited loss of heterozygosity (LOH) for DNA sequences located on chromosome arm 17p. There was no significant correlation between LOH and methylation status (P = 0.19). Methylation in tumors beyond that seen in normal brain predicted poor overall survival independent of clinical risk category (P = 0.014). The results of our study show that methylation of the CpG island that contains the HIC-1 gene is common in medulloblastoma and, together with LOH of 17p, may be a critical event in the formation and aggressiveness of this tumor.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/13/4411

The OGG1 and MYH DNA glycosylases prevent the accumulation of DNA 8-hydroxyguanine. In Myh-/- mice, there was no time-dependent accumulation of DNA 8-hydroxyguanine in brain, small intestine, lung, spleen, or kidney. Liver was an exception to this general pattern. Inactivation of both MYH and OGG1 caused an age-associated accumulation of DNA 8-hydroxyguanine in lung and small intestine. The effects of abrogated OGG1 and MYH on hepatic DNA 8-hydroxyguanine levels were additive. Because there is an increased incidence of lung and small intestine cancer in Myh-/-/Ogg1-/- mice, these findings support a causal role for unrepaired oxidized DNA bases in cancer development.

Sales, K. J., A. A. Katz, et al. (2002). "Cyclooxygenase-1 Is Up-Regulated in Cervical Carcinomas:

http://cancerres.aacrjournals.org/cgi/content/abstract/62/2/424

This study was designed to investigate the expression and molecular signaling of cyclooxygenase-1 (COX-1) in cervical carcinomas. Real-time quantitative reverse transcription-polymerase chain reaction and Western blot analysis confirmed enhanced expression of COX-1 RNA, and protein in squamous cell carcinomas and adenocarcinoma of the cervix. COX-1 expression in all carcinoma tissues was associated with enhanced expression of COX-2 RNA and protein. The site of COX-1 expression was localized by immunohistochemistry to the neoplastic epithelial cells in all squamous cell carcinomas and adenocarcinomas studied. Minimal COX-1 immunoreactivity was detected in normal cervix. To explore events associated with COX-1 up-regulation, we developed a doxycycline-regulated expression system in HeLa (cervical carcinoma) cells. Overexpression of COX-1 in HeLa cells resulted in induced expression of cyclooxygenase-2 (COX-2) and prostaglandin E synthase (PGES) concomitant with increased prostaglandin E2 (PGE2) synthesis. Treatment of HeLa cells overexpressing COX-1 with the dual COX enzyme inhibitor indomethacin or selective COX-2 inhibitor NS-398 significantly reduced PGE2 synthesis. Indomethacin, but not NS-398, treatment abolished the up-regulation of expression of COX-2 and PGES in HeLa cells, suggesting that the observed up-regulation of COX-2 and PGES was mediated by COX-1-enzyme products. To assess whether enhanced PGE2 synthesis after COX-1 induction would act in an autocrine/paracrine manner, we investigated the effect of COX-1 on the expression of the different isoforms of PGE2 receptors (EP1-EP4). We found that the cAMP-linked PGE2 receptors were significantly up-regulated by COX-1 overexpression coincident with enhanced cAMP responsiveness of these cells to exogenous PGE2 ligand. Finally, overexpression of COX-1 was associated with enhanced expression of the angiogenic factors basic fibroblast growth factor, vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2. This up-regulation of angiogenic factor expression was abolished by indomethacin and partially reduced by NS-398. These data indicate that COX-1 up-regulation modulates the expression of factors that may act in an autocrine/paracrine manner to enhance and sustain tumorigenesis in neoplastic cervical epithelial cells. It is likely that similar mechanisms may act in vivo to modulate tumorigenesis of cervical carcinomas.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/21/7530

Bladder cancer is associated with smoking, occupational exposures, and glutathione S-transferase (GST) M1 and N-acetyltransferase (NAT) 2 polymorphisms that may influence carcinogen metabolism, but somatic p53mutations are often CpG dinucleotide G:C-A:T transitions that can occur spontaneously. We conducted a case-control study to determine whether p53mutation characteristics might distinguish cases with environmental versus endogenous causes. p53exons 4-9 were amplified from 146 bladder tumors by PCR, screened by single-strand conformational polymorphism analysis, and sequenced. Thirty-one cases were p53-positive, and 112 were p53-negative (germ line or silent). G:C-A:T transitions were also subclassified as CpG or non-CpG. Cases and 215 clinic controls were interviewed. GSTM1, NAT1, and NAT2 polymorphisms were assayed from peripheral blood. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic and polytomous regression. Case-control ORs for smoking, occupations, and NAT1*10genotype were similar for p53-positive and p53-negative cases. Associations with GSTM1-null and NAT2-slow genotypes were somewhat stronger for p53-positive [OR, 3.3; CI, 1.4-7.8 (GSTM1 null); OR, 1.8; CI, 0.8-4.0 (NAT2 slow)] than p53-negative cases [OR, 1.5; CI:0.9-2.3 (GSTM1 null); OR, 0.9; CI, 0.6-1.4 (NAT2 slow)]. Smoking was strongly associated with CpG G:C-A:T (OR, 15.3; CI:3.6-65) versus other G:C-A:T
(OR, 1.8; CI, 0.3-9.8). NAT2 slow genotypes were also associated with CpG G:C-A:T (OR, 6.2; CI:0.7-52), whereas GSTM1 null was associated with non-CpG G:C-A:T (OR, 7.8; CI, 0.9-65). Associations were not substantially different for case subtypes defined by p53 mutation status alone. Estimates for p53 subtypes were imprecise but support in vitro evidence that some CpG G:C-A:T transitions may be caused by smoking and other environmental mutagens.


Of 14 chemokine receptors investigated, only CXCR4 was expressed on ovarian cancer cells [C. J. Scotton et al., Cancer Res., 61: 4961-4965, 2001]. To further understand the role of this chemokine receptor in ovarian tumor biology, we studied the action of its ligand, CXCL12 (stromal cell-derived factor 1), on the CXCR4-expressing ovarian cancer cell lines IGROV. Ligand stimulation of the CXCR4 receptor resulted in sustained activation of Akt/protein kinase B and biphasic phosphorylation of p44/42 mitogen-activated protein kinase in IGROV. When IGROV cells were cultured under suboptimal conditions, CXCL12 stimulated their in vitro growth, an effect that was abrogated by neutralizing antibodies to CXCR4. This increase in cell number was attributable to stimulation of DNA synthesis, not protection from apoptosis. CXCL12 treatment of IGROV cells also induced mRNA and protein for tumor necrosis factor alpha, a cytokine that is expressed by tumor cells in ovarian cancer biopsies. IGROV cells invaded through Matrigel toward a CXCL12 gradient. Invasion was abrogated by the broad spectrum matrix metalloproteinase and TNF{alpha} converting enzyme inhibitor Marimastat and was partially inhibited by neutralizing antitumor necrosis factor {alpha} antibodies. These effects were not limited to the IGROV cell line. They could also be demonstrated in the CAOV-3 ovarian cancer cell line and primary ovarian tumor cells isolated from ovarian ascites. These biological effects of CXCL12 on IGROV cells were also inhibited by the small molecular weight CXCR4 antagonist AMD3100. Finally, we found abundant intracellular CXCL12 protein in tumor cells in 15 of 18 ovarian cancer biopsies but not in epithelial cells from normal ovary or borderline disease. The chemokine CXCL12 may have multiple biological effects in ovarian cancer, stimulating cell migration and invasion through extracellular matrix, as well as DNA synthesis and establishment of a cytokine network in situations that are suboptimal for tumor cell growth.


Development of basal cell carcinomas (BCCs) in skin is associated with uncontrolled Sonic hedgehog (Shh) signaling, which operates primarily through the Gli family of transcription factors. Gli2 is a mediator of physiological Shh signaling in skin and is sufficient to produce BCCs when overexpressed by use of a Keratin 5 (K5) promoter. Analysis of Gli protein deletion mutants has identified an NH2-terminal transcription repressor domain in Gli2 but not Gli1. To assess the potential involvement of the Gli2 repressor domain in skin tumor development, we overexpressed the Gli2(Delta)N2 mutant in transgenic mice by use of the K5 promoter. K5-Gli2(Delta)N2 mice developed a variety of skin tumors resembling human trichoblastomas, cylindromas, basaloide follicular hamartomas, and rarely, BCCs. In striking contrast, K5-Gli2 mice overexpressing wild-type Gli2 developed only BCCs. Other differences between tumors arising in these two sets of transgenic mice included their gross appearance, growth rate, and predilection for specific body sites. However, the expression levels of Shh target genes, which reflect the magnitude of Shh pathway activation, were not dramatically different. Tumors from K5-Gli2(Delta)N2 mice, unlike
human or mouse BCCs, gave rise to cell lines that constitutively expressed Shh target genes in vitro and were tumorigenic in nude mice. Interestingly, the phenotype of K5-Gli2(ΔN2) mice was strikingly similar to that reported after K5 promoter-driven overexpression of GLI1, which lacks an NH2-terminal region homologous to the Gli2 repressor domain. These results underscore the qualitative difference in oncogenicity of GLI1 and Gli2 when overexpressed in skin, and reveal a previously unanticipated role for the Gli2 NH2 terminus in defining tumor phenotype.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/9/2164

We developed a novel microarray system to assess gene expression, DNA methylation, and histone acetylation in parallel, and to dissect the complex hierarchy of epigenetic changes in cancer. An integrated microarray panel consisting of 1507 short CpG island tags located at the 5'-end regions (including the first exons) was used to assess effects of epigenetic treatments on a human epithelial ovarian cancer cell line. Treatment with methylation (5-aza-2'-deoxycytidine) or deacetylation (trichostatin A) inhibitors alone resulted in up-regulation of 1.9 or 1.1% of the genes analyzed; however, the combined treatment resulted in synergistic reactivation of more genes (10.4%; P < 0.001 versus either treatment alone). On the basis of either primary or secondary responses to the treatments, genes were identified as methylation-dependent or -independent. Synergistic reactivation of the methylation-dependent genes by 5-aza-2'-deoxycytidine plus trichostatin A revealed a functional interaction between methylated promoters and deacetylated histones. Increased expression of some methylation-independent genes was associated with enhanced histone acetylation, but up-regulation of most of the genes identified using this technology was because of events downstream of the epigenetic cascade. We demonstrate proof of principle for using the triple microarray system in analyzing the dynamic relationship between transcription factors and promoter targets in cancer genomes.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/1/262

To identify novel tumor suppressor genes involved in ovarian carcinogenesis, we generated four down-regulated suppression subtraction cDNA libraries from two early-stage (stage I/II) and two late-stage (stage III) primary ovarian tumors, each subtracted against cDNAs derived from normal ovarian epithelial cell brushings. Approximately 600-700 distinct clones were sequenced from each library. Comparison of down-regulated clones obtained from early- and late-stage tumors revealed genes that were unique to each library which suggested tumor-specific differences. We found 45 down-regulated genes that were common in all four libraries. We also identified several genes, the role of which in tumor development has yet to be elucidated, in addition to several under expressed genes, the potential role of which in carcinogenesis has been described previously (Bagnoli et al., Oncogene, 19: 4754-4763, 2000; Yu et al., Proc. Natl. Acad. Sci. USA, 96: 214-219, 1999; Mok et al., Oncogene, 12: 1895-1901, 1996). The differential expression of a subset of these genes was confirmed by semiquantitative reverse transcription-PCR using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control in a panel of 15 stage I and 15 stage III tumors of mixed histological subtypes. Chromosomal sorting of library sequences revealed that several of the genes mapped to known regions of deletion in ovarian cancer. Loss of heterozygosity (LOH) analysis revealed multiple genomic regions with a high frequency of loss
in both early- and late-stage tumors. To determine whether loss of expression of some of the genes corresponds to loss of an allele by LOH, we used a microsatellite marker for one of the novel genes on 8q and have shown that loss of expression of this novel gene correlates with loss of an allele by LOH. In conclusion, our analysis has identified down-regulated genes, which map to known as well as novel regions of deletions and may represent potential candidate tumor suppressor genes involved in ovarian cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/8/2359

We have shown recently that about half of the human TGCTs reveal DNA copy number increases affecting two distinct regions on chromosome arm 17q. To identify potential target genes with elevated expressions attributable to the extra copies, we constructed a cDNA microarray containing 636 genes and expressed sequence tags from chromosome 17. The expression patterns of 14 TGCTs, 1 carcinoma in situ, and 3 normal testis samples were examined, all with known chromosome 17 copy numbers. The growth factor receptor-bound protein 7 (GRB7) and junction plakoglobin (JUP) were the two most highly overexpressed genes in the TGCTs. GRB7 is tightly linked to ERBB2 and is coamplified and coexpressed with this gene in several cancer types. Interestingly, the expression levels of ERBB2 were not elevated in the TGCTs, suggesting that GRB7 might be the target for the increased DNA copy number in TGCTs. Because of the limited knowledge of altered gene expression in the development of TGCTs, we also examined the expression levels of 512 additional genes located throughout the genome. Several genes novel to testicular tumorigenesis were consistently up- or down-regulated, including POV1, MYCL1, MYBL2, MXI1, and DNMT2. Additionally, overexpression of the proto-oncogenes CCND2 and MYCN were confirmed from the literature. The overexpressions were for some of the target genes closely associated to either seminoma or nonseminoma TGCTs, and hierarchical cluster analysis of the gene expression data effectively distinguished among the known histological subtypes. In summary, this focused functional genomic characterization of TGCTs has lead to the identification of new gene targets associated with a common genomic rearrangement as well as other genes with potential importance to testicular tumorigenesis.


http://cancerres.aacrjournals.org/cgi/content/abstract/65/4/1459

Identifying new effective therapeutic treatments for lung cancer is critical to improving overall patient survival. We have targeted both the estrogen receptor (ER) and the epidermal growth factor receptor (EGFR) pathways using an ER antagonist, fulvestrant ("Faslodex"), and the selective EGFR tyrosine kinase inhibitor, gefitinib ("Iressa"), in non-small cell lung cancer (NSCLC) cells. Rapid activation of phospho-EGFR and phospho-p44/p42 mitogen-activated protein kinase by estrogen was observed, indicating nonnuclear ER transactivation of EGFR. Additionally, EGFR protein expression was down-regulated in response to estrogen and up-regulated in response to fulvestrant in vitro, suggesting that the EGFR pathway is activated when estrogen is depleted in NSCLC cells. Cell growth and apoptosis were examined in several NSCLC lines that express varying amounts of ER(beta), EGFR, and Neu but no full-length ER(alpha). One cell line contained an EGFR mutation. Cells were exposed to 10 nmol/L estrogen and 10 ng/mL EGF and either 1 {micro}mol/L fulvestrant or 1 {micro}mol/L gefitinib alone or in
combination. In all cell lines, the drug combination decreased cell proliferation up to 90% and increased apoptosis 2-fold. The relative responses to gefitinib and fulvestrant were similar regardless of ER and EGFR expression and mutation status. In an in vivo lung tumor xenograft model, the drug combination decreased tumor volume in severe combined immunodeficient mice by ~60% compared with 49% and 32% for gefitinib and fulvestrant treatment alone, respectively. Antitumor effects of the combination therapy were accompanied by biochemical and histologic evidence of increased apoptosis, decreased phospho-p44/p42 mitogen-activated protein kinase expression, and increased Ki-67 expression compared with individual treatment. These studies provide evidence of a functional interaction between the ER and the EGFR pathways in NSCLC.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/15/5347

Current therapies for gliomas often fail to address their infiltrative nature. Conventional treatments leave behind small clusters of neoplastic cells, resulting in eventual tumor recurrence. In the present study, we have evaluated the antitumor activity of neural progenitor cells against gliomas when stereotactically injected into nucleus Caudatus of Fisher rats. We show that the rat neural progenitor cell lines HiB5 and ST14A, from embryonic hippocampus and striatum primordium, respectively, are able to prolong animal survival and, in 25% of the cases, completely inhibit the outgrowth of N29 glioma compared with control animals. Delayed tumor outgrowth was also seen when HiB5 cells were inoculated at the site of tumor growth 1 week after tumor inoculation or when a mixture of tumor cells and HiB5 cells were injected s.c. into Fisher rats. HiB5 cells were additionally coinoculated together with two alternative rat gliomas, N32 and N25. N32 was growth inhibited, but rats inoculated with N25 cells did not show a prolonged survival. To evaluate the possibility of the involvement of the immune system in the tumor outgrowth inhibition, we show that HiB5 cells do not evoke an immune response when injected into Fisher rats. Furthermore, the rat neural progenitor cells produce all transforming growth factor (beta) isotypes, which could explain the observed immunosuppressive nature of these cells. Hence, some neural progenitor cells have the ability to inhibit tumor outgrowth when implanted into rats. These results indicate the usefulness of neural stem cells as therapeutically effective cells for the treatment of intracranial tumors.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/11/3871

It is well established that DNA hypermethylation of tumor suppressor and tumor-related genes can occur in cancer cells and that each cancer subtype has specific gene sets that are commonly susceptible to methylation and silencing. Glutathione S-transferase (GSTP1) is one example of a gene that is hypermethylated and inactivated in the majority of prostate cancers. We previously reported that hypermethylation of the GSTP1 CpG island promoter in prostate cancer cells is initiated by a combination of transcriptional gene silencing (by removal of the Sp1 sites) and seeds of methylation that, instead of being constantly removed because of demethylation associated with transcription, acts as a catalyst for the spread of methylation across the CpG island. In this study, we now demonstrate that the seeds of DNA methylation also play an important role in initiating chromatin modification. Our results address a number of central questions about the temporal relationship between gene expression, DNA hypermethylation, and chromatin modification in cancer cells. We find that for the GSTP1 gene, (a) histone acetylation is
independent of gene expression, (b) histone deacetylation is triggered by seeds of DNA methylation, (c) the spread of DNA hypermethylation across the island is linked to MBD2 and not MeCP2 binding, and (d) histone methylation occurs after histone deacetylation and is associated with extensive DNA methylation of the CpG island. These findings have important implications for understanding the biochemical events underlying the mechanisms responsible for abnormal hypermethylation of CpG island-associated genes in cancer cells.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/23/8682

The identification of membrane-associated and secreted genes that are differentially expressed is a useful step in defining new targets for the diagnosis and treatment of cancer. Extracting information on the subcellular localization of genes represented on DNA microarrays is difficult and is limited by the incomplete sequence and annotation that is available in existing databases. Here we combine a biochemical and bioinformatic approach to identify membrane-associated and secreted genes expressed in the MCF-7 breast cancer cell line. Our approach is based on the analysis of differential hybridization levels of RNAs that have been physically separated by virtue of their association with polysomes on the endoplasmic reticulum. This approach is specifically applicable to oligonucleotide microarrays such as Affymetrix, which use single-color hybridization instead of dual-color competitive hybridizations. Assignment to membrane-associated and secreted class membership is based on both the differential hybridization levels and an expression threshold, which are calculated empirically from data collected on a reference set of known cytoplasmic and membrane proteins. This method enabled the identification of 755 membrane-associated and secreted probe sets expressed in MCF-7 cells for which this annotation did not previously exist. The data were used to filter a previously reported expression dataset to identify membrane-associated and secreted genes which are associated with poor prognosis in breast cancer and represent potential targets for diagnosis and treatment. The approach reported here should provide a useful tool for the analysis of gene expression patterns, identifying membrane-associated or secreted genes with biological relevance that have the potential for clinical applications in diagnosis or treatment.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/6/2199

NY-ESO-1 is a cancer/testis antigen expressed in normal adult tissues solely in the testicular germ cells of normal adults and in various cancers. It induces specific humoral and cellular immunity in patients with NY-ESO-1-expressing cancer. The aim of this study was to determine the frequency of NY-ESO-1 mRNA and protein expression in malignant and benign breast tumors. NY-ESO-1 mRNA expression was detected by conventional reverse transcription-PCR and real-time PCR, and that of the protein expression by immunohistochemistry and Western blot analysis. Expression of NY-ESO-1 mRNA was detected in 37 of 88 (42%) cancer specimens, whereas that of the NY-ESO-1 protein was detected only in 1 mRNA-positive specimen. In the latter case, expression level of NY-ESO-1 mRNA relative to that in the testis was relatively high (75% of testicular expression) and to the other among breast cancer specimens. In benign breast lesions, 21 of 31 (68%) specimens expressed low levels of NY-ESO-1 mRNA. In 1 case of fibroadenoma, NY-ESO-1 mRNA was 8% of the testicular level, and protein was detected by Western blot analysis. Only 1 breast cancer patient had detectable antibody at time of surgery, which disappeared within 2 years. Tumor specimen from this patient was both NY-ESO-1 mRNA
and protein positive, and NY-ESO-1-specific CD8 T cells were detected in this patient by IFN-γ enzyme-linked immunospot assay using NY-ESO-1 recombinant adenovirus vaccine. A higher rate of NY-ESO-1 expression was noted in breast cancer with high histological grade and negative hormone receptor status, suggesting NY-ESO-1 as a potential tumor antigen for immunotherapy in patients with breast cancer and poor prognosis.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/7/1961

Frameshifts in short mononucleotide tracts (SMT) in genes, such as TGFβRII and BAX, are common in gastrointestinal tumors of the microsatellite mutator phenotype (MMP). The significance of less common mutations has been recently challenged because frequencies as high as 50% were reported in some noncoding SMTs in MMP colon cancer cell lines (L. Zhang, et al., Cancer Res., 61: 3801-3805, 2001). We did not confirm these findings after examining >50 MMP gastrointestinal cancers for mutations in eight SMT loci with the highest reported frequencies. In three of these loci, no clonal mutations were detected, and they were infrequent (2.9-6.7%) in the other five. Length polymorphisms are frequent (25.7-43.9%) in one-half of these SMTs, suggesting an explanation for the discrepancy. Because of the peculiar features of MMP tumors, low prevalence of mutations in cancer genes may not be a disqualifying criterion for their functionality.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/22/7913

Recent studies show that antagonists of growth hormone-releasing hormone (GH-RH) inhibit proliferation of various cancers indirectly through blockage of the endocrine GH-insulin-like growth factor (IGF) I axis and directly by an action on tumor cells involving the suppression of autocrine/paracrine IGF-I, IGF-II, or GH-RH. The effectiveness of therapy with GH-RH antagonist JV-1-38 and its mechanisms of action were investigated in NCI-H838 non-small cell lung carcinoma (NSCLC) xenografted s.c. into nude mice and in vitro. Treatment with GH-RH antagonist JV-1-38 significantly (P < 0.05-0.001) inhibited tumor growth as demonstrated by a 58% decrease in final tumor volume, 54% reduction in tumor weight, and the extension of tumor-doubling time from 8.5 +/- 1.38 to 12 +/- 1.07 days as compared with controls. Using ligand competition assays with 125I-labeled GH-RH antagonist JV-1-42, specific high-affinity binding sites for GH-RH were found on tumor membranes. Reverse transcription-PCR revealed the expression of mRNA for GH-RH and splice variant 1 (SV1) of GH-RH receptor in H838 tumors. Reverse transcription-PCR analysis also demonstrated that H838 tumors express IGF-I and IGF-I receptors. Tumoral concentration of IGF-I and its mRNA expression were significantly decreased by 25% (P = 0.05) and 65% (P < 0.001), respectively, in animals receiving JV-1-38, whereas serum IGF-I levels remained unchanged. In vitro studies showed that H838 cells secreted GH-RH and IGF-I into the medium. The growth of tumor cells in vitro was stimulated by IGF-I and inhibited by GH-RH antagonist JV-1-38 and a GH-RH antiserum. Our results extend the findings on the involvement of IGF-I in NSCLC and suggest that GH-RH may be an autocrine growth factor for H838 NSCLC. The antitumorigenic action of GH-RH antagonists could be partly direct and mediated by SV1 of tumoral GH-RH receptors. The finding of GH-RH and SV1 of GH-RH receptors in NSCLC provides a new approach to the treatment of this malignancy based on the use of antagonistic analogues of GH-RH.

http://cancerres.aacrjournals.org/cgi/content/abstract/64/8/2846

CD40 is expressed on B-cell malignancies, including human multiple myeloma (MM) and a variety of carcinomas. We examined the potential therapeutic utility of SGN-40, the humanized anti-CD40 monoclonal antibody, for treating human MM using MM cell lines and patient MM cells (CD138++, CD40+). SGN-40 (0.01-100 (micro)g/ml) induces modest cytotoxicity in MM cell lines and patient MM cells. In the presence of de novo protein synthesis inhibitor cycloheximide, SGN-40 significantly induced apoptosis in Dexamethasone (Dex)-sensitive MM.1S and Dex-resistant MM.1R cells and in patient MM cells. SGN-40-mediated cytotoxicity is associated with up-regulation of cytotoxic ligands of the tumor necrosis factor family (Fas/FasL, tumor necrosis factor-related apoptosis-inducing ligand, and tumor necrosis factor (alpha)). SGN-40 treatment also induces a down-regulation of CD40 dependent on an endocytic pathway. Consequently, pretreatment of MM cells with SGN-40 blocked sCD40L-mediated phosphatidylinositol 3'-kinase/AKT and nuclear factor (kappa)B activation. Importantly, pretreatment of MM.1S and MM.1R cells with SGN-40 inhibited proliferation triggered by interleukin 6 (IL-6) but not by insulin-like growth factor-I. In addition, SGN-40 pretreatment of MM.1S cells blocked the ability of IL-6 to protect against Dex-induced inhibition of DNA synthesis. This was associated with a 2-4-fold reduction of IL-6 receptor at protein and mRNA levels in SGN-40-treated MM.1S cells and patient MM cells. Taken together, these results provide the preclinical rationale for the evaluation of SGN-40 as a potential new therapy to improve patient outcome in MM.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/2/441

Cutaneous melanomas have been found to express several immunogenic differentiation melanoma-associated antigens (MAAs) that have been suggested to play an important role in disease outcome. Adaptive host immunity to MAAs has shown some level of control on melanoma progression. To date, there has been no definitive report correlating the level of differentiated MAAs gene expression in melanomas with overall disease outcome. Metastasis of melanoma to distant visceral organ sites usually indicates a survival of less than 1 year; however, a subset of patients who undergo cytoreductive surgery of distant metastases survive for a longer period. We hypothesized that the gene expression level of differentiation MAAs in metastatic melanoma (AJCC stage IV) lesions would be predictive of survival. We focused on three known differentiation MAAs: tyrosinase (TYR), TYR-related protein 2 (TRP-2), and melanoma antigen recognized by T cells 1 (MART-1); all three of them are known to induce immune responses in melanoma patients and are frequently expressed in melanomas. A quantitative reverse-transcriptase RealTime PCR (qRT) assay was developed for these MAAs to assess mRNA expression in metastatic melanoma tumors obtained from cytoreductive surgery of AJCC stage IV melanoma patients (n = 35). Patients were followed up for over 60 months. There was a variation in mRNA copy levels for individual MAAs in melanoma tumors. Elevated MAA mRNA copy levels of TYR and TRP-2 significantly (P < 0.03 and < 0.009, respectively) correlated with improved overall survival. Patients having at least one MAA expressed in their tumors had a significantly (P = 0.01) better overall survival (median 16 months). These studies demonstrate that levels of differentiated MAA mRNA expression of advanced-stage metastatic melanomas can be used as molecular predictive factors of disease outcome. The studies also imply that an assessment of
melanoma tumor MAAs may provide a stratification factor targeted for active-specific immunotherapy.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/22/6606

CWR22 has been a valuable xenograft model for the study of prostate cancer progression from an androgen-dependent tumor to one that grows in castrated animals. Herein, we report the identification and characterization of a novel androgen receptor (AR) mutation occurring in a relapsed tumor (CWR22R-2152) and in the CWR22Rv1 cell line established from it. The mutation was not detected in the original, hormone-dependent CWR22 xenograft, indicating that this change occurred during the progression to androgen independence. It is characterized by an in-frame tandem duplication of exon 3 that encodes the second zinc finger of the AR DNA-binding domain. Accordingly, immunoblot analyses demonstrated the expression of an AR species having an approximately 5-kDa increase in size relative to the LNCaP AR. This was accompanied by a COOH-terminally truncated AR species migrating with a relative mass of 75-80 kDa, referred to as AR(Δ)LBD because it lacks the ligand-binding domain. By recreating the exon 3 duplication mutation in a wild-type AR expression construct, the generation of AR(Δ)LBD could be recapitulated. Whereas AR(Δ)LBD exhibited constitutive nuclear localization and DNA binding, these functions in the full-length AR remained androgen dependent. The CWR22Rv1 AR repertoire displayed dose-dependent, androgen-responsive transcriptional transactivation in reporter assays, albeit to a lesser extent in comparison with LNCaP. This cell line also expressed low levels of prostate-specific antigen mRNA and did not express or secrete detectable levels of prostate-specific antigen protein in androgen-depleted medium or in response to physiological androgenic stimulation. In summary, the CWR22Rv1 cell line displays both androgen-responsive and androgen-insensitive features due, at least in part, to a novel insertional mutation of the AR.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/6/1172

Overexpression of the epidermal growth factor receptor (egfr) gene is a common feature in breast cancer. We demonstrated recently that the expression of EGFR in breast cancer strongly correlates with the length of a CA simple sequence repeat within the first 2000 bases in intron 1 of the egfr gene [CA simple sequence repeat (CA-SSR) I; H. Buergers et al., Cancer Res., 60: 854-857, 2000]. Using a standardized semiautomated method of microsatellite analysis for loss of heterozygosity detection, we identified an allelic imbalance (AI) at the egfr locus in 55 of 163 primary breast cancer cases. Fine mapping of the chromosomal region at 7p12-15 around the egfr gene using 10 CA-SSR markers showed that mutations of egfr in breast cancer are frequently restricted to the first intron of egfr. Thereby, the simple sequence repeat CA-SSR I in intron 1 was affected in 84% of the patients with AI. Reverse transcription-PCR analysis of 23 breast cancer tissues with AI excluded the presence of in-frame deletions between exon 2 and exon 7. For additional characterization of the underlying phenomenon leading to the detection of an AI in microsatellite analysis, a quantitative 5′-nuclease assay for the first CA-SSR I in intron 1 was established. In breast cancer cases with AI the presence of amplifications of this sequence was shown. Kaplan-Meier analysis revealed a statistically significant worse prognosis for patients with AI in the cancer tissue at the egfr locus compared with patients without AI. Interestingly, 75%
of the patients bearing AI of CA-SSR I in the tumor also showed AI at normal, nontumorous breast tissue. Our data strongly support the assumption that distinct amplifications in intronic sequences of the egfr gene, which enhance the basic transcription activity of the gene, represent one of the first steps in breast carcinogenesis. Furthermore, they point to the presence of prognosis-associated markers for breast cancer already in morphological normal breast tissue.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/17/4938

To clarify modes of silencing of p16 and their concerns to the development and progression of esophageal squamous cell carcinomas (ESCCs), we examined immunoreactivity of p16, loss of heterozygosity (LOH) at six microsatellite loci in 9p13-22, and the methylation status of the p16 promoter in 42 cases of the ESCC at various stages. The samples taken from step sections in and around the tumors were examined to map heterogeneity of those changes. Thereby at least one focus of dysplasia was detected in each case. No immunexpression of p16 was detected in the ESCCs of 38 cases (90.5%) and in dysplasias of 34 cases (81%), whereas the histologically normal epithelia adjacent to the ESCC showed the p16 expression even in the presence of p16 methylation. Of the ESCCs/dysplasias without p16 expression (38/34), 16/0 showed both p16 methylation and LOH at the near-p16 loci (+/+), 14/30 did only methylation (+/-) and 8/4 did only LOH (-/+). The presence of LOH with/without homozygous deletion (HD) at the near-p16 loci correlated with the advanced tumor stages (P < 0.001). The mapping of +/+ cases indicated that the +/+ carcinomas were included in the +/- carcinomas, which were, in turn, surrounded by the +/- dysplasias and/or by the +/- normal-looking epithelia, whereas the -/+ dysplasias were always accompanied by the -/+ carcinomas. These results suggest that the mode of p16 silencing either through methylation or through LOH and possible mutation is determined in each patient before the occurrence of ESCCs and dysplasia. The mapping of HD and p16 expression suggest that the HD is a later event than the p16 silencing.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/10/2578

Uveal melanoma is the most common intraocular malignancy. About 50% of patients die of metastases, which almost exclusively originate from primary tumors that have lost one chromosome 3 (monosomy 3). To gain insight into the biological mechanisms that underlie the various metastasizing potential of uveal melanoma, we have determined gene expression levels in 20 primary tumors using oligonucleotide microarrays containing 12500 probe sets. The expression measurements of those 7902 genes that were expressed in more than 10% of tumors were analyzed using two different statistical approaches. We used a modified Wilcoxon rank-sum test to identify genes differentially expressed between tumors with and without monosomy 3. Seven genes showed complete loss of expression in tumors with monosomy 3 but were expressed in tumors with disomy 3. Two of them, CHL1 and fts485, are located within or close to the uveal melanoma susceptibility locus UVM2 at 3p25. However, mutation analysis of both genes in eight tumors with monosomy 3 did not reveal structural or epigenetic alteration. To identify tumor classes, we performed unsupervised hierarchical cluster analysis; this approach separated uveal melanomas into two groups. We found that this classification is strikingly robust because, when tested by "resampling," the same grouping is obtained from 47 of 50 subsamples of genes. In clusterings of the three remaining subsamples, the grouping of only one tumor does
not conform with the original classification. Excluding this tumor, cluster analyses of subsamples containing as few as 300 randomly chosen genes consistently result in the same classification, thus indicating that the difference between the two tumor classes is pervasive. Interestingly, all of the tumors in one of the groups have disomy 3, whereas all of the others have monosomy 3. Our findings suggest that there are two distinct entities of uveal melanoma that were previously unrecognized because they are not obviously distinguishable by clinicopathological features.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/3/647

Glycine N-methyltransferase (GNMT), a multifunctional protein involved in the maintenance of the genetic stability, is often down-regulated in hepatocellular carcinoma (HCC). Using genotypic characterization of GNMT in hepatoma cell lines and in a Taiwanese population with a high incidence of liver cancer we have investigated the role of this gene in the progression of liver cancer. Six novel polymorphisms, including two short tandem repeats, one 4-nucleotide insertion/deletion polymorphism, and three single nucleotide polymorphisms, in GNMT were identified in this study. The rates of loss of heterozygosity at the GNMT locus in pairs of normal and tumor tissue from the HCC patients were approximately 36-47%. In addition, the observed heterozygosity of GNMT decreases in tumor adjacent liver DNA from HCC patients compared with that observed in blood DNA from normal individuals and HCC patients. This may result from the early event of loss of heterozygosity within the GNMT gene in the liver tissues of HCC patients. However, in this study, we did not observe the association of polymorphic GNMT alleles as inherited risk factors for HCC. We also elucidated the functional impact of genetic markers in the GNMT promoter by performing luciferase reporter gene and gel mobility shift assays. The results indicate that two polymorphisms, short tandem repeat 1 and insertion/deletion polymorphism, in the promoter region could cause allelic specific effects on the transcriptional activity of GNMT. The risk genotypes of GNMT, which presumably have a lower expression level, as estimated from in vitro functional studies, are over-represented in tumor-adjacent tissues from HCC patients. In summary, our results suggest that GNMT alteration may be an early event in HCC development and that GNMT could be a new tumor susceptibility gene for HCC.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/10/2957

The von Hippel-Lindau tumor suppressor protein acts as the substrate recognition component of a ubiquitin E3 ligase that targets hypoxia-inducible factor (HIF){(alpha)} subunits for proteolysis. Stabilization of HIF{(alpha)} subunits has been described in VHL-defective cell lines, leading to HIF activation and up-regulation of hypoxia-inducible mRNAs. Mutations of the von Hippel-Lindau tumor suppressor protein are found in most clear cell renal cell carcinomas (CC-RCCs) but not other renal tumors, raising a question about the importance of activation of the HIF pathway in CC-RCC development. To address this question, we have examined the expression of HIF{(alpha)} subunits in 45 primary renal tumors and related this to tumor subtype, the presence of VHL mutations, and measures of angiogenesis. We show that HIF{(alpha)} is up-regulated in the majority of CC-RCCs, and that the pattern of expression is biased toward the HIF-2{(alpha)} isoform. Expression of HIF{(alpha)} proteins was associated significantly with up-regulation of VEGF mRNA and protein and increased microvessel density. Up-regulation of HIF{(alpha)} in CC-RCC was found to involve increased mRNA as well as protein expression, suggesting that both
VHL-dependent and VHL-independent mechanisms are involved. These results suggest that activation of the HIF pathway is functionally important in CC-RCC development and might provide a new therapeutic target.


Thymidylate synthase (TS) is a key enzyme in folate metabolism and the primary target of 5-fluorouracil. A repeat polymorphism in the TS promoter enhancer region (2rpt versus 3rpt of 28 bp) is associated with decreased expression, and a 6-bp deletion in the 3'untranslated region may affect RNA stability. We investigated the role of TS polymorphisms in a case control study of adenomatous polyps (510 cases and 604 polyp-free controls). Multivariate-adjusted odds ratios (ORs; 95% confidence interval) for TSER 2rpt/3rpt and 2rpt/2rpt compared with 3rpt/3rpt were 0.8 (0.6-1.2) and 0.9 (0.6-1.3), respectively. We observed a significant gene-nutrient interaction between the TSER polymorphism and folate intake: among 3rpt/3rpt individuals (greater expression), folate intake > 440 [micro]g/day (highest tertile) versus [<=]440 [micro]g/day was associated with a 2-fold decreased risk [ORs 1.0 (reference group) versus 0.5 (0.3-0.9)]. However, among 2rpt/2rpt individuals, high folate intake was associated with a 1.5-fold increased risk [ORs 0.6 (0.4-0.9) versus 0.9 (0.5-1.5; P for interaction = 0.03)]. Vitamin B12 showed a similar trend (P = 0.08). No clear pattern was seen with the TS 1494del6 polymorphism. These findings raise questions regarding the molecular pathways linking folate metabolism and colorectal carcinogenesis, including whether high folate is beneficial in the presence of all metabolic genotypes.


The serum of cancer patients often harbors increased free DNA levels, which can potentially be used for cancer detection. Because genetic and epigenetic alterations of the adenomatous polyposis coli (APC) gene are common events in gastrointestinal tumor development, we sought to investigate the frequency and level of aberrant APC promoter methylation in primary tumors and paired preoperative serum or plasma samples of lung cancer patients by semiquantitative methylation-specific fluorogenic real-time PCR. We detected methylation of APC in 95 of 99 (96%) primary lung cancer tissues. Forty-two of 89 (47%) available serum and/or plasma samples from these cases carried detectable amounts of methylated APC promoter DNA. In contrast, no methylated APC promoter DNA was detected in serum samples from 50 healthy controls. A highly elevated APC methylation level in lung cancer tissue was the only independent factor predicting inferior survival in this cohort (P = 0.015). APC methylation analysis appears to be promising as a prognostic factor in primary lung cancer and as a noninvasive tumor marker in plasma and/or serum DNA.

We profiled the expression of genes in benign and untreated human prostate cancer tissues using oligonucleotide microarrays. We report here 50 genes with distinct expression patterns in metastatic and confined tumors (Gleason score 6 and 9; lymph node invasive and noninvasive). Validation of expression profiles of 6 genes by quantitative PCR revealed a strong inverse correlation in the expression of zinc finger protein 185 (ZNF185), bullous pemphigoid antigen gene (BPAG1), and prostate secretory protein (PSP94) with progression of prostate cancer. Treatment of prostate cancer cell lines with 5-aza-2’-deoxycytidine (5-Aza-CdR), an inhibitor of DNA methylation, restored ZNF185 expression levels. Moreover, methylation-specific PCR confirmed methylation of the 5’CpG islands of the ZNF185 gene in all of the metastatic tissues and 44% of the localized tumor tissues, as well as in the prostate cancer cell lines tested. Thus, transcriptional silencing of ZNF185 by methylation in prostate tumor tissues implicates the ZNF185 gene in prostate tumorigenesis.


Frequent BRAF mutations were reported recently in a variety of human malignancies, including colorectal cancer. In this study, we screened 293 colorectal cancers for mutations in exons 11 and 15, two regions containing hotspots for BRAF mutation. Of the 293 cancers, 170 had normal mismatch repair, and 123 had defective mismatch repair (originating from both somatic as well as germ-line mutations in several of the mismatch repair genes). A total of 63 exonic mutations (22%) were detected, 60 of which were V599E, and one each of D593G, G468E, and D586A. Of the tumors with defective mismatch repair, 34% (42 of 123) had a mutation in BRAF, whereas only 12% (21 of 170) of tumors with proficient mismatch repair demonstrated a mutation (P < 0.0001). Interestingly, BRAF mutations were found most often in cases with an hMLH1
abnormality (35 of 60) and rarely in cases with an hMSH2 abnormality (1 of 39; \( P < 0.0001 \)). More interestingly, of the 31 hMLH1 cases with a BRAF mutation, 30 occurred in tumors known to have hypermethylation of hMLH1 promoter. Only 1 of the 15 cases with a germ-line mutation in hMLH1 had a mutation in BRAF. In this series, BRAF mutations occurred rarely in tumors with defective mismatch repair attributable to the presence of germ-line mutation in either hMLH1 or hMSH2. Furthermore, BRAF mutations were strongly associated with the epigenetic alteration of hMLH1. Overall, these data suggest that BRAF mutations are not a consequence of defective mismatch repair per se.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/22/7646

Hepatocellular carcinoma (HCC) is one of the most common fatal cancers in the world. However, the underlying molecular mechanisms contributing to hepatocarcinogenesis are still unclear. A putative tumor suppressor gene, namely DLC-1 (frequently deleted in liver cancer) was identified and mapped at chromosome 8p21.3-22, a recurrently deleted region in human cancers. The gene exerts inhibitory effects on the cell proliferation of HCC cells. In this study, we investigated the biological function, and genetic and epigenetic status of this gene in human HCC. With in vitro GTPase activating proteins activity assay, we established that DLC-1 protein was a GTPase-activating protein specific for RhoA and Cdc42. Deletion of the DLC-1 gene was frequent in human HCC, as revealed by loss of heterozygosity analysis performed on 100 human HCC cases with markers mapped at the DLC-1 locus, and allelic losses ranging from 44% to 50% of the informative cases. However, somatic mutations of the DLC-1 gene were rare. Moreover, with real-time quantitative PCR, we found that DLC-1 mRNA was significantly underexpressed in HCCs when compared with the corresponding nontumorous livers (\( P < 0.0001 \)). In addition, the CpG island 5' to the DLC-1 gene was methylated in 3 of 7 HCC cell lines and in 6 (24%) of 25 primary HCCs. These data suggest that transcriptional silencing by hypermethylation may contribute to the inactivation of the DLC-1 gene. Taken together, the results of our study suggest that both genetic and epigenetic alterations play an important role in inactivation of the DLC-1 gene in hepatocarcinogenesis.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/19/6178

Aberrant DNA methylation of promoter CpG islands is associated with transcriptionally repressive heterochromatin in neoplasia. The dynamics of this epigenetic process in mediating the transition from an active to an inactive state of transcription remains to be elucidated, however. Here, we used the methylation-specific oligonucleotide microarray to map the methylation patterns of a CpG island, located within the promoter and the first exon regions of RASSF1A, in normal breast tissue controls, primary tumors, and breast cancer cell lines. Oligonucleotide pairs, spaced along the CpG island region, were designed to discriminate between methylated and unmethylated alleles of selected sites. The methylation-specific oligonucleotide data indicate that the majority of test samples show widespread methylation in the first exon of RASSF1A. In contrast, the promoter area was usually undermethylated in normal controls and in 32% of the primary tumors tested, whereas the rest of the primary tumors and breast cancer cell lines showed various degrees of methylation in the region. Methylation profiling of individual tumors further suggest that DNA methylation progressively spreads from the first exon into the promoter area of this gene. Functional analysis indicates that increased density of RASSF1A promoter methylation is
associated with altered chromatin, marked by a depletion of acetylated histones and methylated histone 3-lysine 4 and an enrichment of methylated histone 3-lysine 9 in the studied area. The combination of these epigenetic modifications may engender a stable silencing of the gene in breast cancer cells. Thus, this study underscores the importance of detailed mapping of methylation patterns within a CpG island locus that may provide insights into the progressive nature of aberrant DNA methylation and its relationship with transcriptional silencing during the neoplastic process.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/16/4990

Mice with a targeted inactivation of both alleles of the cyclin-dependent kinase inhibitor p27kip1 developed both small and large intestinal adenomas when fed a control AIN-76A diet. A Western-style diet that is high in fat and phosphate and low in calcium and vitamin D was also able to initiate adenoma formation in wild-type mice. The combination of p27kip1 inactivation and the Western-style diet was additive in terms of tumor incidence, frequency and size, and in reducing the life span of the mice. The genetic and dietary combination also resulted in development of adenocarcinoma. Tumor formation was linked to a disruption in homeostasis of the intestinal mucosa, involving increased cell proliferation and decreased apoptosis. There was also decreased goblet cell differentiation as assessed by alcian blue staining and expression of the Muc2 gene, especially in mice fed the Western-style diet, although this differentiation lineage was still present as indicated by expression and staining for intestinal trefoil factor. The inactivation of p27kip1 and the consequent disruption of normal colonic cell maturation in the mucosa were associated with modestly elevated c-myc, cdk4, and cyclin D1 expression. These data establish a fundamental role for p27kip1 in maintenance of intestinal cell homeostasis and in suppressing tumor formation. The data also emphasize the critical role that dietary factors can have in both tumor initiation and progression through interaction with pathways that normally maintain intestinal homeostasis.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/6/1975

Aberrant DNA methylation patterns may be the earliest somatic genome changes in prostate cancer. Using real-time methylation-specific PCR, we assessed the extent of hypermethylation at 16 CpG islands in DNA from seven prostate cancer cell lines (LNCaP, PC-3, DU-145, LAPC-4, CWR22Rv1, VCaP, and C42B), normal prostate epithelial cells, normal prostate stromal cells, 73 primary prostate cancers, 91 metastatic prostate cancers, and 25 noncancerous prostate tissues. We found that CpG islands at GSTP1, APC, RASSF1a, PTGS2, and MDR1 were hypermethylated in >85% of prostate cancers and cancer cell lines but not in normal prostate cells and tissues; CpG islands at EDNRB, ESR1, CDKN2a, and hMLH1 exhibited low to moderate rates of hypermethylation in prostate cancer tissues and cancer cell lines but were entirely unmethylated in normal tissues; and CpG islands at DAPK1, TIMP3, MGMT, CDKN2b, p14/ARF, and CDH1 were not abnormally hypermethylated in prostate cancers. Receiver operator characteristic curve analyses suggested that CpG island hypermethylation changes at GSTP1, APC, RASSF1a, PTGS2, and MDR1 in various combinations can distinguish primary prostate cancer from benign prostate tissues with sensitivities of 97.3-100% and specificities of 92-100%. Hypermethylation of the CpG island at EDNRB was correlated with the grade and
stage of the primary prostate cancers. PTGS2 CpG island hypermethylation portended an increased risk of recurrence. Furthermore, CpG island hypermethylation patterns in prostate cancer metastases were very similar to the primary prostate cancers and tended to show greater differences between cases than between anatomical sites of metastasis.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/4/878

WWOX (WW domain containing oxidoreductase), a putative tumor suppressor gene that maps to the common fragile site FRA16D on chromosome 16q23.3-24.1, is altered in breast, esophageal, and ovarian cancer. Because the FRA3B/FHIT locus at 3p14.2 is a preferential target for genetic changes caused by tobacco smoke, we intended to evaluate the status of the FRA16D/WWOX gene in non-small cell lung cancer; we have analyzed 27 paired normal and tumor lung tissues and 8 lung cancer cell lines for WWOX alterations by reverse transcriptase-PCR, loss of heterozygosity, and mutation analysis. Transcripts missing WWOX exons were detected in 7 primary tumors (7 of 27; 25.9%) and 5 of 8 cell lines. In addition, loss of heterozygosity at the WWOX locus was observed in 10 primary tumors (10 of 27; 37.0%). We conclude that WWOX alterations occur in a significant fraction of lung cancers and may contribute to the pathogenesis of non-small cell lung cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/19/6170

Ferredoxin reductase (FDXR) is a putative contributor to TP53-mediated apoptosis from 5-fluorouracil chemotherapy through the generation of oxidative stress. With TaqMan real-time quantitative reverse transcription-PCR, this study established a significant difference in FDXR relative RNA expression level between tumor (median, 212.9 units) and normal tissues (median, 113.8 units) from 51 colorectal cancer patients (P < 0.001). Seven single nucleotide polymorphisms (SNPs) in the FDXR gene were discovered, with no significant difference in variant allele frequency between colon tumor and normal tissues (P > 0.05), and the common haplotypes for FDXR were not different between colon tumor and normal samples. No correlation was observed between FDXR genotype and RNA expression implying that the polymorphisms described in this study are not regulating FDXR expression in colon cancer. This genomic characterization provides the foundation for pharmacogenetic analysis of the impact of FDXR on chemotherapy for colorectal cancer.


http://cancerres.aacrjournals.org/cgi/content/abstract/63/14/4174

ARHI is a maternally imprinted tumor suppressor gene that maps to a site on chromosome 1p31 where loss of heterozygosity has been observed in 40% of human breast and ovarian cancers. ARHI is expressed in normal ovarian and breast epithelial cells, but ARHI expression is lost in a
majority of ovarian and breast cancers. Expression of ARHI from the paternal allele can be down-regulated by multiple mechanisms in addition to loss of heterozygosity. This article explores the role of DNA methylation in silencing ARHI expression. There are three CpG islands in the ARHI gene. CpG islands I and II are located in the promoter region, whereas CpG island III is located in the coding region. Consistent with imprinting, we have found that all three CpG islands were partially methylated in normal human breast epithelial cells. Additional confirmation of imprinting has been obtained by studying DNA methylation and ARHI expression in murine A9 cells that carry either the maternal or the paternal copy of human chromosome 1. All three CpG islands were methylated, and ARHI was not expressed in A9 cells that contained the paternal allele. Conversely, CpG islands were not methylated and ARHI was expressed in A9 cells that contained the paternal allele of human chromosome 1. Aberrant methylation was found in several breast cancer cell lines that exhibited decreased ARHI expression. Hypermethylation was detected in 67% (6 of 9) of breast cancer cell lines at CpG island I, 33% (3 of 9) at CpG island II, and 56% (5 of 9) at CpG island III. Hypomethylation was observed in 44% (4 of 9) of breast cancer cell lines at CpG island II. When methylation of CpG islands was studied in 20 surgical specimens, hypermethylation was not observed in CpG island I, but 3 of 20 cases exhibited hypermethylation in CpG island II (15%), and 4 of 20 cases had hypermethylation in CpG island III (20%). Treatment with 5-aza-2'-deoxycytidine, a methyltransferase inhibitor, could reverse aberrant hypermethylation of CpG island I, II and III and partially restore ARHI expression in some, but not all of the cell lines. Treatment with 5-aza-2'-deoxycytidine partially reactivated ARHI expression in cell lines with hypermethylation of CpG islands I and II but not in cell lines with partial methylation or hypomethylation of these CpG islands. To test the impact of CpG island methylation on ARHI promoter activity more directly, constructs were prepared with the ARHI promoter linked to a luciferase reporter and transfected into SKBr3 and human embryo kidney 293 cells. Methylation of the entire construct destroyed promoter activity. Selective methylation of CpG island II alone or in combination with CpG island I abolished ARHI promoter activity. Methylation of CpG I alone partially inhibited promoter activity of ARHI. Thus, hypermethylation of CpG island II in the promoter region of ARHI is associated with the complete loss of ARHI expression in breast cancer cells. Other epigenetic modifications such as hypermethylation in CpG island III may also contribute to the loss of ARHI expression.


http://cancerres.aacrjournals.org/cgi/content/abstract/62/13/3743

Uroplakins (UPs) are a group of integral membrane proteins that are synthesized as the major differentiation products of mammalian urothelium. UPII gene expression is bladder specific and differentiation dependent, but very little is known about its transcription response elements. To identify the promoter elements, a DNA fragment of 2239 bp upstream of the UPII gene was amplified by PCR and linked to a promoterless firefly luciferase reporter gene. Transient transfection experiments showed that the DNA segment located between -1809 and +1 bp resulted in preferential expression in bladder carcinoma cells with negligible expression in nonurothelial cells. This promoter was engineered into adenovirus (Ad) type 5 to drive the expression of the E1A and E1B genes and to create an attenuated replication-competent Ad variant, termed CG8840. Viral replication and the cytopathic effect of CG8840 were evaluated by virus yield and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in bladder transitional cell carcinoma (TCC) cell lines RT4 and SW780; nonbladder cancer cell lines G361 (melanoma), LNCaP (prostate cancer), PA-1 (ovarian cancer), and U118 (brain cancer); and human primary cells including lung fibroblasts, bladder smooth muscle cells, and mammary epithelial cells. CG8840 replicated in and eliminated bladder TCC efficiently with high specificity (10,000:1) in comparison with nonbladder cells. The antitumor activity of CG8840 was examined in BALB/c nu/nu mice carrying s.c. human TCC xenografts. Intratumoral and i.v. administration of
CG8840 in RT4 human bladder cancer xenografts caused significant (P < 0.01) inhibition of tumor growth. Synergistic antitumor efficacy was observed when CG8840 was combined with docetaxel, resulting in significant regression of RT4 bladder cancer xenograft tumors within 6 weeks after i.v. administration of CG8840 (3.33 x 10^9 plaque-forming units/animal on day 1) and docetaxel (20 mg/kg on days 2, 6, and 9). These results demonstrate the utility of the UPII promoter in the generation of urothelium-specific adenoviral vectors and provide a potential foundation for the development of bladder tumor-specific oncolytic viral therapies.


http://cancerres.aacrjournals.org/cgi/content/abstract/64/6/2000

The human FHIT (fragile histidine triad) gene is a putative tumor suppressor gene located at chromosome region 3p14.2. Previous studies have shown that loss of heterozygosity, homozygous deletions, and abnormal expression of the FHIT gene are involved in several types of human malignancies. A CpG island is present in the 5’ promoter region of the FHIT gene, and methylation in this region correlates with loss of FHIT expression. To test whether aberrant methylation of the FHIT gene may play a role in pediatric leukemia, we assessed the FHIT methylation status of 10 leukemia cell lines and 190 incident population-based cases of childhood acute lymphocytic and myeloid leukemias using methylation-specific PCR. Conventional and fluorescence in situ hybridization cytogenetic data were also collected to examine aneuploidy, t(12, 21), and other chromosomal rearrangements. Four of 10 leukemia cell lines (40%) and 52 of 190 (27.4%) bone marrows from childhood leukemia patients demonstrated hypermethylation of the promoter region of FHIT. Gene expression analyses and 5-aza-2’-deoxycytidine treatment showed that promoter hypermethylation correlated with FHIT inactivation. Among primary leukemias, hypermethylation of FHIT was strongly correlated with acute lymphoblastic leukemia (ALL) histology (P = 0.008), high hyperdiploid (P < 0.0001), and translocation-negative (P < 0.0001) categories. Hyperdiploid B-cell ALLs were 23-fold more likely to be FHIT methylated compared with B-cell ALL harboring TEL-AML translocations. FHIT methylation was associated with high WBC counts at diagnosis, a known prognostic indicator. These results suggest that hypermethylation of the promoter region CpG island of the FHIT gene is a common event and may play an important role in the etiology and pathophysiology of specific cytogenetic subtypes of childhood ALL.

**Carbohydrate Polymers** (1)


http://www.sciencedirect.com/science/article/B6TFD-4DFK86B-3/2/91d8797f7a5e708ca00e5de45ee0d028

To evaluate the mechanism for the biological activity of a natural polysaccharide isolated from the mucus of the loach, Misgurnus anguillicaudatus (MAP), the immunomodulatory of MAP was investigated by the methods of molecular biology and cellular biology. The results showed that
MAP enhanced proliferation of T lymphocyte, IL-2 expression of Th1 cells, and IL-4 expression of Th2 cells. Time dependence of the secretion of cytokines showed that Th1 cell was the primary cellular target affected by MAP on T lymphocyte. However, MAP did not increase directly the proliferation of B cells and enhanced less IgM antibody production. Moreover, MAP improved the viability of peritoneal macrophages, stimulated TNF-[alpha] and IL-6 production and induced the inducible nitric oxide synthase (iNOS) transcription in macrophages. In addition, MAP exerted its immunomodulating activity at an optimal dose of 30 [mu]g/ml. At this concentration, MAP promoted farthest proliferation of spleen lymphocyte and macrophages. Consequently, MAP enhanced the immune system functions. In conclusion, the biological activity of the loach, which was as traditional Chinese medicine in folk remedies for the treatments of hepatitis, osteomyelitis, carbuncles, inflammations and cancers, as well as for the restoration to health in debilities by various pathogens and aging, may mainly result from MAP selectively activating T cells and macrophages and stimulating secretion of some cytokines.

**Carbohydrate Research** (1)


http://www.sciencedirect.com/science/article/B6TFF-42HFP1G-6/2/24e34f21062d14aadb2c9bdeab327808

The cDNA of Chinese hamster ovary (CHO) cell cytosolic sialidase was amplified by RT-PCR and cloned into the pGEX-2T plasmid vector encoding for glutathione S-transferase (GST). Screening revealed transformed Escherichia coli clones with the constructed plasmid encoding the CHO cell sialidase sequence. After isopropyl-[beta]-thiogalacto-pyranoside (IPTG) induction, SDS-PAGE of the total protein extracts revealed a new protein of about 70 kDa, correlating with the molecular weight of a fusion protein composed of the GST (26 kDa) and the cloned cytosolic CHO cell sialidase (43 kDa). A soluble fusion protein was purified from sonified E. coli homogenates by one-step affinity chromatography on Glutathione Sepharose 4B, which showed sialidase activity towards 4-methyl-umbelliferyl-[alpha]-N-acetylneuraminic acid (MUF-Neu5Ac) substrate. Induction of cells with 0.1, 0.5, and 1.0 mM IPTG revealed highest total protein amounts after induction with 1.0 mM IPTG, but highest specific activity for affinity chromatography purified eluates from cultures induced with 0.1 mM IPTG. Therefore, large scale production was performed by inducing cells during exponential growth in a 25 L bioreactor for 3 h with 0.1 mM IPTG after chilling the cell suspension to 25[deg]C. The amount of 26.46 mg of 40-fold purified GST-sialidase with a specific activity of 0.999 U/mg protein was obtained from crude protein extracts by one-step affinity chromatography. 2-Deoxy-2,3-dehydro-N-acetylneuraminic acid (Neu5Ac2en) and Neu5Ac were competitive inhibitors for the sialidase, the former being the more effective one using MUF-Neu5Ac as the substrate. The cytosolic sialidase is capable of desialylating a wide spectrum of different types of gangliosides using a thin-layer chromatography overlay kinetic assay without detergents. This is the subject of the accompanying paper (Muthing, J.; Burg, M. Carbohydr. Res. 2001, 330, 347-356).

http://carcin.oupjournals.org/cgi/content/abstract/25/5/749

Mutations of the N- and K-ras genes occur in ~15-30% of acute myeloid leukaemia patients. The role of the oncogenic ras in leukaemogenesis remains unclear. Few studies have revealed that mutations in the ras oncogene family are more probably found in acute myeloid leukaemia patients with previous exposure to toxic agents. A case-case study was conducted in the areas of Florence and Turin, Italy, to investigate whether the presence of N- and K-ras mutations in acute myeloid leukaemia patients was related to a higher frequency of exposure to chemicals. During a 3-year period, 111 acute myeloid leukaemia patients were enrolled. All the patients were interviewed using a semi-structured questionnaire collecting data on residential history, occupation, personal habits and pathological history. The presence of N- and K-ras mutations was analysed by amplification and synthetic oligonucleotide probes and by the so-called polymerase chain reaction amplification for specific alleles technique. A total of 34 (30.6%) patients were found to harbour ras mutations in N-ras and/or K-ras. Fourteen patients (12.6%) had a single ras mutation and 20 patients (18%) had two ras mutations. A positive association between a priori at risk jobs and ras mutations was found, based on nine exposed cases; the odds ratio, adjusted by age, sex and previous X-ray and/or chemotherapy was 2.8 (95% confidence intervals: 0.9-9.0). When considering only subjects with two ras mutations the odds ratio was 4.8 (95% confidence intervals: 1.2-18.8). The odds ratio for a previous X-ray and/or chemotherapy was 16.2 (95% confidence intervals: 1.8-755.9); when only subjects with two ras mutations were considered, the odds ratio was 26.1 (95% confidence intervals: 2.5-1248.9). In conclusion, our data suggest that ras oncogene mutations might identify a group of leukaemia in people with previous X-ray/chemotherapy or with exposure to chemical agents in the work environment.


http://carcin.oupjournals.org/cgi/content/abstract/23/8/1399

Glucoraphanin in Brassica vegetables breaks down to either sulforaphane or sulforaphane nitrile depending on the conditions, and sulforaphane can be further conjugated with glutathione. Using a high-throughput microtitre plate assay and TaqMan real time quantitative RT-PCR to measure mRNA, we show that sulforaphane and its glutathione conjugate, but not the nitrile, increased significantly (P < 0.05) both UGT1A1 and GSTA1 mRNA levels in HepG2 and HT29 cells. These changes were accompanied by an increase in UGT1A1 protein, as assessed by immunoblotting, and a 2.8-fold increase in bilirubin glucuronidation. When treated together, the nitrile derivative did not affect sulforaphane induction. The induction of UGT1A1 and GSTA1 mRNA by sulforaphane was time and concentration dependent. The results show a functional induction of glucuronidation by sulforaphane but not sulforaphane nitrile, and show that the pathway of metabolism of glucosinolates in Brassica vegetables is important in determining the resulting biological and anticarcinogenic activities.
diagnosis lung cancer among never smokers: results from a population-based study."
Carcinogenesis 26(2): 381-386.

http://carcin.oupjournals.org/cgi/content/abstract/26/2/381

The NAD(P)H:quinone oxidoreductase 1 gene, NQO1, contains a C to T transition at amino acid
codon 187, which results in very low enzymatic activity. Previous studies of the association
between NQO1 genotype and lung cancer have had mixed findings. This population-based case
control study examines the association between NQO1 genotype and lung cancer in the largest
sample of never smokers (<100 cigarettes, lifetime) to date. Cases (n = 161) were identified
through the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program,
and 5-year age- and race-matched population-based controls (n = 173) were identified using
random digit dialing. Allele frequencies of C and T, respectively, were 0.79 and 0.21 in
Caucasians, and 0.84 and 0.16 in African Americans. Among those diagnosed aged \( \geq \)50
years, C/T and T/T genotyped individuals had 0.48 times lower lung cancer risk than individuals
with C/C genotype (95% CI: 0.27-0.87). There was a non-significant suggestion of a protective
effect associated with the T allele among those with a history of environmental tobacco smoke
exposure (OR = 0.57, 95% CI: 0.32-1.03) but not among those without (OR = 0.98, 95% CI: 0.41-
2.38). Sex, race, family history of lung cancer and histologic type did not modify the effect
of NQO1 genotype on lung cancer risk. The observed risk reductions may be attributable to the
greatly reduced procarcinogen activating of NAD(P)H:quinone oxidoreductase 1 in individuals
with at least one copy of the T allele.

changes in immature ovariectomized mice following exposure to ethynyl estradiol."
Carcinogenesis 25(7): 1277-1291.

http://carcin.oupjournals.org/cgi/content/abstract/25/7/1277

Temporal- and dose-dependent changes in hepatic gene expression were examined in immature
ovariectomized C57BL/6 mice gavaged with ethynyl estradiol (EE), an orally active estrogen. For
temporal analysis, mice were gavaged every 24 h for 3 days with 100 \( \mu \)g/kg EE or vehicle
and liver samples were collected at 2, 4, 8, 12, 24 and 72 h. Gene expression was monitored
using custom cDNA microarrays containing 3067 genes/ESTs of which 393 exhibited a change at
one or more time points. Functional gene annotation extracted from public databases associated
temporal gene expression changes with growth and proliferation, cytoskeletal and extracellular
matrix responses, microtubule-based processes, oxidative metabolism and stress, and lipid
metabolism and transport. In the dose-response study, hepatic samples were collected 24 h
following treatment with 0, 0.1, 1, 10, 100 or 250 \( \mu \)g/kg EE. Thirty-nine of the 79 genes
identified as differentially regulated at 24 h in the time course study exhibited a dose-response
relationship with an average ED50 value of 47 \(+/-\) 3.5 \( \mu \)g/kg. Comparative analysis
indicated that many of the identified temporal and dose-dependent hepatic responses are similar
to EE-induced uterine responses reported in the literature and in a companion study using the
same animals. Results from these studies confirm that the liver is a highly estrogen responsive
tissue that exhibits a number of common responses shared with the uterus as well as distinct
estrogen-mediated profiles. These data will further aid in the elucidation of the mechanisms of
action of estrogens in the liver as well as in other classical and non-classical estrogen responsive
tissues.

and retinoid X receptors in non-small cell lung cancer: implications for tumor development and
Retinoic acid receptors (RARs) and retinoid X receptors (RXRs) are important in regulating the development, growth and differentiation of cells and have inhibitory effects on non-small cell lung cancer (NSCLC) cell growth. A comprehensive analysis of all RAR and RXR subtypes mRNA expression in a large series of patients with NSCLC and their role in the development and progression of this disease is lacking. Using a quantitative real-time RT-PCR method, we analyzed the mRNA expression of all retinoid receptor subtypes in tumor and matching normal-appearing tissues of 88 patients with NSCLC. Gene expression in tumor tissues was detected with the following frequencies: RAR{alpha} 100%, RAR{beta} 94%, RAR{gamma} 94%, RXR{alpha} 100%, RXR{beta} 100% and RXR{gamma} 92%. Levels of mRNA expression in tumor tissues compared with matching normal-appearing tissue were equal or reduced with the following frequencies: RAR{alpha} 76.1%, RAR{beta} 59.1%, RAR{gamma} 39.8%, RXR{alpha} 67.1%, RXR{beta} 54.5% and RXR{gamma} 88.6%, and were significantly associated with any one other subtype. The probability of survival was significantly different among patients with low gene expression in no or any two subtypes, any three or four subtypes or any five or six subtypes (P = 0.004, log rank test). Multivariate analysis confirmed low gene expression status as a significant independent unfavorable prognostic factor (P = 0.015). Our results show that decreased expression of all RAR and RXR receptor subtypes is a frequent event in NSCLC. Widely co-regulated down-regulation of expression of all retinoid subclasses suggests a fundamental dysregulation of the retinoid pathway in this cancer. Quantitation of RAR and RXR mRNA expression levels in tumor tissue is a candidate prognostic marker and surrogate biomarker for chemopreventive trials in NSCLC.


Inherited mutations of Patched (PTCH) in the nevoid basal cell carcinoma syndrome (NBCCS) lead to several developmental defects and contribute to tumor formation in a variety of tissues. PTCH mutations have been also identified in sporadic tumors associated with NBCCS including basal cell carcinoma (BCC) and medulloblastoma. Mice heterozygous for Pch recapitulate the typical developmental symptoms of NBCCS and develop rhabdomyosarcoma (RMS) and medulloblastoma. PTCH is assumed to act as a tumor suppressor gene although inactivation of both alleles has been demonstrated only in a fraction of tumors. We have investigated the status of Pch in RMS of heterozygous Pch neo67/+ mice. Although the wild-type Pch allele was retained in tumor tissue, the high levels of Pch mRNA in these tumors result from overexpression of the mutant Pch transcript. Our results suggest that the wild-type Pch allele might be selectively silenced in RMS tissue or, alternatively, that haploinsufficiency of Pch is sufficient to promote RMS formation in mice.

Betel quid (BQ) chewing, a popular habit in numerous Asian countries including India and Taiwan, has a strong correlation with an increased risk of oral squamous cell carcinoma (OSCC). While substantial efforts have been made to test the cytotoxic, genotoxic and mutagenic effects of BQ extract and its components, the disease mechanisms underlying BQ-induced oral carcinogenesis remain obscure. Here, we show that a neuronal protein, microtubule-associated protein 2 (MAP2), was induced by BQ extract in cultured normal human oral keratinocytes (NHOKs). Subsequent analyses demonstrated that such induction was more eminent and consistent in the high-molecular-weight isoform of MAP2 (hmw-MAP2) than that in its low-molecular-weight counterpart (lmw-MAP2). Furthermore, we analyzed expression of hmw-MAP2 protein in 88 oral specimens consisting of clinicopathologically pre-malignant (leukoplakia) and malignant (OSCC) lesions, along with their adjacent normal mucosa. Immunohistochemistry revealed that, with the exposure to BQ, the hmw-MAP2 was over-expressed in 41.2% (7/17) of OSCC, 11.2% (1/9) of leukoplakia and none (0/19) of normal mucosa. In contrast, expression of the hmw-MAP2 was barely detected in BQ-free OSCC. These results suggest a significant correlation between expression of the hmw-MAP2 and BQ-associated progression of oral carcinogenesis (P = 0.0046). Interestingly, the hmw-MAP2 was found to preferentially express in histopathologically less differentiated OSCC (P = 0.014); the percentages of positive staining in poorly, moderately and well differentiated OSCC were 62.5, 21.4 and 7.1%, respectively. However, BQ chewing appeared to have marginal correlation with such propensity. Finally, we show that the majority of hmw-MAP2-positive poorly differentiated lesions were also histopathologically invasive. Taken together, these findings suggest the possibility that the hmw-MAP2 may be a diagnostic marker for BQ-chewing lesions and a potential therapeutic target. To our knowledge, this study has provided the first clinical implication that closely links a cytoskeletal protein to BQ-associated oral cancer.


http://carcin.oupjournals.org/cgi/content/abstract/23/1/123

A rat surgical esophageal adenocarcinoma (EAC) model induced by esophagogastroduodenal anastomosis was recently established in our laboratory. This model mimics mixed reflux of gastric and duodenal contents in human patients and produces EAC without treatment with any carcinogen. We compared the protein expression pattern between rat EAC and normal tissues by 2-dimensional protein gel electrophoresis. The overexpressed protein spots of the tumor sample were cut out and analyzed by matrix-assisted laser desorption/ionization mass spectrometry. Several stress proteins (Grp94, Grp78, calnexin, Hsp90(beta) and ER61) were identified by this method. Western blotting and RT-PCR further confirmed overexpression of Grp94 in rat EAC. Immunohistochemical staining also revealed expression of Grp94 in the epithelial cells of columnar lined esophagus and EAC. Similar to the rat model, well-differentiated human EAC and gastric cardia adenocarcinomas were also found to overexpress Grp94, but esophageal squamous cell carcinomas did not. We also characterized apoptosis, cell proliferation and oxidative DNA damage in the rat tissues. Since Grp94 is known to inhibit apoptosis by maintaining intracellular Ca2+ homeostasis, our data suggest a possible correlation between oxidative stress, Grp94 overexpression and apoptosis regulation in esophageal adenocarcinogenesis.


http://carcin.oupjournals.org/cgi/content/abstract/26/4/811
Polymorphisms in GSTM1, GSTT1 and GSTP1 genes in humans are associated with the reduction of enzymatic activity toward several substrates, including those in tobacco smoke. To investigate the potential role these polymorphisms have, as modulators of early-onset lung cancer risk, a population-based case-control study involving early-onset lung cancer cases was performed. Biological samples were available for 350 individuals diagnosed <50 years of age identified from the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program and 410 cases of age, race and sex-matched controls ascertained through random digit dialing. African Americans carrying at least one G allele at the GSTP1 locus were 2.9-fold more likely to have lung cancer compared with African Americans without a G allele after adjustment for age, sex, pack years of smoking and history of lung cancer in a first-degree relative (95% CI 1.29-6.20). African Americans with either one or two risk genotypes at the GSTM1 and GSTP1 loci were at increased risk of having lung cancer compared with those having fully functional GSTM1 and GSTP1 genes (OR = 2.8, 95% CI 1.1-7.2 and OR = 4.0, 95% CI 1.3-12.2, respectively). No significant single gene associations between GSTM1, GSTT1 or GSTP1 and early-onset lung cancer were identified in Caucasians, after adjusting for age, sex, pack years and family history of lung cancer. However, our results suggest that specific combinations of glutathione S-transferase polymorphisms increase the risk of early-onset of lung cancer. Joint analysis of these genotypes may identify individuals who are at a higher risk of developing early-onset lung cancer with a greater certainty than single gene studies.


http://carcin.oupjournals.org/cgi/content/abstract/23/11/1933

The stearoyl-CoA desaturase 1 (Scd1) gene is involved in the synthesis and regulation of unsaturated fatty acids. Its expression is increased by several treatments/conditions that are associated with hepatocarcinogenesis (peroxisome proliferators, iron overload, dichloroacetic acid). We found that the Scd1 gene is differentially expressed, showing >10-fold higher mRNA levels in the normal liver tissue of C3H/He mice, which are genetically susceptible to hepatocarcinogenesis, than of BALB/c mice, which are resistant. Similarly, Scd1 mRNA expression was 4-fold higher in the normal liver of F344 rats, which are susceptible to hepatocarcinogenesis, than in Brown Norway (BN) rats, which are resistant. The chromosomal location of the Scd1 locus, both in mice and rats, excludes Scd1 candidacy as a hepatocellular tumor-modifier gene, as the Scd1 locus did not show allele-specific effects in a BALB/cxC3H/He intercross or in a BNxF344 backcross and intercross. No Scd1 coding polymorphisms were detected in the mouse and the rat strains showing elevated Scd1 expression. These results suggest that the Scd1 gene represents a downstream target of hepatocellular tumor-modifier loci in two rodent species.


http://carcin.oupjournals.org/cgi/content/abstract/25/12/2451

We reported previously that subjects homozygous for the cytochrome P450 2A6 (CYP2A6) *4 have a lower risk of lung cancer. The purpose of this study was to clarify whether or not the alterations of smoking behavior and risk for lung cancer could be found in subjects possessing novel CYP2A6 variants discovered recently. An epidemiological study was performed with 1094 cases and 611 controls in male Japanese smokers. It was found that the amounts of daily
cigarette consumption in subjects who harbored CYP2A6*4/*7, *4/*10, *7/*7, *7/*9 and *4/*4 genotypes were significantly less than those in subjects carrying the *1/*1 genotype (P < 0.01). Even after adjustment with cigarette consumption, the adjusted odds ratios (ORs) for lung cancer were significantly lower in subjects who harbored CYP2A6*1/*4, *1/*7, *1/*9, *1/*10, *4/*4, *4/*7, *4/*9, *7/*7 and *7/*9 genotypes than those who possessed the *1/*1 genotype (P < 0.05). When participants were classified into four groups according to the CYP2A6 genotypes, group 1 (*1/*1), group 2 (heterozygotes for the *1 and a variant allele), group 3 (heterozygotes and homozygotes for variant alleles except for *4/*4) and group 4 (*4/*4), lung cancer risk was found to be less in subjects with the variant of CYP2A6 alleles (group 2, OR of 0.59 [95% confidence interval (CI), 0.44-0.79]; group 3, OR of 0.52 [95% CI, 0.37-0.72]; group 4, OR of 0.30 [95% CI, 0.16-0.57]). The reduced risk for lung cancer was seen more clearly in heavy smokers than in light smokers. Additional stratification analysis showed that the ORs for squamous cell carcinoma (OR of 0.07) and small cell carcinoma (OR of 0.10) were lower than that of adenocarcinoma (OR of 0.39) in group 4. These results suggest that the CYP2A6 is one of the principal determinants affecting not only smoking behavior but also susceptibility to tobacco-related lung cancer.


http://carcin.oupjournals.org/cgi/content/abstract/24/3/435

Mutational activation of {beta}-catenin and cyclin D1 over-expression are a frequent change in mouse hepatic tumors. Although activated {beta}-catenin may bind to T cell factor (TCF) family members and transcriptionally activate the cyclin D1 gene, either {beta}-catenin or cyclin D1 may be activated by various pathways independently of {beta}-catenin mutations. In this study, we investigated {beta}-catenin activation and mutations, cyclin D1 expression, H-ras mutations and phosphorylation of extracellular signal regulated protein kinases 1/2 (ERK1/2), Akt and glycogen synthetase kinase 3{beta} (GSK3{beta}) in mouse hepatic carcinogenesis. Nuclear/cytoplasmic staining of {beta}-catenin, a sign of {beta}-catenin activation, was frequently observed in association with the high nuclear cyclin D1 labeling index in the hepatic tumors at the late stage of carcinogenesis. The {beta}-catenin activation was further suggested by the fact that all hepatocellular carcinoma (HCC) cell lines examined showed the nuclear {beta}-catenin/TCF4 complex together with cyclin D1 over-expression. However, the fact that only 31.8% (7/22) of the lesions with the nuclear/cytoplasmic {beta}-catenin staining showed {beta}-catenin mutations indicated that {beta}-catenin was activated not only by its own mutations but also by other reason(s). On the other hand, there was no correlation between the {beta}-catenin/cyclin D1 activation and the H-ras mutations or phosphorylation of Akt, GSK3{beta} and ERK1/2, although GSK3{beta} was frequently over-expressed in the tumors. These results indicate that, although {beta}-catenin and cyclin D1 activation are well correlated, the Akt/GSK3{beta} and ras/ERK1/2 pathways may not play a major role in the {beta}-catenin/cyclin D1 activation.


http://carcin.oupjournals.org/cgi/content/abstract/25/12/2379

It has been shown that the matrix metalloproteinase (MMP)-1 promoter polymorphism 1G/2G is associated with an increased risk of developing various cancers including renal cell carcinoma (RCC), and is in linkage disequilibrium (LD) with the MMP-3 promoter polymorphism 5A/6A. These two genes are localized in 11q22 adjacent to each other. However, the relationship between the MMP-3 5A/6A polymorphism and susceptibility to cancer remains ambiguous. In this
In a study, we genotyped eight polymorphisms in the region containing the MMP-1 and MMP-3 genes in 177 healthy subjects, and explored the relationships between RCC and these polymorphisms or haplotypes in 156 RCC cases and 230 age- and gender-matched controls. All the subjects studied were of Japanese descent. There were three polymorphisms that showed stronger LD with the MMP-1 1G/2G promoter variant than with the MMP-3 5A/6A promoter variant. One of these three polymorphisms was present in exon 2 of the MMP-3 gene and caused an amino acid change, Glu45Lys (G/A). When the genotype distribution of Glu45Lys was compared between RCC patients and controls, the frequency of the G/G genotype was significantly higher in the patients [age- and gender-adjusted odds ratio (OR) = 1.81, 95% confidence interval (CI) = 1.20-2.74]. A significant increase in the frequency of the 2G/2G genotype of the MMP-1 1G/2G polymorphism was also observed in the patients (age- and gender-adjusted OR = 1.86, CI = 1.23-2.82), whereas there was no significant difference for the MMP-3 5A/6A polymorphism. As expected based on these genotype-level results, the frequency of the 2G-G haplotype of MMP-1 1G/2G and MMP-3 Glu45Lys (G/A) polymorphisms was significantly higher in the patients than in the controls (crude OR = 1.95, CI = 1.31-2.91). These findings suggest that this haplotype of MMP-1 and MMP-3 variants may be associated with the risk of developing RCC.


http://carcin.oupjournals.org/cgi/content/abstract/24/4/757

We hypothesized that the mouse liver tumor response to non-genotoxic carcinogens would involve some common early gene and protein expression changes that could ultimately be used to predict chemical hepatocarcinogenesis. In order to identify a panel of genes to test, we analyzed global differences in gene and protein expression in livers from B6C3F1 mice following dietary treatment with two rodent carcinogens, the benzodiazepine anti-anxiety drug oxazepam (2500 p.p.m.) and the hypolipidemic agent Wyeth (Wy)-14,643 (500 p.p.m.) compared with livers from untreated mice. Male mice were exposed for 2 weeks and 1, 3 or 6 months to oxazepam or Wy-14,643 in an age-matched study design. By histopathological evaluation, no liver preneoplastic foci or tumors were detected at 6 months in treated or control groups. By cDNA microarray analysis [NIEHS Mouse Chip (8700 genes); n = 3 individual livers/group, four hybridizations/sample], expression of 36 genes or 220 genes were changed relative to control livers following 6 months of oxazepam or Wy-14,643 treatment, respectively. To obtain a more comprehensive picture of gene/protein expression changes, we also conducted a proteomics study by 2D-gel electrophoresis followed by matrix assisted laser desorption/ionization-mass spectrometry on cytoplasmic, nuclear, and microsomal subcellular fractions of the same liver samples utilized for the cDNA microarray analysis. Real-time PCR, western blot analysis and immunohistochemistry were utilized for validation and to expand the results to other time points. Cyp2b20, growth arrest- and damage-inducible gene \{beta\} (Gadd45(betah)), tumor necrosis factor \{alpha\}-induced protein 2 and insulin-like growth factor binding protein 1 (Igfbp5) genes and proteins were upregulated by oxazepam, and Cyp2b20, Cyclin D1, proliferating cell nuclear antigen, Igfbp5, Gadd45(betah) and cell death-inducing DNA fragmentation factor \{alpha\} subunit-like effector A exhibited higher expression after Wy-14,643 treatment. Most of these genes/proteins were also deregulated at 2 weeks. There appeared to be more distinct than common changes in the expression of carcinogenesis-related genes/proteins between the two compounds, suggesting that the major carcinogenic pathways are different for these compounds and may be distinct for different chemical classes.

Human beings are exposed to a multitude of carcinogens in their environment, and most cancers are considered to be chemically induced. Here we examined differences in genetic alterations in rat forestomach tumors induced by repeated exposure to a genotoxic carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or N-methylN-nitosurethane (MNUR), and chronic treatment with a non-genotoxic carcinogen, butylated hydroxyanisole (BHA) or caffeic acid (CA). A total of 132, 6-week-old male F344 rats were employed. Forty rats were treated with MNNG by intragastric administration at a dose of 20 mg/kg body wt once a week for 32 weeks, and 20 rats received 20 p.p.m. MNUR in their drinking water for 48 weeks. Further groups of 20 animals were administered 2% BHA or 2% CA in the diet for 104 weeks. The remaining rats were maintained without any supplement as controls. Multiple forestomach tumors were observed in all rats of the MNNG-, MNUR-, BHA- and CA-treated groups. Histopathologically, MNUR- and CA-treated groups showed almost the same pattern. On polymerase chain reaction-single strand conformation polymorphism analysis, H-ras and p53 gene mutations were observed at high and relatively low frequencies, respectively, in forestomach tumors induced by MNNG and MNUR. Most H-ras gene mutations were G[-&gt;]A transitions in codons 7 and 12 of exon 1. On the other hand, forestomach tumors due to the non-genotoxic carcinogens, BHA and CA, had almost no mutations of the H-ras and p53 genes. Moreover, relative overexpression of cyclin D1 and p53 was detected in forestomach tumors induced by the genotoxic carcinogens, while their non-genotoxic counterparts had a tendency to show low expression of those molecules. Mutations of the {beta}-catenin gene were not detected in any group. The present study demonstrates that rat forestomach tumors induced by genotoxic and non-genotoxic carcinogens have different underlying genetic alterations, even if their pathological features are similar.


The von Hippel-Lindau (VHL) tumor suppressor gene plays a prominent role in the development of renal cell carcinoma (RCC) in humans. VHL functions as a ubiquitin E3 ligase, controlling the stability of hypoxia inducible factor (HIF) and tumor angiogenesis. Alterations in this tumor suppressor gene are rarely observed in spontaneous or chemically induced RCC that arise in conventional strains of rodents and Vhl knockout mice (Vhl+/-) do not develop spontaneous RCC. We tested whether Vhl knockout mice exhibited increased susceptibility to renal carcinogenesis using the well-characterized renal carcinogen streptozotocin. No differences were observed between wild-type and Vhl+/- animals in the frequency or type of renal lesions induced by 50-200 mg/kg streptozotocin. Carcinogen-induced RCC that developed in Vhl heterozygotes and wild-type mice did not contain mutations in the wild-type Vhl, as determined by direct sequencing of the primary tumors. While Vhl+/- mice exhibited no increase in renal lesions in response to streptozotocin, heterozygous animals did develop vascular proliferative lesions of the liver, uterus, ovary, spleen and heart. These lesions, ranging from angiectasis to hemangiosarcoma, were most prominent in the livers of Vhl+/- mice, where they were found in high incidence and high multiplicity. Wild-type mice developed a low-frequency of liver angiectasis (7-15%) only at the highest doses of carcinogen used (150 and 200 mg/kg, respectively) while Vhl+/- mice exhibited angiectasis, hemangioma and hemangiosarcomas with a frequency ranging from 19 to 46% at 50-200 mg/kg streptozotocin. Untreated Vhl+/- mice had a spontaneous incidence of hepatic vascular lesions of 21%. Furthermore, vascular lesions of the uterus, ovary, spleen and heart were observed only in Vhl+/- mice, with an incidence of (5-28%). Taken together, the data indicate that heterozygosity at the Vhl locus predisposes mice to a vascular phenotype ranging from angiectasis to hemangiosarcoma, consistent with the ability of this tumor suppressor gene to control the stability of HIF and regulate key proteins that participate in angiogenesis.

http://carcin.oupjournals.org/cgi/content/abstract/23/8/1281

Bile acids have been suggested to play an important role in the etiology of colon and gastric cancer after gastrectomy, but the molecular biology of these effects is poorly understood. We evaluated the effect of different bile acids on human gastric and colon carcinoma cells and identified genes by RNA arbitrarily primed PCR for differential display that are modulated following treatment with hydrophobic bile acids. Thioredoxin reductase (TR) mRNA was upregulated after treatment with taurochenodeoxycholic acid (TCDDA) in St 23132 cells. This raised the question whether deoxycholic acid (DCA) would have regulative effects on TR in HT-29 cells. After an incubation time of 6 h with DCA, TR mRNA expression was increased up to threefold. Ursodeoxycholic acid had no influence on TR mRNA expression. The upregulation of TR after DCA incubation was almost identical to incubation with 12-O-tetradecanoylphorbol-13-acetate. This implies that hydrophobic bile acids mediate oxidative stress in gastrointestinal cancer cells, which was confirmed by measurement of oxidative burst after treatment with DCA. The results suggest that hydrophobic bile acids induce oxidative stress in gastrointestinal cancer resulting in a compensatory upregulation of TR mRNA, one of the key components in the complex anti-oxidant defense system within eukaryotic cells. The activation of at least parts of the redox signaling system is potentially related to the cytotoxicity and the stimulation of the cell death machinery induced by toxic bile acids.


http://carcin.oupjournals.org/cgi/content/abstract/23/11/1885

The maternally expressed H19 gene is transcribed as an untranslated RNA that serves as a riboregulator. We have previously reported that this transcript accumulates in epithelial cells in ~10% of breast cancers. To gain further insight on how the overexpression of the H19 gene affects the phenotype of human breast epithelial cells, we investigated the oncogenic potential of RNA that was abundantly expressed from MDA-MB-231 breast cancer cells stably transfected with the genomic sequence of the human H19 gene. The amount of H19 RNA did not affect cell proliferation capacity, timing of cell cycle phases or anchorage-dependent ability of H19-transfected clones in vitro. But in anchorage-independent growth assays the H19-recombined cells formed more and larger colonies in soft-agar versus control cells. To explore this phenotypic change, we analysed tumour development after subcutaneous injection of H19-recombined cells into scid mice. Results showed that H19 overexpression promotes tumour progression. These data support the hypothesis that an overload of H19 transcript is associated with cells exhibiting higher tumorigenic phenotypes and therefore we conclude that the H19 gene has oncogenic properties in breast epithelial cells.


http://carcin.oupjournals.org/cgi/content/abstract/26/3/597

The pattern of somatic mutations in TP53 is distinct for particular cancers and carcinogenic
exposures, providing clues to disease etiology, e.g. G:C\[-\gt\]T:A mutations in TP53 are more frequently observed in smoking-associated lung cancers. In order to investigate possible causes and mechanisms of lung cancer susceptibility differences, the TP53 gene was sequenced in a case-only study of lung cancers (206 men and 103 women). Our primary hypothesis was that the TP53 mutation spectrum is influenced by polymorphisms in genes involved in DNA repair and apoptosis. We observed a TP53 mutation frequency in exons 5-8 of 25%. Functional polymorphisms in XPD (Asp312Asn, rs1799793 and Lys751Gln, rs1052559), a protein required for nucleotide excision repair and with roles in p53-mediated apoptosis, were modestly associated with G:C\[-\gt\]T:A mutations in TP53 in lung tumors [Asp/Asn312 + Asn/Asn312 and/or Lys/Gln751 + Gln/Gln751 versus Asp/Asp312 + Lys/Lys751; odds ratio (OR) 2.73, 95% confidence interval (CI) 0.98-7.61], consistent with the role of this protein in repair of bulky carcinogen-DNA adducts. In addition, a TP53 polymorphism (Arg72Pro, rs1042522) with a known role in the efficiency of apoptosis was also associated with the presence of a TP53 mutation (Pro/Arg72 or Pro/Pro72 versus Arg/Arg72; OR 2.25, 95% CI 1.21-4.17) or a G:C\[-\gt\]T:A mutation in TP53 (Pro/Arg72 or Pro/Pro72 versus Arg/Arg72; OR 2.42, 95% CI 0.97-6.04). An interaction between the XPD variant alleles (Asn312 and Gln751) and the TP53 Pro72 allele was observed for TP53 mutations (any TP53 mutation Pint = 0.027, G:C\[-\gt\]T:A TP53 mutation Pint = 0.041). The statistical interaction observed in our study is consistent with the observed biological interaction for XPD and p53 in nucleotide excision repair and apoptosis. In conclusion, differences in TP53 mutation spectra in lung tumors are associated with several genetic factors and may reflect differences in lung cancer susceptibility and carcinogenesis.


http://carcin.oupjournals.org/cgi/content/abstract/24/10/1615

Previously, we demonstrated that connexins (Cxs) showed aberrant localization and expression in most endometrial hyperplasia and carcinoma samples, indicating that during endometrial carcinogenesis, loss of gap junctional intercellular communication (GJIC) may occur at relatively early stages. In the present study, we focused on the correlations between GJIC and the expression of the E-cadherin and its 5' CpG island methylation in endometrial cancer cells and tissues to investigate their roles in the carcinogenesis and tumor progression of endometrial cancer. In this study, three of the 10 cell lines investigated, Ishikawa, RL-952 and KLE, in which both Cxs and E-cadherin mRNA were expressed, exhibited GJIC by scrape-loading/dye transfer. On the other hand, the other seven cell lines, in which either or both Cxs and E-cadherin mRNA were negative or weakly expressed, did not show GJIC. HEC-50, HEC-1B and HEC-108, in which Cxs were positively expressed but E-cadherin was negatively expressed, showed cytoplasmic localization of Cxs by immunohistochemistry. All five lines, which showed the weak expression of E-cadherin, had E-cadherin 5' CpG island methylation. By immunohistochemistry of 56 endometrial carcinomas, 13 of 27 methylated samples showed weak expression of Cx26 and the other 14 showed diffuse localization in cytoplasm. On the other hand, of 29 unmethylated samples, two showed cell-cell localization, 25 weak expression and two diffuse localization. Furthermore, E-cadherin expression was revealed to be drastically down-regulated by E-cadherin antisense oligonucleotides that post-transcriptionally down-regulated E-cadherin expression and in the cell, the localization of Cxs were changed from the cell-cell borders to the cytoplasm, and GJIC also decreased. The results indicated that 5' CpG island methylation, which caused loss of E-cadherin expression, indirectly caused the suppression of GJIC by aberrant localization of Cxs in endometrial carcinoma cells.

Palli, D., G. Masala, et al. (2004). "The effects of diet on DNA bulky adduct levels are strongly modified by
Frequent consumption of fresh fruit and vegetables, and polymorphisms in the detoxifying enzyme glutathione S-transferase M1 (GSTM1) and other metabolic genes have been shown to modulate cancer risk at some sites. We have shown recently that DNA adducts, a reliable indicator of genotoxic damage and, possibly, of cancer risk, are modulated by plasma levels of selected micronutrients. Here we further investigate the association between DNA adduct levels and consumption of major food groups and foods, and the estimated dietary intake of nutrients, taking into account the possible modifying effect of metabolic polymorphisms, in a larger sample of 634 healthy adults enrolled in a prospective study in Italy. DNA adducts and five polymorphic metabolic genotypes (GSTM1, GSTT1, NAT2, CYP1A1 and MTHFR) were determined in peripheral leukocytes by using 32P-postlabeling technique and PCR methods. DNA bulky adducts (mean: 7.82 +/- 0.40/109 nt) were detected in 482/634 samples (76.0%). Overall, DNA adduct levels were significantly and inversely associated with the intake of raw leafy vegetables (P = 0.02), non-citrus fruits (P = 0.04), potassium (P = 0.01) and beta-carotene (P = 0.05). No association was evident with the five genotypes. Stratification by GSTM1 genotype showed strong inverse associations of DNA adduct levels with increasing consumption of all vegetables combined (P = 0.04), leafy vegetables (P = 0.004), raw leafy vegetables (P = 0.002) and fish (P = 0.03) among 307 GSTM1-null subjects; strong inverse associations also emerged with estimated dietary intakes of beta-carotene (P = 0.004), vitamin E (P = 0.004), niacin (P = 0.02) and potassium (P = 0.01). In contrast, no association emerged among 295 subjects with a GSTM1-wild genotype. Overall, statistically significant interactions in predicting DNA adduct levels were observed between the GSTM1-null genotype and consumption of leafy vegetables (P = 0.01), white meat (P = 0.04), and intake of vitamin C (P = 0.04), vitamin E (P = 0.05) and beta-carotene (P = 0.02). Our results suggest that the role of a diet rich in antioxidants in preventing or reducing DNA adduct formation is restricted to subjects lacking the detoxifying activity of GSTM1 isoenzyme ([~]50% of the general population).


http://carcin.oupjournals.org/cgi/content/abstract/24/4/739

DNA adducts, a reliable indicator of internal dose exposure to genotoxic agents and, possibly, of cancer risk, have been shown to be modulated by diet, particularly by the consumption of fresh fruit and vegetables, and by the intake of antioxidants (Palli et al., 2000, Int. J. Cancer, 87, 444-451). We have therefore investigated the association between DNA adducts in peripheral leukocytes and plasma levels of selected micronutrients, also taking into account the role of metabolic polymorphisms and smoking history, in a large independent random sample of volunteers enrolled in the prospective study EPIC-Italy ([~]110 subjects from each of the three main geographical study areas, Northern, Central and Southern Italy). DNA adducts and five polymorphic metabolic genotypes were determined in peripheral leukocytes using the 32P-postlabelling technique and PCR methods. Plasma levels of six carotenoids, retinol and {alpha}- and {gamma}-tocopherol were determined in the same blood sample. Among 331 subjects, 78.3% had detectable levels of DNA adducts (mean 7.46 +/- 0.48 per 109 nucleotides). Vitamin supplementation was reported by only a few subjects (3.9%). Strong inverse associations emerged between levels of DNA adducts and plasma retinol (P = 0.02), {alpha}-tocopherol (P = 0.04) and {gamma}-tocopherol (P = 0.03), but not carotenoids (except a borderline inverse association with {beta}-carotene, P = 0.08). An inverse significant association with plasma levels of retinol and {gamma}-tocopherol persisted in the subgroup of non-smokers, whereas a negative association with {alpha}-tocopherol emerged only in smokers. DNA adduct levels did not show any significant variation according to analyzed genotypes. Stratification by GSTM1 genotype,
however, showed a significant negative association between DNA adduct levels and plasma levels of (alpha)- (P = 0.02) and (beta)-carotene (P = 0.02) in subjects with the GSTM1 null genotype. Our results confirm that biomarkers of dietary intake of antioxidants significantly modulate DNA adducts and suggest specific inverse associations between DNA adduct levels and antioxidant concentrations among GSTM1 null subjects and smokers.


http://carcin.oupjournals.org/cgi/content/abstract/23/6/1057

Infection with human papillomavirus (HPV) of specific high-risk type triggers a series of events in target cells, which will eventually lead to development of genital neoplasia. The integration of high-risk HPV DNA into the cell genome has been regarded as a crucial event in tumor progression. With respect to different HPV types, the knowledge of HPV integrated loci is still limited. We have now determined the genomic variation and chromosomal location of HPV 33 DNA in the cell line UT-DEC-1, established from a vaginal mild dysplasia lesion. The viral sequence of the cell line was determined, and a variant of the prototype HPV 33 strain was identified, showing nucleotide substitutions resulting in amino acid changes in the E2, L2 and E4 open reading frames. In late passage UT-DEC-1 cells, a deletion of more than half of the 3' part of E1 and major parts of the E2 and E4 genes provided evidence for integration. The flanking sequences of the integration site were completely homologous to published sequences from chromosomal band 5p14, and remained unchanged in all subclones established from late passage cells. There were no chromosomal deletions or gross rearrangements at the integration site, and only a single heterozygotic copy of HPV 33 was detected. The karyotype of late passage cells showed only minor changes compared with early passage cells. During passaging of the cell line, there were progressive changes towards a malignant phenotype, and in parallel to this, the cells carrying episomal HPV 33 of the early passages was completely superseded by cells containing the integrated virus. Thus, our results show that this single copy heterozygote integration of HPV 33 into chromosome band 5p14 appears to be associated with emergence of cells escaping senescence, and with growth advantage compared with cells carrying episomal virus.


http://carcin.oupjournals.org/cgi/content/abstract/23/5/735

Sodium butyrate (NaB), a short-chain fatty acid naturally present in the human colon, is able to induce cell cycle arrest, differentiation and apoptosis in colon cancer cells. In addition to these effects, we investigated the effect of NaB on two angiogenesis-related proteins in a colon carcinoma cell line (HT29): vascular endothelial growth factor (VEGF), the most potent angiogenic factor, and hypoxia-inducible factor (HIF)-1(alpha), the main transcription activator of the VEGF gene, which are both constitutively expressed at high levels in HT29 also in normoxic conditions. NaB treatment had a different effect on VEGF165 and HIF-1(alpha) expression. In fact, it induced a dose-dependent down regulation of the VEGF165 protein level that was not paralleled by a concomitant down regulation of the corresponding mRNA, suggesting a post-translational regulation of the factor. Conversely, after 24 h of treatment all the tested NaB concentrations reduced the HIF-1(alpha) protein level, whereas after a longer time of exposure HIF-1(alpha) level increased in the presence of a high NaB concentration (2 mM) with a concomitant increase in HIF-1(alpha) mRNA. These results indicate that NaB, besides regulating
other fundamental cellular processes, is able to modulate the expression of two important angiogenesis-related molecules and suggested a further possible clinical application of this short-chain fatty acid as an anti-angiogenic compound in association with conventional chemotherapeutic agents.


http://carcin.oupjournals.org/cgi/content/abstract/24/9/1533

Cytochrome P450 1B1 (CYP1B1) is active in the metabolism of estrogens to reactive catechols and of different procarcinogens. Several studies have investigated the relationship between genetic polymorphisms of CYP1B1 and breast cancer risk, however, with inconsistent results. We investigated such an association in postmenopausal Swedish women, with special emphasis on long-term menopausal hormone users, in a large population-based case-control study. We genotyped 1521 cases and 1498 controls for the CYP1B1 single nucleotide polymorphisms (SNPs) m2, m3 and m4 and reconstructed haplotypes. The frequencies of CYP1B1*1, CYP1B1*2, CYP1B1*3 and CYP1B1*4 alleles among controls were estimated to be 0.087, 0.293, 0.444 and 0.175, respectively. It thus appeared that very few haplotypes contained combinations of SNPs at two or three loci and that single SNP genotype data effectively represented haplotypes. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from logistic regression models. We found no overall association between any CYP1B1 genotype and breast cancer risk. The data indicated, however, that women who had used menopausal hormones for 4 years or longer, and carried the CYP1B1*3/*3 genotype may be at increased risk of breast cancer, OR 2.0 (95% CI 1.1-3.5), compared with long-term users without this genotype. We explored the effect of CYP1B1 genotype on breast cancer risk in subgroups defined by body mass index, family history, smoking and catechol-O-methyl transferase genotype, but found no convincing evidence for interaction. In summary, our results strongly indicate that the studied CYP1B1 gene polymorphisms do not influence breast cancer risk overall but may modify the risk after long-term menopausal hormone use.


http://carcin.oupjournals.org/cgi/content/abstract/24/2/335

There is abundant epidemiological evidence that arsenic is an environmental carcinogen related to human cancers of the skin, lung, liver and urinary bladder, in particular. Dimethylarsinic acid (DMA) has also been reported to act as a carcinogen/or a promoter in rat models. To elucidate molecular mechanisms, we conducted an 18 month carcinogenicity study of DMA in p53 heterozygous (+/-) knockout mice, which are susceptible to early spontaneous development of various types of tumors, and wild-type (+/+) C57BL/6J mice. Totals of 88-90 males, 7-8 weeks of age, were divided into three groups each administered 0, 50 or 200 p.p.m. DMA in their drinking water for 18 months. Mice that were found moribund or died before the end of the study were autopsied to evaluate the tumor induction levels, as well as those killed at the end. Both p53+/- knockout and wild-type mice demonstrated spontaneous tumor development, but lesions were more prevalent in the knockout case. Carcinogenic effect of DMA was evident by significant early induction of tumors in both treated p53+/- knockout and wild-type mice, significant increase of the tumor multiplicity in 200 p.p.m.-treated p53+/- knockout mice, and by significant increase in the incidence and multiplicity of tumors (malignant lymphomas) in the treated wild-type mice. By the end of 80 weeks, tumor induction, particularly malignant lymphomas and sarcomas, were similar in treated and control p53+/- knockout mice. No evidence for organ-tumor specificity of DMA was
obtained. Molecular analysis using PCR-SSCP techniques revealed no p53 mutations in lymphomas from either p53+/− knockout or wild-type mice. In conclusion, DMA primarily exerted its carcinogenic effect on spontaneous development of tumors with both of the animal genotypes investigated here.


http://carcin.oupjournals.org/cgi/content/abstract/24/9/1455

Genetic susceptibility to breast cancer is influenced by high- and low-penetrance genes. The low-penetrance genes contributing to increased and decreased risk likely exist at appreciable frequencies in the human population. To identify high-frequency, low-penetrance modifier genes, we are using a rat genetic model. Eight quantitative trait loci, named mammary carcinoma susceptibility (Mcs) loci, have been genetically identified in two rat strains, Wistar-Kyoto (WKy) and Copenhagen. These strains are resistant to developing mammary cancer compared with susceptible Wistar-Furth (WF) female rats. Here we provide physical evidence of the existence of Mcs5 in the resistant WKy rat and further narrow the candidate region defining the QTL. Two congenic rat lines (C and D) containing large segments of the WKy Mcs5 QTL on chromosome 5 were generated on a WF background. The minimal WKy interval from markers D5Wox7 and D5Uwm37 (line C) conferred resistance to developing 7,12-dimethylbenz-[a]anthracene (DMBA)-induced mammary carcinomas. Line C females that were homozygous for the WKy allele at this interval averaged 1.1{+/-}0.3 carcinomas/rat compared with 6.9{+/-}0.4 average carcinomas/rat for WF control females (P<0.01). Line D females containing the minimal WKy interval from D5Rat26 to D5Uwm42, were as susceptible to developing mammary carcinomas as WF controls (5.7{+/-}0.6 versus 6.9{+/-}0.4, respectively). The WKy region in common to these lines from D5Rat26 to D5Uwm37 is thus not expected to contain Mcs5-associated genes. Based on results presented here, the Mcs5 locus has been physically located within a congenic interval on rat chromosome 5 between markers D5Uwm8 and D5Rat26.


http://carcin.oupjournals.org/cgi/content/abstract/26/4/855

p33ING1b is a candidate tumor suppressor gene and a nuclear protein. We investigated whether genetic and epigenetic mechanisms affect p33ING1b expression in ovarian cancer thus contributing toward its pathogenesis. A total of 111 ovarian cancers collected from Beijing and Hong Kong were used for this study. Weak or negative p33ING1b protein expression was demonstrated by immunohistochemistry on tissue microarray in 28/111 cases. Real-time quantitative RT-PCR also showed overall significant reduction of p33ING1b mRNA expression (P = 0.0137), with 53.1% (17/32) cases showing 2- to 5-fold reduction and absence of expression. The reduction of mRNA expression in cancer correlated with decreased p33ING1b protein expression (P < 0.0001). While no p33ING1b mutation was found, allelic loss at the p33ING1b locus was demonstrated in 25% (8/32) cases. The allelic loss profiles also showed statistical significant correlation with reduction of p33ING1b protein and mRNA expression (P = 0.031 and 0.030). Promoter methylation as assessed by methylation specific PCR was found in 23.9% (21/88) cases analyzed. Bisulfite sequencing results confirmed the p33ING1b promoter methylation status of these methylation positive cases. Statistical significant correlation between methylation and mRNA expression (P = 0.006) was demonstrated. Treatment with demethylating drug, 5'-aza-2'-deoxycytidine, resulted in dosage-dependent elevated mRNA expression of p33ING1b in ovarian cancer cell lines. This is the first study reporting epigenetic mechanism
regulating the p33ING1b expression. Our findings support that genetic and epigenetic alteration of p33ING1b are likely to contribute towards the pathogenesis of ovarian cancers.


http://carcin.oupjournals.org/cgi/content/abstract/25/12/2311

Oxidized DNA base lesions, such as thymine glycol (Tg) and 8-hydroxyguanine, are often toxic and mutagenic and have been implicated in carcinogenesis. To clarify whether NEIL1 protein, which exhibits excision repair activity towards such base lesions, is involved in gastric carcinogenesis, we examined 71 primary gastric cancers from Japanese patients and four gastric cancer cell lines for mutations and genetic polymorphisms of the NEIL1 gene. We also examined 20 blood samples from Chinese patients for NEIL1 genetic polymorphisms. Three mutations (c.82_84delGAG:p.Glu28del, c.936G > A and c.1000A > G:p.Arg334Gly) and two genetic polymorphisms were identified. When the excision repair activity towards double-stranded oligonucleotide containing a Tg:A base pair was compared among six types of recombinant NEIL1 proteins, p.Glu28del-type NEIL1, found in a primary case, was found to exhibit an extremely low activity level. Moreover, c.936G > A, located in the last nucleotide of exon 10 and detected in the KATO-III cell line, was shown to be associated with a splicing abnormality using an in vivo splicing assay. An immunofluorescence analysis showed that the wild-type NEIL1 protein, but not the truncated protein encoded by the abnormal transcript arising from the c.936G > A mutation, was localized in the nucleus, suggesting that the truncated protein is unlikely to be capable of repairing nuclear DNA. An expression analysis revealed that NEIL1 mRNA expression was reduced in six of 13 (46%) primary gastric cancer specimens that were examined. These results suggest that low NEIL1 activities arising from mutations and reduced expression may be involved in the pathogenesis in a subset of gastric cancers.


http://carcin.oupjournals.org/cgi/content/abstract/23/1/61

Several epidemiological studies have suggested a modulatory effect of dietary folate intake on the risk of colorectal cancer. The molecular basis for this inverse association is not clearly understood, but may involve alterations in DNA methylation. In this study, we examined the levels of methylation intermediates [S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH)] and of global DNA methylation in the pre-neoplastic small intestine of Min (multiple intestinal neoplasia) mice. We also studied the effect of folate/choline deficiency on these parameters and on tumor multiplicity in this animal model. In folate-adequate Min mice, we identified positive linear correlations between SAM or SAH and tumor numbers (R² = 0.38, P < 0.005; R² = 0.26, P = 0.025, respectively). A positive correlation between global DNA hypomethylation and tumor multiplicity was also observed (R² = 0.29, P = 0.014). These three biochemical determinants (SAM, SAH and DNA hypomethylation) may, therefore, serve as early markers of cell transformation. Folate/choline deficiency, however, did not produce a consistent effect on tumor numbers in three separate experiments. As an increase in tumor numbers was observed only in folate- and choline-deficient mice with low levels of SAM and DNA hypomethylation, the modulatory role of folate may be dependent on the transformation state of the cell.

http://carcin.oupjournals.org/cgi/content/abstract/25/9/1629

The isothiocyanate, sulforaphane and the flavonoid, apigenin modulate gene expression including phase II detoxifying enzymes, such as glutathione S-transferases (GST) and UDP-glucuronosyltransferases (UGT). Using undifferentiated CaCo-2 cells, we have examined the interactions between sulforaphane and apigenin in the regulation of UGT and GST expression. We show that apigenin induces UGT1A1 transcription (4-fold) but not GSTA1, and that sulforaphane induces both UGT1A1 (3.7-fold) and GSTA1 (2.8-fold) transcription in both dose- and time-dependent manners. The combination of sulforaphane and apigenin resulted in a synergistic induction of UGT1A1 mRNA up to 12-fold, although this interaction was not seen for GSTA1. Nuclear factor kappa B (NF-{kappa}B) mRNA was induced by apigenin and sulforaphane (2.5- and 2-fold, respectively). NF-{kappa}B translocation inhibitor SN50 and phosphatidylinositol 3-kinase (PI3) inhibitor LY294002 decreased the induction of GSTA1 by sulforaphane almost to baseline level. However, the MEK inhibitor PD98059 enhanced significantly the induction of GSTA1 by sulforaphane. This suggests that NF-{kappa}B and PI3-kinase signaling pathways play a role in GSTA1 gene expression. Conversely, the induction of UGT1A1 transcription by sulforaphane was totally abolished by PD98059, although PD98059 slightly enhanced (20%) the induction of UGT1A1 by apigenin implying that the induction of UGT1A1 by sulforaphane is mediated by MAPK/extracellular signal-regulated kinase kinase, whereas UGT1A1 induction by apigenin may be associated with NF-{kappa}B translocation since the NF-{kappa}B translocation inhibitor, SN50 enhanced the induction of UGT by apigenin. The results show that UGT1A1 and GSTA1 are regulated by sulforaphane through different signal transduction pathways and the differences in the mechanisms of modulation of UGT1A1 transcription by sulforaphane and apigenin resulted in a synergistic effect between these two compounds in the induction of UGT1A1.


http://carcin.oupjournals.org/cgi/content/abstract/24/1/39

Although a number of studies have suggested that diets with low intake of folate, an important methyl donor, are associated with increased risks of colon cancer and its precursor the adenomatous polyp, the underlying mechanisms are poorly understood. Dysregulation and instability of DNA methylation and alterations in the levels of the predominant DNA methylating enzyme, DNA (cytosine-5)-methyltransferase 1 (Dnmt1), have also been linked to tumorigenesis. We have used a combination of genetic and dietary manipulation to assess the effects of reduced Dnmt1 expression with and without folate deficiency on tumor induction in the ApcMin mouse. ApcMin mice with a reduction in Dnmt1 expression (ApcMin/+Dnmt1C+) had significantly lower tumor numbers than ApcMin mice with normal Dnmt1 (ApcMin+/+Dnmt1+/+). Dietary folate deficiency from weaning to 13 weeks of age did not affect tumor number or size in ApcMin/+Dnmt1C+ mice. However, in ApcMin/+Dnmt1C+ mice with high baseline tumor numbers (41 +/- 4), folate deficiency was associated with a decreased absolute number of tumors (27 +/- 3), but a higher proportion of larger tumors as compared with mice on the control diet. In the repeat experiment, ApcMin/+Dnmt1C+ mice had low baseline tumor numbers (20 +/- 2) and folate deficiency did not affect tumor number (23 +/- 4) or size as compared with the same mice on the control diet. These results suggest that, in the presence of Dnmt1 deficiency, the effects of folate deficiency on tumor number and size may depend on the stage of adenoma development when folate deficiency is initiated. We also show that folate deficiency with or without reductions in Dnmt1 did not affect overall genomic DNA methylation or the methylation levels of two candidate genes, E-cadherin or p53, in normal or neoplastic intestinal tissue. In
conclusion, genetic deficiency in Dnmt1 with or without folate deficiency decreases tumor number in the ApcMin mouse model, but this effect may not be mediated by changes in SAM or SAH levels, nor by alterations in global methylation in the pre-neoplastic intestinal tissue.


http://carcin.oupjournals.org/cgi/content/abstract/25/3/419

To investigate a possible link between bile acids and the pathogenesis of pancreatic cancer, we determined whether conjugated or unconjugated bile acids induced cyclooxygenase-2 (COX-2) in two human pancreatic cancer cell lines, BxPC-3 and SU 86.86. Bile acids are known promoters of gastric and colon cancer. We demonstrated previously that COX-2, an enzyme that catalyzes the synthesis of prostaglandins, is over-expressed in human pancreatic adenocarcinoma. Both human pancreatic cell lines were treated with conjugated and unconjugated bile acids. COX-2 mRNA and protein were determined. In addition, prostaglandin E2 (PGE2) synthesis was measured. Treatment with conjugated or unconjugated bile acids for 3 h up-regulated COX-2 mRNA. Chenodeoxycholate (CD) or deoxycholate at concentrations ranging from 12.5 to 100 {micro}M caused a dose-dependent induction of COX-2 protein with a maximal effect at 100 {micro}M. Induction of COX-2 protein by CD and deoxycholate was detected after treatment for 6 h with maximal induction at 12 h. Taurochenodeoxycholate, a conjugated bile acid, also caused dose-dependent induction of COX-2 but higher concentrations of bile acid (200-1200 {micro}M) were required. Levels of cyclooxygenase-1 were unaffected by bile acid treatment. Unconjugated and conjugated bile acids caused 7- and 4-fold increases in PGE2 production, respectively. Taken together, these findings suggest a possible role for bile acids in the pathogenesis of pancreatic cancer.


http://carcin.oupjournals.org/cgi/content/abstract/25/10/1795

Genetic instability is a prominent feature in multiple myeloma and progression of this disease from monoclonal gammopathy of uncertain significance (MGUS) and smouldering myeloma (SMM) is associated with increasing molecular and chromosomal abnormalities. The DNA mismatch repair (MMR) pathway is a post-replication DNA repair system that maintains genetic stability by repairing mismatched bases and insertion/deletion loops mistakenly incorporated during DNA replication. Deficiencies in proteins pivotal to this pathway result in a higher mutation rate, particularly at regions of microsatellite DNA. We have investigated the proficiency of the MMR pathway in clinical samples and myeloma cell lines. Microsatellite analysis showed instability at one or more of nine loci examined in 15 from 92 patients: 7.7% of MGUS/SMM, 20.7% of MM/plasma cell leukaemia (PCL) and 12.5% of relapsed MM/PCL. An in vitro heteroduplex G/T repair assay found reduced repair in two cell lines, JIM1 and JIM3, and in two of four PCL cases and was associated with aberrant expression of at least one mismatch repair protein. Thus we show that MMR defects are found in plasma cell dyscrasias and the increased frequency during more active stages of the disease suggests a contributory role in disease progression.

Estrogen is involved in breast carcinogenesis. Hypotheses have been raised that its effect is modified by enzymes such as catechol-O-methyltransferase (COMT) that deactivate potentially genotoxic estrogen metabolites. We have investigated the association between the functional genetic Val108/158Met polymorphism in COMT and breast cancer risk in a large population-based case-control study performed in the genetically homogeneous Swedish population. We determined COMT genotype in 1534 women with invasive breast cancer and in 1504 control women and calculated odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models. There was no overall association between COMT genotype and breast cancer risk. However, the L allele was associated with an increased risk for lobular breast cancer, with OR 2.0 (95% CI 1.2-3.5) for HL and 1.7 (95% CI 0.9-3.0) for LL. In exploratory subset analyses, we found no statistically significant interaction, but some indication of a positive association between HL and LL genotypes and breast cancer among women with diabetes mellitus and a negative association among nulliparous women. Based on our findings, COMT activity alone does not seem to play a major role in breast carcinogenesis, but may be of importance in certain histotypes or in conjunction with other exposures.


Arsenic is a well-documented human carcinogen, and contamination with this heavy metal is of global concern, presenting a major issue in environmental health. However, the mechanism by which arsenic induces cancer is unknown, in large part due to the lack of an appropriate animal model. In the present set of experiments, we focused on dimethylarsinic acid (DMA), a major metabolite of arsenic in most mammals including humans. We provide, for the first time, the full data, including detailed pathology, of the carcinogenicity of DMA in male F344 rats in a 2-year bioassay, along with the first assessment of the genetic alteration patterns in the induced rat urinary bladder tumors. Additionally, to test the hypothesis that reactive oxygen species (ROS) may play a role in DMA carcinogenesis, 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation in urinary bladder was examined. In experiment 1, a total of 144 male F344 rats at 10 weeks of age were randomly divided into four groups that received DMA at concentrations of 0, 12.5, 50 and 200 p.p.m. in the drinking water, respectively, for 104 weeks. From weeks 97-104, urinary bladder tumors were observed in 8 of 31 and 12 of 31 rats in groups treated with 50 and 200 p.p.m. DMA, respectively, and the preneoplastic lesion, papillary or nodular hyperplasias (PN hyperplasia), was noted in 12 and 14 rats, respectively. DMA treatment did not cause tumors in other organs and no urinary bladder tumors or preneoplastic lesions were evident in the 0 and 12.5 p.p.m.-treated groups. Urinary levels of arsenicals increased significantly in a dose-responsive manner except for arsenobetaine (AsBe). DMA and trimethylarsine oxide (TMAO) were the major compounds detected in the urine, with small amounts of monomethylarsonic acid (MMA) and tetramethylarsonium (TeMa) also detected. Significantly increased 5-bromo-2'-deoxyuridine (BrdU) labeling indices were observed in the morphologically normal epithelium of the groups treated with 50 and 200 p.p.m. DMA. Mutation analysis showed that DMA-induced rat urinary bladder tumors had a low rate of H-ras mutations (2 of 20, 10%). No alterations of the p53, K-ras or (beta)-catenin genes were detected. Only one TCC (6%) demonstrated nuclear accumulation of p53 protein by immunohistochemistry. In 16 of 18 (89%) of the TTCs and 3 of 4 (75%) of the papillomas, decreased p27kip1 expression could be demonstrated. Cyclin D1 overexpression was observed in 26 of 47 (55%) PN hyperplasias, 3 of 4 (75%) papillomas, and 10 of 18 (56%) TCCs. As a molecular marker of oxidative stress, increased COX-2 expression was noted in 17 of 18 (94%) TCCs, 4 of 4 (100%) papillomas, and 39 of 47 (83%) PN hyperplasias. In experiment 2,
8-OHdG formation in urinary bladder was significantly increased after treatment with 200 p.p.m. DMA in the drinking water for 2 weeks compared with the controls. The studies demonstrated DMA to be a carcinogen for the rat urinary bladder and suggested that DMA exposure may be relevant to the carcinogenic risk of inorganic arsenic in humans. Diverse genetic alterations observed in DMA-induced urinary bladder tumors imply that multiple genes are involved in stages of DMA-induced tumor development. Furthermore, generation of ROS is likely to play an important role in the early stages of DMA carcinogenesis.


http://carcin.oupjournals.org/cgi/content/abstract/26/2/395

Glutathione S-transferases detoxify polycyclic aromatic hydrocarbons found in tobacco smoke by glutathione conjugation. Polymorphisms within the GSTM1, GSTT1 and GSTP1 genes, coding for enzymes with deficient or reduced activity, have been studied as potential modifiers of lung cancer risk. It is hypothesized that risk associated with potential susceptibility gene polymorphisms might be most evident at low levels of exposure. Never smokers developing lung cancer represent a highly susceptible subset of the population, exposed to tobacco carcinogens only through environmental tobacco smoke. This population-based case-control study examines the association between GSTM1, GSTT1 and GSTP1 genotypes and lung cancer in one of the largest samples of never smokers to date. Cases (n = 166) were identified through the metropolitan Detroit Surveillance, Epidemiology and End Results (SEER) program and age- and race-matched population-based controls (n = 181) were identified using random digit dialing. Overall, there was no significant association between single or combinations of genotypes at GSTM1, GSTT1 or GSTP1 and lung cancer risk after adjustment for age, race, sex and household ETS exposure in years. However, in never smokers exposed to 20 or more years of household ETS, carrying the GSTM1 null genotype was associated with a 2.3-fold increase in risk [95% confidence interval (CI) 1.05-5.13]. Individuals in this high ETS exposure category carrying the GSTM1 null and the GSTP1 Val allele were at over 4-fold increased risk of developing lung cancer (OR = 4.56, 95% CI: 1.21-17.21). These findings suggest that in the presence of ETS, the GSTM1 genotype both alone and in combination with the GSTP1 genotype alters the risk of developing lung cancer among never smokers.


http://carcin.oupjournals.org/cgi/content/abstract/25/10/1935

Multiple enzymes with overlapping functions and shared substrates in the glutathione (GSH) metabolic pathway have been associated with host susceptibility to tobacco smoke carcinogens and in lung cancer etiology. However, few studies have investigated the differing and interacting roles of GSH pathway enzymes with tobacco smoke exposure on lung cancer risk in young (<50 years of age) and old (>80 years of age) populations. Between 1997 and 2001, 237 primary lung cancer patients (170 young, 67 old) and 234 controls (165 young, 69 old) were enrolled at the Mayo Clinic. Using PCR amplification of genomic DNA, polymorphic markers for {gamma}GCS, GPX1, GSTP1 (I105V and A114V), GSTM1 and GSTT1 were genotyped. Recursive partitioning and logistic regression models were used to build binary classification trees and to estimate odds ratios (OR) and 95% confidence intervals for each splitting factor. For the young age group, cigarette smoking had the greatest association with lung cancer (OR = 3.3). For never smokers, the dividing factors of recursive partitioning were GSTT1 (OR = 1.7), GPX1 (OR = 0.6) and
GSTM1 (OR = 4.3). For the old age group, smoking had the greatest association with lung cancer (OR = 3.6). For smokers, the dividing factors were GPX1 (OR = 3.3) and GSTP1 (I105V) (OR = 4.1). Results from logistic regression analyses supported the results from RPART models. GSH pathway genes are associated with lung cancer development in young and old populations through differing interactions with cigarette smoking and family history. Carefully evaluating multiple levels of gene-environment and gene-gene interactions is critical in assessing lung cancer risk.

Cardiovascular Pathology  (1)


http://www.sciencedirect.com/science/article/B6T13-3YVMYPJ-14/2/a3401c4d91caf866a77835241ddd6581

Viral infection of the myocardium is implicated in the pathogenesis of myocarditis and dilated cardiomyopathy (DCM). Enteroviruses have been considered the most common viral etiologic agents, based on peripheral culture and serologic methods. Recently, polymerase chain reaction (PCR) has been shown to be useful in the detection of viral genomes from various infected organs and body fluids. In this study, myocardial samples from autopsy specimens (formalin fixed and fresh frozen) were examined for enteroviral and DNA viral (adenovirus, herpes simplex virus [HSV], and cytomegalovirus [CMV]) genome by PCR. The specimens studied were from 58 patients with myocarditis, 28 patients with DCM and endocardial fibroelastosis [EFE], and 22 controls. Viral genome was detectable in 34 of the 58 (59%) autopsy-proven myocarditis samples (18 adenovirus, 12 enterovirus, 2 CMV, 2 HSV) and 6 of the 28 samples from patients with DCM and EFE (6 adenovirus). We conclude that PCR is effective in the rapid amplification of virus from frozen and formalin-fixed myocardial samples and that adenovirus is an important etiologic agent in viral myocarditis as well as DCM with EFE.

Cardiovascular Research  (13)


http://www.sciencedirect.com/science/article/B6T14-3TCFMDY-N/2/855e6599f570a441be0195db7d10ef44

Objective: Methylation of cytosine in CG dinucleotides within regulatory elements is believed to silence gene expression. These dinucleotides occur in certain important regulatory elements in the promoter region of the human [beta]-myosin heavy chain ([beta]-MHC) gene. We therefore
investigated whether methylation of these elements correlates with [beta]-MHC gene transcription in human ‘expressing’ (right atrial) and ‘non-expressing’ (peripheral blood leucocytes) cells. Methods: We employed 2 techniques to assess promoter methylation: (i) analysis of the susceptibility to digestion of a particular CCGG restriction site in the promoter region when genomic DNA is cleaved with the restriction endonucleases MspI (methylation-insensitive) and HpaII (methylation-sensitive), and (ii) the bisulphite-PCR method to examine in detail the methylation patterns of 3 important regulatory elements that contain CG dinucleotides. [beta]-MHC mRNA expression in right atrium and leucocytes was assessed using reverse-transcription-PCR with specific primers that do not detect [alpha]-MHC cDNA. Results: The digestion pattern observed with MspI or HpaII indicated that the CCGG site was almost completely methylated in leucocytes, but relatively unmethylated in atrial myocardium from the same patients. When methylation was examined with the bisulphite-PCR method we found a reciprocal relationship between the level of [beta]-MHC mRNA expression in leucocytes and atrial myocardium and the degree of methylation of CG dinucleotides in the 5' regulatory elements of the gene. Conclusions: Tissue-specific methylation of the human [beta]-MHC gene promoter may play a role in determining the pattern of expression of this gene. Furthermore, alteration of the level of methylation may underlie the changes in transcription of this gene that occur, for example, when atrial or ventricular myocardium hypertrophies.

Objectives: Increasing evidence suggests that vascular calcification is a regulated process. We studied the vascular expression pattern of a key factor in mineralization and a counteracting, protective factor. Based on the phenotype of null mice, Core binding factor [alpha]-1 (Cbfa-1) plays a pivotal role in bone formation, whereas Matrix Gla Protein (MGP) is a potent inhibitor of vascular calcification. Methods: We investigated the expression of MGP and Cbfa-1 in cultured, human monocytic cells, endothelial cells and smooth muscle cells (SMC), as well as in normal and atherosclerotic vessel specimens. Results: In cultured cells MGP is expressed in endothelial cells and SMC, whereas Cbfa-1 mRNA is predominantly present in macrophages and to a lesser extent in SMC. In the normal vessel wall MGP expression is high at the luminal side and declines toward the center of the media, whereas Cbfa-1 is absent. Moderate, diffuse calcification of the aorta media was observed only in those regions where MGP is low or absent. In atherosclerotic lesions MGP is expressed in endothelial cells and SMC that form fibrous caps, but is never present in macrophages. Cbfa-1 is synthesized in regions without MGP, it is associated with calcified areas and Cbfa-1 may be considered a marker for osteoprogenitor-like cells in the vessel wall. Conclusions: Our observations on MGP expression confirm and extend published data and are consistent with a protective function of MGP. Cbfa-1 expression is absent in normal medial SMC and co-localizes with neointimal macrophages and focal calcifications.


Objective: While natriuretic peptides can inhibit growth of vascular smooth muscle cells (VSMC), controversy exists as to whether this effect is mediated via the guanylate cyclase-coupled
receptors, NPR-A and NPR-B, or the clearance receptor, NPR-C. The original aim of this study was to examine the mechanism by which the NPR-C receptor regulates growth. Methods: Rat VSMC were characterized with regard to natriuretic peptide receptor expression by RT/PCR and radioligand binding studies. The effect on growth following addition of the peptides and the ligands for NPR-C was measured by [3H]thymidine incorporation. Cyclic guanosine monophosphate (cGMP) levels were determined by radioimmunoassay and mitogen activating protein kinase activity was based on the phosphorylation of myelin basic protein. Results: In rat VSMC, passages 4-12, both atrial natriuretic peptide (ANP) and C-type natriuretic peptide (CNP) dose-dependently inhibited serum and PDGF-induced VSMC growth. In contrast, NPR-C specific ligands alone had no effect on cell growth but enhanced growth inhibition when co-administered with ANP and CNP. ANP and CNP also decreased PDGF-BB-stimulated MAP kinase activity. Once again, NPR-C specific ligands alone had no effect but enhanced the effects of ANP. Furthermore, a cGMP specific phosphodiesterase inhibitor dose-dependently inhibited VSMC growth and markedly enhanced natriuretic-peptide-induced inhibition at low peptide concentrations. To examine a potential mechanism for the controversy concerning the NPR-C, we investigated the autocrine expression of ANP and CNP by VSMC and found that mRNA encoding both peptides could be detected by RT/PCR. Conclusion: Our findings indicate that the guanylyl-cyclase-linked receptors mediate the antiproliferative actions of the natriuretic peptides on vascular smooth muscle cell growth. Moreover, we hypothesize that the apparent inhibition of growth by NPR-C specific ligands reported by others may be due to stabilization of natriuretic peptides produced by the cultured VSMC and subsequent action of these peptides at guanylyl-cyclase-linked receptors.


http://www.sciencedirect.com/science/article/B6T14-3YGv5CF-13/2/65d3595d32fcee1770afca9795a10868c

Objective: The function of angiotensin converting enzyme (ACE) at cell sites of high collagen turnover, such as heart valves, is uncertain. The aim of this study was to assess ACE and kininase-II-like activities and collagen turnover in cultured valvular interstitial cells of the adult rat heart. Methods: The valvular interstitial cell phenotype was determined by immunolabelling (rhodamine phalloidin, desmin, and Griffonia simplicifolia lectin), and the presence of ACE mRNA and protein was confirmed by reverse transcriptase-polymerase chain reaction analysis, ACE monoclonal antibody and in vitro autoradiography, respectively. ACE and kininase-II-like activities in valvular interstitial cells were analysed by high performance liquid chromatography. Angiotensin II (AT1) and bradykinin receptors in valvular interstitial cell membranes were examined by western immunoblotting and binding assay. Type I collagen and collagenase in valvular interstitial cell culture media were determined by and zymography, respectively. Type I collagen mRNA expression in cultured valvular interstitial cells was determined by northern blot analysis and in situ hybridisation. Results: In intact valvular interstitial cells or their cell membrane we found: (1) actin microfilaments, but not desmin or lectin labelling; (2) ACE mRNA expression and binding activity; (3) conversion of angiotensin I to angiotensin II, which was completely inhibited by 50 [mu]M lisinopril, while kininase-II-like activity exceeded ACE activity and was not inhibited by lisinopril; (4) AT1 and bradykinin receptors in valvular interstitial cell membrane preparations; (5) type I collagen mRNA expression and collagenase activity; and (6) angiotensin II induced increase in type I collagen synthesis and mRNA expression. Conclusions: Cultured valvular interstitial cells represent a non-endothelial, non-smooth-muscle cell type that expresses mRNA for ACE and type I collagen. ACE and kininase-II-like activities in valvular interstitial cells may be involved in the regulation of peptides that influence collagen turnover. Angiotensin II stimulates type I collagen synthesis and mRNA expression in these cells.
Objective: Congestive heart failure (CHF) is accompanied by enhanced peripheral sympathetic nerve activity, increased vascular resistance and impaired peripheral blood flow. Besides noradrenaline and neuropeptide Y, the sympathetic nervous system also releases ATP, which has contractile effects mediated by different subtypes of P2-receptors on the vascular smooth muscle cells. The present study was designed to examine postsynaptic changes of the contractile responses to ATP and other extracellular nucleotides in CHF. Methods: CHF was induced by left coronary artery ligation resulting in a reproducible myocardial infarction in Sprague-Dawley rats. Contractile responses were examined in cylindrical segments of aorta and the mesenteric artery after endothelium removal. To determine if an altered response was regulated on the transcriptional level, competitive reverse transcription polymerase chain reaction (RT-PCR) was used to estimate the amount of P2X1-receptor mRNA. Results: ATP, which is both a P2X1- and a P2Y-receptor agonist, induced a weaker contraction in the mesenteric artery from CHF as compared to sham operated rats. A decrease in both potency and maximum contraction was shown for the selective P2X1-receptor agonist, [alpha][beta]-MeATP, in the mesenteric artery (pEC50=6.04 vs. 5.76, Cmax=57% vs. 33%, sham vs. CHF operated rats), but not in the aorta. Competitive RT-PCR also revealed decreased P2X1-receptor mRNA levels in CHF operated rats in the mesenteric artery (9106.103 vs. 714.103 molecules/[mu]g, sham vs. CHF operated rats), while it remained unaltered in the aorta. To study the P2Y-receptor induced contractile effects, the P2X1-receptors were first desensitised with [alpha][beta]-MeATP (10-5 M for 8 min). After P2X1-receptors desensitisation, UTP and UDP induced strong contractions in both the mesenteric artery and in the aorta, while ATP and ADP were much less effective. These contractions were not altered by CHF, indicating that vascular contraction mediated by P2Y-receptors are unaffected by CHF. Conclusion: CHF induces downregulation of P2X1-receptor stimulated contraction in the mesenteric artery depending on decreased mRNA synthesis for the receptor, while the P2Y-receptor activity remains unchanged. Downregulation of P2X1-receptors appears to be specific for peripheral resistance arteries. This may represent a compensatory response to enhanced peripheral sympathetic nerve activity and increased vascular resistance in CHF.

Objective: Inflammatory cells invade the fibrotic myocardium of spontaneously hypertensive rats at the same sites as where fibroblasts are produced. The role of these inflammatory cells in myocardial fibrogenesis was studied in the present work. Methods: The production and distribution of proteins that may be implicated in inflammation was examined by immunohistochemistry of sections of left ventricles from 1-month and 4-month renovascular hypertensive and age-matched control rats using antibodies against ICAM-1, LFA-1, TGF[beta]1, PDGF-A, T and H kininogens, IgG, IgM, C3, and C5b-9. Infiltrating inflammatory cells were phenotyped by immunohistochemistry. The TGF[beta]1 and PDGF-A mRNA levels were checked by RT-PCR. Results: Infiltrating cells were mainly T helper lymphocytes and macrophages, and there were more inflammatory cells in hypertensive rats than in control rats, localized especially around coronary arteries and in microscars. There were more ICAM-1 and LFA-1 in the ventricles of hypertensive than in control rats at 1 month, but the ICAM-1 expressions in hypertensive and control rats were similar at 4 months. TGF[beta]1 and PDGF-A mRNA steady states increased in
4-month hypertensive rats, but there was no labeling for TGF[beta] or PDGF by
immunohistochemistry. There was only faint labeling for T and H kininogens, and it was not
increased in hypertensive rats. There were deposits of IgM and C5b-9 only in hypertensive rats.
Conclusion: Thus, inflammatory cells infiltrate the cardiac tissue of renovascular hypertensive rats
as in the case of spontaneously hypertensive rats and these cells may use the ICAM-1/LFA-1
system to infiltrate, but neither TGF[beta] and PDGF-A, nor the kininogen system seem to be
associated with cardiac fibrogenesis. Otherwise, the complement system could act as
arteriosclerotic and/or leukocyte mobilizing factors.

manner and induces GLUT4 up-regulation in the late phase of cardioprotection." Cardiovascular
Research 61(3): 610.

Objective: The aim of this study was to determine the role of AMP-activated protein kinase
(AMPK) and its link to protein kinase C (PKC) in the late phase of cardioprotection afforded by
ischemic preconditioning (PC) against myocardial stunning. Methods and results: Rabbits were
instrumented with a balloon occluder around a coronary artery and with a Doppler sensor to
monitor the thickening fraction (TF). Conscious rabbits underwent five cycles of 5-min ischemia/5-
min reperfusion (I/R) on 2 consecutive days (days 1 and 2). Reduction of TF after I/R was
significantly less and recovery of TF was faster on day 2, indicating a late PC effect. PC provoked
translocation of PKC-epsiv from the cytosol to the membrane and significantly increased AMPK
activity by 100% immediately after PC. The mRNA level of GLUT4, a glucose transporter, was
elevated by 150% at 3 h after PC, and the total protein level of GLUT4 was increased by 107% at
24 h after PC. The level of sarcolemmal GLUT4 protein after I/R on day 2 was 41% higher than
its level after I/R on day 1. AMPK activation and up-regulation of GLUT4 by PC were abrogated
by pre-treatment with PKC inhibitors. Conclusion: PC activated AMPK and up-regulated GLUT4
expression in a PKC-dependent manner. This GLUT4 up-regulation at 24 h after PC may
contribute to attenuation of myocardial stunning.

atherosclerosis." Cardiovascular Research 36(2): 256.

Objective: Analysis of T-cell receptor (TCR) [beta]-chain gene expression in atherosclerotic
lesions of human aorta. Methods: TCR diversity was studied using non-radioactive polymerase
chain reaction for quantitative assessment of TCRBV gene transcripts, together with size and
sequence analysis of the [beta]-chain third complementarity-determining region (CDR3). Samples
represent a wide range of atheromatous histology, allowing evaluation of the T-cell repertoire at
different stages of disease. Results: Diverse TCRBV family usage was observed in the majority of
the samples, as the 25 different TCRBV products were detected at levels exceeding background.
The data also showed that TCRBV transcripts expressed in the diseased aorta tissue displayed
considerable size heterogeneity and no repetition of CDR3 nucleotide motifs. Conclusions: The
eyear presence of T-lymphocytes in the atheromatous blood vessel has been interpreted as an
indication of specific immunological reactions operating during the course of the atherosclerotic
process. Although a T-cell infiltrate characterized by limited usage of TCRAV genes cannot be
excluded, the unrestricted usage of TCRBV genes argues against a local T-cell clonal expansion
in atherogenesis.

http://www.sciencedirect.com/science/article/B6T14-460MB58-7/2/439344a52a4f1c2ad6ac6e7e0dd6876c

Objective: Two major isoforms of smoothelin have been reported, a 59-kDa smoothelin-A in visceral smooth muscle cells and a 110-kDa smoothelin-B in vascular smooth muscle cells. The present study was undertaken to investigate the expression of these smoothelin isoforms in different smooth muscle tissues and to determine how they are generated. Methods: Western blotting with a new, well-defined, smoothelin antibody was used to confirm the existence of two major smoothelin isoforms. Northern blotting, RT-PCR, primer extension and 5’RACE were applied to analyse the expression of these isoforms in human and mouse. Promoter reporter assays were carried out to establish the existence of a dual promoter system governing the expression pattern of the gene. Results: Antibody C6G confirmed the existence of two smoothelin proteins. Northern blotting showed that in vascular tissues a larger smoothelin transcript is generated than in visceral tissue. The cDNA of this larger smoothelin-B was cloned. Computer analysis of the open reading frame suggests an [alpha]-helical structure of 130 amino acids at the amino terminus of smoothelin-B. The smoothelin gene was cloned and sequenced. It comprises about 25 kb and contains 21 exons. The translational start of smoothelin-B is located in exon 2, whereas transcription and translation of the previously described smoothelin-A starts inside exon 10. Smoothelin-A and -B were demonstrated to be generated by two physically separated promoters. Splice variants within the calponin homology domain at the 3’ end of the gene were found for both isoforms. Conclusions: Two major smoothelin isoforms are generated from a single gene by a dual promoter system in a tissue specific manner. Further variation in the smoothelin proteins is achieved by alternative splicing in the calponin homology domain.


http://www.sciencedirect.com/science/article/B6T14-436F05F-8/2/bb7433bf5a271f7d092d1c5e3b79c9a9

Objective: Cardiotrophic growth factors with anti-cell death actions on cardiac myocytes have gained attention for treatment of patients with myocardial infarction. Hepatocyte growth factor (HGF) plays a role in tissue repair and protection from injuries, however, the physiological role of HGF in the myocardium has not been well defined. We asked if HGF would afford to the infarcted myocardium. Methods and results: Mature cardiac myocytes prepared from adult rats expressed barely detectable levels of the c-Met/HGF receptor, however, c-Met receptor expression increased during cultivation, which meant that cardiac myocytes are potential targets of HGF. Addition of hydrogen peroxide remarkably decreased the number of viable mature cardiac myocytes in primary culture, whereas treatment with HGF enhanced survival of the cells subjected to the oxidant stress. Although very low levels of c-Met/HGF receptor and HGF mRNA expression were seen in normal rat hearts, both c-Met/HGF receptor and HGF mRNA levels rapidly increased to much higher levels than normal, when the rats were subjected to myocardial infarction. Immunohistochemical analysis of the c-Met receptor indicated that this receptor was expressed in cardiomyocytes localized in the border regions of the viable myocardium and in non-infarcted regions following myocardial infarction. Conclusion: The c-Met/HGF receptor is induced in cardiomyocytes following myocardial infarction and HGF exhibits protective effect on cardiomyocytes against oxidative stress. Our working hypothesis is that HGF may afford myocardial protection from myocardial infarction.

http://www.sciencedirect.com/science/article/B6T14-4DS6KGM-2/2/f87217ba5a5216edb73e787984bd34ca

Objective: Defects in myocardial mitochondrial structure and function have been associated with heart failure in humans and animal models. Mice lacking the muscle LIM protein (MLP) develop morphological and clinical signs resembling human dilated cardiomyopathy and heart failure. We tested the hypothesis that defects in the cytoskeleton lead to dilated cardiomyopathy through mitochondrial dysfunction in the MLP mouse model. Methods and results: Oxidative phosphorylation activity was determined in left ventricles of MLP knockout (KO) mice and control littermates by measuring complex activities of the electron transport chain (I-IV) and ATP synthase (complex V). All complexes and citrate synthase (CS) showed decreased activities in the KO mice, although activity per amount of CS, a measure for mitochondrial density, was normal. Light and electron microscopy revealed a disorganization of mitochondria and a dramatic decrease in mitochondrial density, even revealing regions completely lacking mitochondria in the KO hearts. Real-time PCR analysis showed decreased transcript levels of mtDNA and nuclear encoded mitochondrial genes and of peroxisome proliferator activated receptor gamma co-activator 1[alpha] (PGC-1[alpha]), a key regulator of mitochondrial biogenesis. MtDNA copy number (ratio mtDNA/nuclear DNA) was slightly increased in the MLP KO mice. Conclusion: Our results show that the absence of MLP causes a local loss of mitochondria. We hypothesize that this is caused by a disturbed interaction between cytoskeleton and mitochondria, which interferes with energy sensing and energy transfer. Recovery of energy depletion by stimulating mitochondrial biogenesis might be a useful therapeutic strategy for improving the energy imbalance in heart failure.


Objective: To test the hypothesis that Vegf-B contributes to the pulmonary vascular remodelling, and the associated pulmonary hypertension, induced by exposure of mice to chronic hypoxia. Methods: Right ventricular systolic pressure, the ratio of right ventricle/[left ventricle+septum] (RV/[LV+S]) and the thickness of the media (relative to vessel diameter) of intralobar pulmonary arteries (o.d. 50-150 and 151-420 [mu]m) were determined in Vegfb knockout mice (Vegfb-/-; n=17) and corresponding wild-type mice (Vegfb+/-; n=17) exposed to chronic hypoxia (10% oxygen) or housed in room air (normoxia) for 4 weeks. Results: In Vegfb+/- mice hypoxia caused (i) pulmonary hypertension (a 70% increase in right ventricular systolic pressure compared with normoxic Vegfb+/- mice; PPPVegfb-/- mice hypoxia did not cause any increase in either right ventricular systolic pressure or pulmonary arterial medial thickness; also right ventricular hypertrophy (41% increase in RV/[LV+S]); PPVegfb+/+ mice. Conclusion: Vegf-B may have a role in the development of chronic hypoxic pulmonary hypertension in mice by contributing to pulmonary vascular remodelling. If so, the effect of Vegf-B appears to be different from that of Vegf-A which is reported to protect against, rather than contribute to, hypoxia-induced pulmonary vascular remodelling.
Objective: The aim was to analyze the early postnatal changes in myocardial density, subsarcolemmal localization and isoform expression of dihydropyridine receptors in rat ventricle and the influence of thyroid status on these changes. Methods: Newborn rats were treated from postnatal day 2 with L-triiodothyronine (T3) or 6-n-propyl-2-thiouracil (PTU) and ventricles were collected on day 1, 7 and 14. Radioligand binding and cell fractionation (density gradient centrifugation) techniques were used to determine the tissue density of various receptors and their subcellular localization. To analyze dihydropyridine receptor [alpha]1 subunit isoform expression, cDNA fragments corresponding to a large portion of motif IV were amplified by reverse transcriptase-polymerase chain reaction and treated with appropriate restriction endonucleases to determine the frequency of splicing events at the level of motif IV. Results: The myocardial density of dihydropyridine receptors increased 3-fold from day 1 to day 14 in control rats, and this increase occurred predominantly in membrane entities equilibrating at high densities in sucrose gradient, that is, presumably, in junctional structures (dyadic couplings). This maturation was delayed after PTU-treatment, and somewhat accelerated by excess T3. The proportion of mRNA variants typical of foetal heart (IVS3A variant and 'deleted' variant, showing a 33-nucleotide deletion at the level of the extracellular loop between IVS3 and IVS4) decreased with age in control rats. This reduction was delayed after treatment with PTU but was not influenced by excess T3. Conclusion: Hypothyroidism impaired the early postnatal maturation of dihydropyridine receptors as regards both their concentration into junctional structures and the decrease in the relative expression of [alpha]1-subunit mRNA variants typical of foetal heart.


As a complementary approach to positional cloning, we used in vivo complementation with bacterial artificial chromosome (BAC) clones expressed in transgenic mice to identify the circadian Clock gene. A 140 kb BAC transgene completely rescued both the long period and the loss-of-rhythm phenotypes in Clock mutant mice. Analysis with overlapping BAC transgenes demonstrates that a large transcription unit spanning ~100,000 base pairs is the Clock gene and encodes a novel basic-helix-loop-helix-PAS domain protein. Overexpression of the Clock transgene can shorten period length beyond the wild-type range, which provides additional evidence that Clock is an integral component of the circadian pacemaking system. Taken together, these results provide a proof of principle that "cloning by rescue" is an efficient and definitive method in mice.

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http://www.sciencedirect.com/science/article/B6WSN-419K2C1-9/2/cd3370dacf8ade7bc6807d12aafbf4f5

[alpha]v integrins have been implicated in many developmental processes and are therapeutic targets for inhibition of angiogenesis and osteoporosis. Surprisingly, ablation of the gene for the [alpha]v integrin subunit, eliminating all five [alpha]v integrins, although causing lethality, allows considerable development and organogenesis including, most notably, extensive vasculogenesis and angiogenesis. Eighty percent of embryos die in midgestation, probably because of placental defects, but all embryos develop normally to E9.5, and 20% are born alive. These liveborn [alpha]v-null mice consistently exhibit intracerebral and intestinal hemorrhages and cleft palates. These results necessitate reevaluation of the primacy of [alpha]v integrins in many functions including vascular development, despite reports that blockade of these integrins with antibodies or peptides prevents angiogenesis.


http://www.sciencedirect.com/science/article/B6WSN-4C6BN8V-4R/2/25297aff588065e88689b366b986f56a9

Two missense mutations occurring in different alleles of the DNA ligase I gene, encoding the major DNA ligase in proliferating mammalian cells, were detected in a human fibroblast strain (46BR). These cells exhibit retarded joining of Okazaki fragments during DNA replication and hypersensitivity to a variety of DNA-damaging agents. 46BR was derived from a patient who displayed symptoms of immunodeficiency, stunted growth, and sun sensitivity. A strongly reduced ability of DNA ligase I to form a labeled enzyme-adenylate intermediate correlated with the genetic defect in 46BR cells. The data indicate that human DNA ligase I is required for joining of Okazaki fragments during lagging-strand DNA synthesis and the completion of DNA excision repair.


http://www.sciencedirect.com/science/article/B6WSN-4C5PF5H-C/2/d9e219bbee9d525105c7f91bafdf4c154e

By the 4-cell stage of C. elegans embryogenesis, a ventral blastomere, called EMS, is already committed to producing pharyngeal and intestinal cell types. Recessive, maternal-effect mutations in the gene skn-1 prevent EMS from producing both pharyngeal and intestinal cells. In skn-1 mutant embryos, EMS instead produces hypodermal cells and body wall muscle cells,
much like its sister blastomere. Genetic analysis suggests that the skn-1 gene product is also required post-embryonically for development of the intestine. We have cloned and sequenced the skn-1 gene and describe sequence similarities to the basic regions of bZIP transcription factors. We propose that the maternally expressed skn-1 gene product acts to specify the fate of the EMS blastomere.


http://www.sciencedirect.com/science/article/B6WSN-4CWYRVJ-10/2/cf7ef12d18ead795124679823cb0b901

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant inherited disorder characterized by degeneration of cerebellar Purkinje cells, spinocerebellar tracts, and selective brainstem neurons owing to the expansion of an unstable CAG trinucleotide repeat. To gain insight into the pathogenesis of the SCA1 mutation and the intergenerational stability of trinucleotide repeats in mice, we have generated transgenic mice expressing the human SCA1 gene with either a normal or an expanded CAG tract. Both transgenes were stable in parent to offspring transmissions. While all six transgenic lines expressing the unexpanded human SCA1 allele had normal Purkinje cells, transgenic animals from five of six lines with the expanded SCA1 allele developed ataxia and Purkinje cell degeneration. These data indicate that expanded CAG repeats expressed in Purkinje cells are sufficient to produce degeneration and ataxia and demonstrate that a mouse model can be established for neurodegeneration caused by CAG repeat expansions.


http://www.sciencedirect.com/science/article/B6WSN-419K1NV-6/2/b1a3c8f42173ef4c603b23239b1f939

To investigate the roles of astroglial cells, we targeted their ablation genetically. Transgenic mice were generated expressing herpes simplex virus thymidine kinase from the mouse glial fibrillary acidic protein (GFAP) promoter. In adult transgenic mice, 2 weeks of subcutaneous treatment with the antiviral agent ganciclovir preferentially ablated transgene-expressing, GFAP-positive glia from the jejunum and ileum, causing a fulminating and fatal jejuno-ileitis. This pathology was independent of bacterial overgrowth and was characterized by increased myeloperoxidase activity, moderate degeneration of myenteric neurons, and intraluminal hemorrhage. These findings demonstrate that enteric glia play an essential role in maintaining the integrity of the bowel and suggest that their loss or dysfunction may contribute to the cellular mechanisms of inflammatory bowel disease.


http://www.sciencedirect.com/science/article/B6WSN-41BD8SK-9/2/607731267ff49ac19c12dde5f71335f2

The Arabidopsis NPR1 gene controls the onset of systemic acquired resistance (SAR), a plant immunity, to a broad spectrum of pathogens that is normally established after a primary exposure
to avirulent pathogens. Mutants with defects in NPR1 fail to respond to various SAR-inducing treatments, displaying little expression of pathogenesis-related (PR) genes and exhibiting increased susceptibility to infections. NPR1 was cloned using a map-based approach and was found to encode a novel protein containing ankyrin repeats. The lesion in one npr1 mutant allele disrupted the ankyrin consensus sequence, suggesting that these repeats are important for NPR1 function. Furthermore, transformation of the cloned wild-type NPR1 gene into npr1 mutants not only complemented the mutations, restoring the responsiveness to SAR induction with respect to PR-gene expression and resistance to infections, but also rendered the transgenic plants more resistant to infection by P. syringae in the absence of SAR induction.


http://www.sciencedirect.com/science/article/B6WSN-4C6BNB9-6V/2/1d64341909ec62e6270842a6dd33d3159

Epidermolytic hyperkeratosis is an autosomal dominant disorder affecting the structural integrity of the suprabasal layers of human epidermis. We have recently documented in one family linkage of the disease phenotype to the cluster of type II keratins. We have now identified a leucine->proline amino acid substitution in the conserved H1 subdomain of keratin 1 that is present only in affected family members. Using a quantitative assay and electron microscopy with synthetic peptides, we show that, whereas the wild-type H1 peptide rapidly disassembles preformed keratin filaments in vitro, the mutant peptide does this far less efficiently. Therefore the mutation in keratin 1 is likely to cause defective keratin filaments and hence a defective cytoskeleton in the epidermal cells in vivo.


http://www.sciencedirect.com/science/article/B6WSN-4C6BNVP-FV/2/fe87d739a947158a7d8359ef96aa17f79

Cells in the mid-body region of the nematode C. elegans develop differently from their anterior or posterior homologs. The gene lin-39 is required for mid-body region-specific development. In lin-39 mutants, mid-body cells express fates characteristic of more anterior or posterior homologs, and the migration of a neuroblast through the mid-body is defective. lin-39 acts cell autonomously in these mid-body cells and in the migrating neuroblast. lin-39 encodes a protein with an Antennapedia class homeodomain, most similar to those of the Drosophila homeotic genes Deformed and Sex combs reduced, and is located in a homeotic gene cluster with two other regional homeotic genes, mab-5 and egl-5. lin-39 and mab-5 function combinatorially in 2 ectodermal cells and have redundant functions in gonad development.


http://www.sciencedirect.com/science/article/B6WSN-4C5PJ3-9C/2/c062065fda13e5018f5cdef3e29c5836

Previously we demonstrated that transgenic mice expressing mutant basal epidermal keratin
genes exhibited a phenotype resembling a group of autosomal dominant human skin disorders known as epidermolysis bullosa simplex (EBS). EBS diseases affect ~1: 50,000 and are of unknown etiology, although all subtypes exhibit blistering arising from basal cell cytolysis. We now demonstrate that two patients with spontaneous cases of Dowling-Meara EBS have point mutations in a critical region in one (K14) of two basal keratin genes. To demonstrate function, we engineered one of these point mutations in a cloned human K14 cDNA, and showed that a K14 with an Arg-125->Cys mutation disrupted keratin network formation in transfected keratinocytes and perturbed filament assembly in vitro. Since we had previously shown that keratin network perturbation is an essential component of EBS diseases, these data suggest that the basis for the phenotype in this patient resides in this point mutation.


http://www.sciencedirect.com/science/article/B6WSN-438GMBP-J/2/76ecfdd0cda872ee8239a0d4a1ea86dd

The "housekeeping" sodium/hydrogen exchanger, NHE1, mediates the electroneutral 1:1 exchange of Na+ and H+ across the plasma membrane. NHE1 is ubiquitous and is studied extensively for regulation of pH i, cell volume, and response to growth factors. We describe a spontaneous mouse mutant, slow-wave epilepsy, (swe), with a neurological syndrome including ataxia and a unique epilepsy phenotype consisting of 3/sec absence and tonic-clonic seizures. swe was fine-mapped on Chromosome 4 and identified as a null allele of Nhe1. Mutants show selective neuronal death in the cerebellum and brainstem but otherwise are healthy. This first example of a disease-causing mutation in an Nhe gene provides a new tool for studying the delicate balance of neuroexcitability and cell survival within the CNS.


http://www.sciencedirect.com/science/article/B6WSN-4C8PS6S-37/2/4cf35773a4e358eb27a236cf2f7683a2

The [sigma]70 subunit of E. coli RNA polymerase is required for sequence-specific recognition of promoter DNA. Genetic studies and sequence analysis have indicated that [sigma]70 contains two specific DNA-binding domains that recognize the two conserved portions of the prokaryotic promoter. However, intact [sigma]70 does not bind to DNA. Using C-terminal and internal polypeptides of [sigma]70, carrying one or both putative DNA-binding domains, we demonstrate that [sigma]70 does contain two DNA-binding domains, but that N-terminal sequences inhibit the ability of intact [sigma]70 to bind to DNA. Thus, we propose that [sigma]70 is a sequence-specific DNA-binding protein that normally functions through an allosteric interaction with the core subunits of RNA polymerase.


http://www.sciencedirect.com/science/article/B6WSN-4C6BNMS-BM/2/5103a260d503ad6c90031ff87e7e71de
The ability of p53 to activate transcription from specific sequences suggests that genes induced by p53 may mediate its biological role as a tumor suppressor. Using a subtractive hybridization approach, we identified a gene, named WAF1, whose induction was associated with wild-type but not mutant p53 gene expression in a human brain tumor cell line. The WAF1 gene was localized to chromosome 6p21.2, and its sequence, structure, and activation by p53 was conserved in rodents. Introduction of WAF1 cDNA suppressed the growth of human brain, lung, and colon tumor cells in culture. Using a yeast enhancer trap, a p53-binding site was identified 2.4 kb upstream of WAF1 coding sequences. The WAF1 promoter, including this p53-binding site, conferred p53-dependent inducibility upon a heterologous reporter gene. These studies define a gene whose expression is directly induced by p53 and that could be an important mediator of p53-dependent tumor growth suppression.


The MAD-related (MADR) family of proteins are essential components in the signaling pathways of serine/threonine kinase receptors for the transforming growth factor [beta] (TGF[beta]) superfamily. We demonstrate that MADR2 is specifically regulated by TGF[beta] and not bone morphogenetic proteins. The gene for MADR2 was found to reside on chromosome 18q21, near DPC4, another MADR protein implicated in pancreatic cancer. Mutational analysis of MADR2 in sporadic tumors identified four missense mutations in colorectal carcinomas, two of which display a loss of heterozygosity. Biochemical and functional analysis of three of these demonstrates that the mutations are inactivating. These findings suggest that MADR2 is a tumor suppressor and that mutations acquired in colorectal carcinomas may function to disrupt TGF[beta] signaling.


Five unrelated children are described with a rare autoimmune lymphoproliferative syndrome (ALPS) characterized by massive nonmalignant lymphadenopathy, autoimmune phenomena, and expanded populations of TCR-CD3+ CD4-CD8- lymphocytes. These findings, suggesting a genetic defect in the ability of T lymphocytes to respond to normal immunoregulatory mechanisms, prompted an evaluation of lymphocyte apoptosis. Each child had defective Fas-mediated T lymphocyte apoptosis associated with a unique, deleterious Fas gene mutation. One mutation appeared to cause a simple loss of function; however, four others had a dominant negative phenotype when coexpressed with normal Fas. Family studies demonstrated the inheritance of the mutant Fas alleles. The occurrence of Fas mutations together with abnormal T
cell apoptosis in ALPS patients suggests an involvement of Fas in this recently recognized disorder of lymphocyte homeostasis and peripheral self-tolerance.


Mutations at the mouse tottering (tg) locus cause a delayed-onset, recessive neurological disorder resulting in ataxia, motor seizures, and behavioral absence seizures resembling petit mal epilepsy in humans. A more severe allele, leaner (tgla), also shows a slow, selective degeneration of cerebellar neurons. By positional cloning, we have identified an [alpha]1A voltage-sensitive calcium channel gene that is mutated in tg and tgla mice. The [alpha]1A gene is widely expressed in the central nervous system with prominent, uniform expression in the cerebellum. [alpha]1A expression does not mirror the localized pattern of cerebellar degeneration observed in tgla mice, providing evidence for regional differences in biological function of [alpha]1A channels. These studies define the first mutations in a mammalian central nervous system-specific voltage-sensitive calcium channel and identify the first gene involved in absence epilepsy.


Fragile X syndrome results from mutations in a (CGG)n repeat found in the coding sequence of the FMR-1 gene. Analysis of length variation in this region in normal individuals shows a range of allele sizes varying from a low of 6 to a high of 54 repeats. Premutations showing no phenotypic effect in fragile X families range in size from 52 to over 200 repeats. All alleles with greater than 52 repeats, including those identified in a normal family, are meiotically unstable with a mutation frequency of one, while 75 meioses of alleles of 46 repeats and below have shown no mutation. Premutation alleles are also mitotically unstable as mosaicism is observed. The risk of expansion during oogenesis to the full mutation associated with mental retardation increases with the number of repeats, and this variation in risk accounts for the Sherman paradox.


Two previously unidentified human cdc25 genes have been isolated, cdc25A and cdc25B. Both genes rescue a cdc25ts mutant of fission yeast. Microinjection of anti-cdc25A antibodies into HeLa cells causes their arrest in mitosis. cdc25A and cdc25B display endogenous tyrosine phosphatase activity that is stimulated several-fold, in the absence of cdc2, by stoichiometric addition of either cyclin B1 or B2 but not A or D1. Association between cdc25A and cyclin B1/cdc2 was detected in the HeLa cells. These findings indicate that B-type cyclins are
multifunctional proteins that not only act as M phase regulatory subunits of the cdc2 protein kinase, but also activate the cdc25 tyrosine phosphatase, of which cdc2 is the physiological substrate. A region of amino acid similarity between cyclins and tyrosine PTPases has been detected. This region is absent in cdc25 phosphatases. The motif may represent an activating domain that has to be provided to cdc25 by intermolecular interaction with cyclin B.


http://www.sciencedirect.com/science/article/B6WSN-4C5PR3D-3V/2/d7e4383a8fa82231a47604d40f9903a3

The dauer larva is a developmentally arrested, non-feeding dispersal stage normally formed in response to overcrowding and limited food. The daf-1 gene specifies an intermediate step in a hierarchy of genes thought to specify a pathway for neural transduction of environmental cues. Mutations in daf-1 result in constitutive formation of dauer larvae even in abundant food. This gene has been cloned by Tc1-transposon tagging, and it appears to encode a new class of serine/threonine kinase. A daf-1 probe detects a 2.5 kb mRNA of low abundance, and the DNA sequence indicates that the gene encodes a 669 amino acid protein, with a putative transmembrane domain and a C-terminal protein kinase domain most closely related to the cytosolic, raf proto-oncogene family. Hence, the daf-1 product appears to be a cell-surface receptor required for transduction of environmental signals into an appropriate developmental response.


http://www.sciencedirect.com/science/article/B6WSN-4CXMRRR-18/2/704128ac73e0449aaff84b978cfab826

Faciogenital dysplasia (FGDY), also known as Aarskog-Scott syndrome, is an X-linked developmental disorder characterized by disproportionately short stature and by facial, skeletal, and urogenital anomalies. Molecular genetic analyses mapped FGDY to chromosome Xp11.21. To clone this gene, YAC clones spanning an FGDY-specific translocation breakpoint were isolated. An isolated cDNA, FGD1, is disrupted by the breakpoint, and FGD1 mutations cosegregate with the disease. FGD1 codes for a 961 amino acid protein that has strong homology to Rho/Rac guanine nucleotide exchange factors (GEFs), contains a cysteine-rich zinc finger-like region, and, like the RasGEF mSos, contains two potential SH3-binding sites. These results provide compelling evidence that FGD1 is responsible for FGDY and suggest that FGD1 is a Rho/RacGEF involved in mammalian development.


http://www.sciencedirect.com/science/article/B6WSN-4CF6DP7-24/2/2b568d1fd438f3aa4d3f270626cd38bd

In C. reinhardtii, the mature psaA mRNA is assembled by a process involving trans-splicing of
three separate transcripts encoded at three widely scattered loci of the chloroplast genome. At least one additional chloroplast locus (tscA) is required for trans-splicing of exons 1 and 2. We have mapped this gene by transformation of a deletion mutant with a particle gun. The 0.7 kb region of the chloroplast genome that is sufficient to rescue tscA function has been subjected to insertion mutagenesis, showing that it does not contain significant open reading frames. We suggest from these experiments that the product of the tscA gene may be a small chloroplast RNA that acts in trans in the first trans-splicing reaction of psaA. A model for the mode of action of this RNA is presented, in which the characteristic structure of group 11 introns is assembled from three separate transcripts.


http://www.sciencedirect.com/science/article/B6WSN-4CYFX42-C/2/e55a5311d0f38996c2597c22f0c4719a

The closely related B. subtilis bacteriophages SPO1 and SP82 have similar introns inserted into a conserved domain of their DNA polymerase genes. These introns encode endonucleases with unique properties. Other intron-encoded "homing" endonucleases cleave both strands of intronless DNA; subsequent repair results in unidirectional gene conversion to the intron-containing allele. In contrast, the enzymes described here cleave one strand on both intron-containing and intronless targets at different distances from their common intron insertion site. Most surprisingly, each enzyme prefers DNA of the heterologous phage. The SP82-encoded endonuclease is responsible for exclusion of the SPO1 intron and flanking genetic markers from the progeny of mixed infections, a novel selective advantage imparted by an intron to the genome in which it resides.


http://www.sciencedirect.com/science/article/B6WSN-41BD7S6-9/2/7a5445c80e140204a462c71064b34722

The nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterized by multiple basal cell carcinomas (BCCs), pits of the palms and soles, jaw keratocysts, a variety of other tumors, and developmental abnormalities. NBCCS maps to chromosome 9q22.3. Familial and sporadic BCCs display loss of heterozygosity in this region, consistent with the gene being a tumor suppressor. A human sequence (PTC) with strong homology to the Drosophila segment polarity gene, patched, was isolated from a YAC and cosmid contig of the NBCCS region. Mutation analysis revealed alterations of PTC in NBCCS patients and in related tumors. We propose that a reduction in expression of the patched gene can lead to the developmental abnormalities observed in the syndrome and that complete loss of patched function contributes to transformation of certain cell types.


http://www.sciencedirect.com/science/article/B6WSN-4CB6NJX-H5/2/f373ff264368d60ef6f2f4ba58b641e
We report the cloning of the gene that encodes the yeast TATA binding protein TFIID. TFIID contains 240 amino acids and has no obvious sequence similarity to other known proteins. TFIID was synthesized in vitro and in two separate assays behaved identically to the protein purified from yeast. TFIID bound to TATA elements from the adenovirus major late promoter (TATAAAA) and the yeast LEU2 promoter (TATTTAA) and formed protein-DNA complexes stable to electrophoresis only in the presence of TFIIA. In vitro-synthesized yeast TFIID also complemented a mammalian in vitro transcription system that lacked TFIID. Comparison of the yeast TFIID gene with the yeast SPT15 gene (suppressor of Ty element insertions) showed that the two genes are identical. This finding indicates that the yeast TFIID activity defined in vitro is responsible for specific transcription in vivo.


The activity of the C. elegans gene ced-9 is required to protect cells that normally survive from undergoing programmed cell death. Here we describe the cloning and molecular characterization of this gene. ced-9 is an element of a polycistronic locus that also contains the gene cyt-1, which encodes a protein similar to cytochrome b560 of complex II of the mitochondrial respiratory chain. ced-9 encodes a 280 amino acid protein showing sequence and structural similarities to the mammalian proto-oncogene bcl-2. Overexpression of bcl-2 can mimic the protective effect of ced-9 on C. elegans cell death and can prevent the ectopic cell deaths that occur in ced-9 loss-of-function mutants. These results suggest that ced-9 and bcl-2 are homologs and that the molecular mechanism of programmed cell death has been conserved from nematodes to mammals.


http://www.sciencedirect.com/science/article/B6WSN-4B3MJM0-7/2/377f809f1586ef711610d191f6c0ea90

The hereditary disease Cockayne syndrome (CS) is characterized by a complex clinical phenotype. CS cells are abnormally sensitive to ultraviolet radiation and are defective in the repair of transcriptionally active genes. The cloned CSB gene encodes a member of a protein family that includes the yeast Snf2 protein, a component of the transcriptional regulator Swi/Snf. We report the cloning of the CSA cDNA, which can encode a WD repeat protein. Mutations in the cDNA have been identified in CS-A cell lines. CSA protein interacts with CSB protein and with p44 protein, a subunit of the human RNA polymerase II transcription factor IIH. These observations suggest that the products of the CSA and CSB genes are involved in transcription.


http://www.sciencedirect.com/science/article/B6WSN-4C592Y4-7D/2/4354ceb647ce4b5b2627f899f858f1b5

Changes in DNA supercoiling in response to environmental signals such as osmolarity, temperature, or anaerobicity appear to play an underlying role in the regulation of gene expression in bacteria. Extensive genetic analyses have implicated the osmZ gene in this regulatory process: osmZ mutations are highly pleiotropic and alter the topology of cellular DNA. We have shown that the product of the osmZ gene is the "histone-like" protein H1 (H-NS). Protein H1 is one of the most abundant components of bacterial chromatin and binds to DNA in a relatively nonspecific fashion. These data imply a regulatory role for one of the major components of bacterial chromatin and provide support for the notion that changes in DNA topology and/or chromatin structure play a role in regulating gene expression.


http://www.sciencedirect.com/science/article/B6WSN-4CXMRNG-G/2/4c7e51b3f5a55303fee6f78f8fe79a3a

A cap-binding protein complex (CBC) present in the nuclei of HeLa cells has been characterized.
Purified CBC consists of two previously identified proteins, CBP80 and CBP20. These proteins are shown to cofractionate to apparent homogeneity and to be coimmunoprecipitable with anti-CBP80 antibodies. Analysis of the inhibition of pre-mRNA splicing in vitro and in vivo by chemically modified analogs of the cap structure, and of the binding of these analogs to CBC in vitro, suggests a role for the complex in splicing. Extracts immunodepleted of CBC do not efficiently splice an adenoviral pre-mRNA owing to blockage of an early step in splicing complex formation. CBC may therefore play a role in pre-mRNA recognition.


Craniosynostosis, the premature fusion of calvarial sutures, is a common developmental anomaly that causes abnormal skull shape. The locus for one autosomal dominant form of craniosynostosis has been mapped to chromosome 5qter. The human MSX2 gene localizes to chromosome 5, and a polymorphic marker in the MSX2 intron segregates in a kindred with the disorder with no recombination. Moreover, a histidine substitutes for a highly conserved proline at position 7 of the MSX2 homeodomain exclusively in affected members. In the mouse, transcripts of the Msx2 gene are localized to calvarial sutures. These results provide compelling evidence that the mutation causes this craniosynostosis syndrome.


The stroke-prone spontaneously hypertensive rat (SHRSP) is a well-characterized model for primary hypertension in humans. High blood pressure in SHRSP shows polygenic inheritance, but none of the loci responsible have previously been identified. To locate genes controlling this quantitative trait, we mapped a large collection of DNA polymorphisms in a cross between SHRSP and the normotensive WKY strain. Here we report strong genetic evidence that a gene, Bp1, having a major effect on blood pressure maps to rat chromosome 10 with a LOD score of 5.10 and is closely linked to the rat gene encoding angiotensin-converting enzyme (ACE), an enzyme that plays a major role in blood pressure homeostasis and is an important target of antihypertensive drugs. We also find significant, albeit weaker, linkage to a locus, Bp2, on chromosome 18. We discuss the implications of genetic dissection of quantitative disease-related phenotypes in mammals.


Variation in internal minisatellite structure can be analyzed by mapping variant repeat units within
amplified alleles. A system capable of distinguishing >1070 allelic states at the human hypervariable locus D1S8 has been developed. Population surveys of internal allelic structure indicate that D1S8 alleles evolve rapidly along haploid chromosome lineages. Internal mapping of deletion mutant alleles physically selected from genomic DNA provides further evidence that germline and somatic mutations altering the number of allelic repeat units seldom if ever arise by unequal exchange between alleles. The existence of low level germline mosaicism for new mutants further indicates that many germline mutation events are premeiotic. Physical selection of new mutants also allows minisatellite mutation rates to be estimated directly in human DNA.


http://www.sciencedirect.com/science/article/B6WSN-41FCWJ3-G/2/110eb0148fc50726e310e7b53b9db279

To explore regulation of potentially lethal responses to bacterial lipopolysaccharide (LPS), we used differential display under LPS-free conditions to compare macrophage cell lines from two strains of mice congenic for a locus affecting LPS sensitivity. LPS- hyporesponsive cells, primary macrophages, and polymorphonuclear leukocytes transcribed secretory leukocyte protease inhibitor (SLPI), a known epithelial cell-derived inhibitor of leukocyte serine proteases. Transfection of macrophages with SLPI suppressed LPS-induced activation of NF-[kappa]B and production of nitric oxide and TNF[alpha]. The ability of interferon-[gamma] (IFN[gamma]) to restore LPS responsiveness is a hallmark of the LPS-hyporesponsive phenotype. IFN[gamma] suppressed expression of SLPI and restored LPS responsiveness to SLPI-producing cells. Thus, SLPI is an LPS-induced IFN[gamma]-suppressible phagocyte product that serves to inhibit LPS responses.


http://www.sciencedirect.com/science/article/B6WSN-4C592K1-1D/2/16777d2f03cee1907d2c6add6d6d0d4eb

When rhesus monkeys were infected with a form of cloned SIVmac239 having a premature stop signal at the 93rd codon of nef, revertants with a coding codon at this position quickly and universally came to predominate in the infected animals. This suggests that there are strong selective forces for open functional forms of nef in vivo. Although deletion of nef sequences had no detectable effect on virus replication in cultured cells, deletion of nef sequences dramatically altered the properties of virus in infected rhesus monkeys. Our results indicate that nef is required for maintaining high virus loads during the course of persistent infection in vivo and for full pathologic potential. Thus, nef should become a target for antiviral drug development. Furthermore, the properties of virus with a deletion in nef suggest a means for making live-attenuated strains of virus for experimental vaccine testing.


http://www.sciencedirect.com/science/article/B6WSN-41CR5SV-C/2/ac02a9769e4963349b93cb9f5155f8fc
Proteases are crucial for cancer metastasis, but due to lack of assays, their role in intravasation has not yet been tested. We have developed a human Alu sequence PCR-based assay to quantitate intravasated cells in an in vivo model. We demonstrated that metalloproteinases (MMPs), and most likely MMP-9, are required for intravasation by showing that marimastat, an inhibitor of MMPs, reduced intravasation by more than 90%, and that only tumor cell lines expressing MMP-9 intravasated. Cells with low surface urokinase plasminogen activator (uPA) and uPA receptor (uPAR) were also incapable of intravasation, despite the presence of high levels of MMP-9. We concluded that breaching of the vascular wall is a rate-limiting step for intravasation, and consequently for metastasis, and that cooperation between uPA/uPAR and MMP-9 is required to complete this step.


http://www.sciencedirect.com/science/article/B6WSN-418PWX6-K/2/4246a16ef70ce25e521117c21f075e38

We used positional cloning to identify the circadian Clock gene in mice. Clock is a large transcription unit with 24 exons spanning ~100,000 bp of DNA from which transcript classes of 7.5 and ~10 kb arise. Clock encodes a novel member of the bHLH-PAS family of transcription factors. In the Clock mutant allele, an A->T nucleotide transversion in a splice donor site causes exon skipping and deletion of 51 amino acids in the CLOCK protein. Clock is a unique gene with known circadian function and with features predicting DNA binding, protein dimerization, and activation domains. CLOCK represents the second example of a PAS domain-containing clock protein (besides Drosophila PERIOD), which suggests that this motif may define an evolutionarily conserved feature of the circadian clock mechanism.


http://www.sciencedirect.com/science/article/B6WSN-4C6BNDX-6Y/2/a9d80b3f9be1d0f41f1174868db0ed79

The mouse short ear gene is required for normal growth and patterning of skeletal structures, and for repair of bone fractures in adults. We have carried out an extensive chromosome walk in the chromosome region that surrounds this locus. Here we show that the short ear region contains the gene for a TGF[beta]-related protein called bone morphogenetic protein 5 (Bmp-5). This gene is deleted or rearranged in several independent mutations at the short ear locus. Mice homozygous for large deletions of the Bmp-5 coding region are viable and fertile. Mutations at the short ear locus provide an important new tool for defining the normal functions of BMPs in mammals. The specific skeletal defects seen in short-eared animals, which occur against a background of otherwise normal skeletal structures, suggest that particular aspects of skeletal morphology may be determined by individual members of a family of signaling factors that can induce the formation of cartilage and bone in vivo.

The mutated gene responsible for the tubby obesity phenotype has been identified by positional cloning. A single base change within a splice donor site results in the incorrect retention of a single intron in the mature tub mRNA transcript. The consequence of this mutation is the substitution of the carboxy-terminal 44 amino acids with 24 intron-encoded amino acids. The normal transcript appears to be abundantly expressed in the hypothalamus, a region of the brain involved in body weight regulation. Variation in the relative abundance of alternative splice products is observed between inbred mouse strains and appears to correlate with an intron length polymorphism. This allele of tub is a candidate for a previously reported diet-induced obesity quantitative trait locus on mouse chromosome 7.


The cloning of double-time (dbt) is reported. DOUBLETIME protein (DBT) is most closely related to human casein kinase I[epsiv]. dbtS and dbtL mutations, which alter period length of Drosophila circadian rhythms, produce single amino acid changes in conserved regions of the predicted kinase. dbtP mutants, which eliminate rhythms of per and tim expression and constitutively overproduce hypophosphorylated PER proteins, abolish most dbt expression. dbt mRNA appears to be expressed in the same cell types as are per and tim and shows no evident oscillation in wild-type heads. DBT is capable of binding to PER in vitro and in Drosophila cells, suggesting that a physical association of PER and DBT regulates PER phosphorylation and accumulation in vivo.


We have cloned the fragile site FRAXE and demonstrate that individuals with this fragile site possess amplifications of a GCC repeat adjacent to a CpG Island in Xq28 of the human X chromosome. Normal individuals have 6-25 copies of the GCC repeat, whereas mentally retarded, FRAXE-positive individuals have >200 copies and also have methylation at the CpG island. This situation is similar to that seen at the FRAXA locus and is another example in which a trinucleotide repeat expansion is associated with a human genetic disorder. In contrast with the fragile X syndrome, the GCC repeat can expand or contract and is equally unstable when passed through the male or female line. These results also have implications for the understanding of chromosome fragility.

A paradigm for control of insulin secretion is that glucose metabolism elevates cytoplasmic [ATP]/[ADP] in [beta] cells, closing KATP channels and causing depolarization, Ca2+ entry, and insulin release. Decreased responsiveness of KATP channels to elevated [ATP]/[ADP] should therefore lead to decreased insulin secretion and diabetes. To test this critical prediction, we generated transgenic mice expressing [beta] cell KATP channels with reduced ATP sensitivity. Animals develop severe hyperglycemia, hypoinsulinemia, and ketoacidosis within 2 days and typically die within 5. Nevertheless, islet morphology, insulin localization, and [alpha] and [beta] cell distributions were normal (before day 3), pointing to reduced insulin secretion as causal. The data indicate that normal KATP channel activity is critical for maintenance of euglycemia and that overactivity can cause diabetes by inhibiting insulin secretion.


SF2 is a protein factor essential for constitutive pre-mRNA splicing in HeLa cell extracts and also activates proximal alternative 5’ splice sites in a concentration dependent manner. This latter property suggests a role for SF2 in preventing axon skipping, ensuring the accuracy of splicing, and regulating alternative splicing. Human SF2 cDNAs have been isolated and overexpressed in bacteria. Recombinant SF2 is active in splicing and stimulates proximal 5’ splice sites. SF2 has a C-terminal region rich in arginine-serine dipeptides, similar to the RS domains of the U1 snRNP 70K polypeptide and the Drosophila alternative splicing regulators transformer, transformer-2, and suppressor-of-white-apricot. Like transformer-2 and 70K, SF2 contains an RNP-type RNA recognition motif.


Regulation of eukaryotic gene expression requires ATP-dependent chromatin remodeling enzymes, such as SWI/SNF, and histone acetyltransferases, such as Gcn5p. Here we show that SWI/SNF remodeling controls recruitment of Gcn5p HAT activity to many genes in late mitosis and that these chromatin remodeling enzymes play a role in regulating mitotic exit. In contrast, interphase expression of GAL1, HIS3, PHO5, and PHO8 is accompanied by SWI/SNF-independent recruitment of Gcn5p HAT activity. Surprisingly, prearresting cells in late mitosis imposes a requirement for SWI/SNF in recruiting Gcn5p HAT activity to the GAL1 promoter, and GAL1 expression also becomes dependent on both chromatin remodeling enzymes. We propose that SWI/SNF and Gcn5p are globally required for mitotic gene expression due to the condensed state of mitotic chromatin.

Batten disease (also known as juvenile neuronal ceroid lipofuscinosis) is a recessively inherited neurodegenerative disorder of childhood characterized by progressive loss of vision, seizures, and psychomotor disturbances. The Batten disease gene, CLN3, maps to chromosome 16p12.1. The so-called 56 chromosome haplotype defined by alleles at the D16S299 and D16S298 loci is shared by 73% of Batten disease chromosomes. Exon amplification of a cosmid containing D16S298 has yielded a candidate gene that is disrupted by a 1 kb genomic deletion in all patients carrying the 56 chromosome. Two separate deletions and a point mutation altering a splice site in three unrelated families have confirmed the candidate as the CLN3 gene. The disease gene encodes a novel 438 amino acid protein of unknown function.


Mice that are homozygous for the autosomal recessive chondrodysplasia (cho) mutation die at birth with abnormalities in cartilage of limbs, ribs, mandible, and trachea. Limb bones of newborn cho/cho mice are wider at the metaphyses than normal bones and only about half the normal length. By linkage analysis, the cho gene and the gene encoding the [alpha]1 (XI) chain of cartilage collagen XI were mapped to the same region of chromosome 3. Deletion of a cytidine residue about 570 nt downstream of the translation initiation codon in cho [alpha]1(XI) mRNA causes a reading frame shift and introduces a premature stop codon. The data demonstrate that collagen XI is essential for normal formation of cartilage collagen fibrils and the cohesive properties of cartilage. The results also suggest that the normal differentiation and spatial organization of growth plate chondrocytes is critically dependent on the presence of type XI collagen in cartilage extracellular matrix.


Charcot-Marie-Tooth disease type 1A (CMT1A) was localized by genetic mapping to a 3 cM interval on human chromosome 17p. DNA markers within this interval revealed a duplication that is completely linked and associated with CMT1A. The duplication was demonstrated in affected individuals by the presence of three alleles at a highly polymorphic locus, by dosage differences at RFLP alleles, and by two-color fluorescence in situ hybridization. Pulsed-field gel electrophoresis of genomic DNA from patients of different ethnic origins showed a novel Sall fragment of 500 kb associated with CMT1A. A severely affected CMT1A offspring from a mating between two affected individuals was demonstrated to have this duplication present on each chromosome 17. We have demonstrated that failure to recognize the molecular duplication can lead to misinterpretation of marker genotypes for affected individuals, identification of false recombinants, and incorrect localization of the disease locus.


The vomeronasal organ of mammals is an olfactory sensory structure that detects pheromones. It contains two subsets of sensory neurons that differentially express G[alpha]o and G[alpha]i2. By comparing gene expression in single neurons, we identified a novel multigene family that codes for a diverse array of candidate pheromone receptors (VRs) expressed by the G[alpha]o+ subset. Different VRs are expressed by different neurons, but those neurons are interspersed, suggesting a distributed mode of sensory coding. Chromosome mapping experiments suggest an evolutionary connection between genes encoding VRs and receptors for volatile odorants. However, a dramatically different structure for VRs and the existence of variant VR mRNA forms indicate that there are diverse strategies to detect functionally distinct sensory stimuli.


http://www.sciencedirect.com/science/article/B6WSN-4194R15-H/2/33abb04c1af5f4728cb647280ce047e9

Autosomal dominant periodic fever syndromes are characterized by unexplained episodes of fever and severe localized inflammation. In seven affected families, we found six different missense mutations of the 55 kDa tumor necrosis factor receptor (TNFR1), five of which disrupt conserved extracellular disulfide bonds. Soluble plasma TNFR1 levels in patients were approximately half normal. Leukocytes bearing a C52F mutation showed increased membrane TNFR1 and reduced receptor cleavage following stimulation. We propose that the autoinflammatory phenotype results from impaired downregulation of membrane TNFR1 and diminished shedding of potentially antagonistic soluble receptor. TNFR1-associated periodic syndromes (TRAPS) establish an important class of mutations in TNF receptors. Detailed analysis of one such mutation suggests impaired cytokine receptor clearance as a novel mechanism of disease.


http://www.sciencedirect.com/science/article/B6WSN-4C590SC-5M/2/2f851708c135c0b27a6b048c0a70835f

We identified and sequenced a cDNA clone encoding a kinesin-like protein from Drosophila. The predicted product of this cDNA has a carboxy-terminal domain that is substantially similar to the motor domain of kinesin heavy chain. The amino-terminal domain is unlike that found in previously identified kinesins or kinesin-like proteins. Analyses of this new sequence suggest that the maximal motor unit in the kinesin superfamily may be as little as 350 amino acids, and that the existence of both kinesin and kinesin-like molecules must be an evolutionarily ancient feature of eukaryotes. We also tested some of the biochemical properties of the protein encoded by this cDNA and found them to be similar to those of kinesin. Finally, the clone we isolated appears to correspond to the non-claret disjunctional (ncd) gene, which when mutant causes defects in meiotic and early embryonic mitotic chromosome segregation, and whose recently determined
sequence predicts a kinesin-like domain.


Telomerase, the ribonucleoprotein enzyme that elongates telomeres, is repressed in normal human somatic cells but is reactivated during tumor progression. We report the cloning of a human gene, hEST2, that shares significant sequence similarity with the telomerase catalytic subunit genes of lower eukaryotes. hEST2 is expressed at high levels in primary tumors, cancer cell lines, and telomerase-positive tissues but is undetectable in telomerase-negative cell lines and differentiated telomerase-negative tissues. Moreover, the message is up-regulated concomitant with the activation of telomerase during the immortalization of cultured cells and down-regulated during in vitro cellular differentiation. Taken together, these observations suggest that the induction of hEST2 mRNA expression is required for the telomerase activation that occurs during cellular immortalization and tumor progression.


Activating mutations of p53 promote tumor progression. The mutant protein adopts a characteristic conformation, which lacks the growth suppressor function of wild-type p53. We show that mutant p53 can drive cotranslated wild-type p53 into the mutant conformation: a similar effect in vivo would block wild-type suppressor function with dominant negative effect. The cotranslational effect of mutant p53 on wild-type conformation depends upon interaction between nascent polypeptides and oligomerization of the full-length proteins. We also show that oligomers of p53 proteins can be induced to change conformation in a cooperative manner. Cell growth stimulation induces a similar conformational change in p53, and our present results indicate that this may involve allosteric regulation.


The translocation of polypeptides across the endoplasmic reticulum is a vectorial process that occurs probably through a protein channel by a mechanism as yet undetermined. Here, we demonstrate bidirectional movement of a 221 residue nascent polypeptide across microsomal membranes and provide evidence suggesting that the retrograde movement is through the translocation channel. Retrograde movement is observed only when the polypeptide is generated from a truncated transcript; addition of a stop codon after codon 221 confers vectorial movement. Retrograde movement can also be prevented by glycosylation of the nascent polypeptide, as well as by inclusion of 32 additional amino acids that may promote folding of the translocated chain.
We propose that the protein translocation channel is a passive pore that does not create a directional bias in polypeptide movement and that vectorial translocation is driven by nascent chain elongation and sustained by posttranslocation events that prevent retrograde movement.


H-NS is an abundant structural component of bacterial chromatin and influences many cellular processes, including recombination, transposition, and transcription. We have studied the mechanism of action of H-NS at the osmotically regulated proU promoter. The interaction of H-NS with a curved DNA element located downstream of the proU promoter is required for normal regulation of expression. Heterologous curved sequences can replace the regulatory role of the proU curve. Hence, the luxAB and lacZ reporter genes, which differ in the presence or absence of a curve, can indicate very different patterns of transcription. H-NS interacts preferentially with these curved DNA elements in vitro. Furthermore, in vivo the interaction of H-NS with curved DNA participates in the control of plasmid linking number. The data suggest that H-NS-dependent changes in DNA topology play a role in the osmoregulation of proU expression.


A variety of cytokines activate receptor-associated members of the Janus family of protein tyrosine kinases (Jaks). To assess the role of Jak2, we have derived Jak2-deficient mice. The mutation causes an embryonic lethality due to the absence of definitive erythropoiesis. Fetal liver myeloid progenitors, although present based on the expression of lineage specific markers, fail to respond to erythropoietin, thrombopoietin, interleukin-3 (IL-3), or granulocyte/macrophage colony-stimulating factor. In contrast, the response to granulocyte specific colony-stimulating factor is unaffected. Jak2-deficient fibroblasts failed to respond to interferon [gamma] (IFN[gamma]), although the responses to IFN[alpha]/[beta] and IL-6 were unaffected. Lastly, reconstitution experiments demonstrate that Jak2 is not required for the generation of lymphoid progenitors, their amplification, or functional differentiation. Therefore, Jak2 plays a critical, nonredundant role in the function of a specific group of cytokines receptors.


The AP-1 transcription factor JunB is a transcriptional regulator of myelopoiesis. Inactivation of JunB in postnatal mice results in a myeloproliferative disorder (MPD) resembling early human chronic myelogenous leukemia (CML). Here, we show that JunB regulates the numbers of hematopoietic stem cells (HSC). JunB overexpression decreases the frequency of long-term HSC
(LT-HSC), while JunB inactivation specifically expands the numbers of LT-HSC and granulocyte/macrophage progenitors (GMP) resulting in chronic MPD. Further, we demonstrate that junB inactivation must take place in LT-HSC, and not at later stages of myelopoiesis, to induce MPD and that only junB-deficient LT-HSC are capable of transplanting the MPD to recipient mice. These results demonstrate a stem cell-specific role for JunB in normal and leukemic hematopoiesis and provide experimental evidence that leukemic stem cells (LSC) can reside at the LT-HSC stage of development in a mouse model of MPD.


http://www.sciencedirect.com/science/article/B6WSN-4C6BMYW-13/2/0e261e7987a3fd58231f7c2cc44f4287

Integration of retroviral DNA can serve as a paradigm for cellular functions that are affected by the packaging of DNA into chromatin. We have used a novel polymerase chain reaction-based assay to survey DNA and chromatin for the precise distribution of many integration sites. Integration into naked DNA targets is nonuniform, implying a nucleotide sequence bias. In chromatin, integration occurs preferentially at positions where the major groove is on the exposed face of the nucleosomal DNA helix, generating a 10 bp periodic spacing of preferred sites. Chromatin assembly enhances the reactivity of many sites, so that integration occurs most frequently at sites in nucleosomal, rather than nucleosome-free, regions of minichromosomes. In contrast, integration is prevented in a region occupied by a site-specific DNA-binding protein. Comparisons of integration events mediated by viral nucleoprotein complexes or by two different retroviral integrases show that the integration machinery also affects target site selection.


http://www.sciencedirect.com/science/article/B6WSN-42DP8H4-4/2/943cb9699881a43ae72759a525a17df

The recessively inherited developmental disorder, cartilage-hair hypoplasia (CHH) is highly pleiotropic with manifestations including short stature, defective cellular immunity, and predisposition to several cancers. The endoribonuclease RNase MRP consists of an RNA molecule bound to several proteins. It has at least two functions, namely, cleavage of RNA in mitochondrial DNA synthesis and nucleolar cleaving of pre-rRNA. We describe numerous mutations in the untranslated RMRP gene that cosegregate with the CHH phenotype. Insertion mutations immediately upstream of the coding sequence silence transcription while mutations in the transcribed region do not. The association of protein subunits with RNA appears unaltered. We conclude that mutations in RMRP cause CHH by disrupting a function of RNase MRP RNA that affects multiple organ systems.


http://www.sciencedirect.com/science/article/B6WSN-4C6BN8V-4W/2/3f65eb9b84148fcee6e9e974436355f56
In mitogenically stimulated and tyrosine kinase--transformed cells, a substantial fraction of the ras GTPase-activating protein (GAP) forms a complex with a protein termed p190. We have cloned several cDNAs encoding the p190 protein. Analysis of the predicted protein sequence reveals three distinct domains with homology to previously described sequences. An N-terminal domain of p190 contains sequence motifs that are found in all of the known GTPases. At the C-terminus of the protein is a domain that contains sequences very similar to those found in the breakpoint cluster region gene product, n-chimerin, and rho GAP, all of which have been shown to possess intrinsic GAP activity on small GTPases. Finally, a 778 aa segment in the middle of p190 is nearly identical in sequence to a recently described transcriptional repressor. This raises the possibility that p190, acting via GAP, can transduce signals from p21ras to the nucleus, perhaps affecting expression of specific cellular genes.


http://www.sciencedirect.com/science/article/B6WSN-4C8PS2T-B/2/e8a2f0510666e6b246e6f8acfc38fe

We mapped the distribution and expression of wild-type and deleted mitochondrial DNA (mtDNA) molecules in skeletal muscle fibers of patients with mitochondrial disease. We show that ragged red fiber segments, which are characteristic histological features of these myopathies, represent focal accumulations of mitochondria containing predominantly deleted mtDNAs and that the mutant genomes are absent or extremely rare in normal fiber segments. This suggests that the mtDNA mutations play a direct role in focal mitochondrial accumulation. Although levels of wild-type mtDNAs and mRNAs in ragged red fiber segments are near normal, mitochondrial function, as revealed by cytochrome oxidase cytochemistry, is severely impaired. This suggests that the presence of mutant mtDNAs interferes with the expression of co-existing wild-type mtDNAs in these segments at a posttranscriptional level.


http://www.sciencedirect.com/science/article/B6WSN-41C26NT-5/2/c3fde4d99a75f8fd544d33bcb3044dfd3

Mice lacking the nuclear bile acid receptor FXR/BAR developed normally and were outwardly identical to wild-type littermates. FXR/BAR null mice were distinguished from wild-type mice by elevated serum bile acid, cholesterol, and triglycerides, increased hepatic cholesterol and triglycerides, and a proatherogenic serum lipoprotein profile. FXR/BAR null mice also had reduced bile acid pools and reduced fecal bile acid excretion due to decreased expression of the major hepatic canalicular bile acid transport protein. Bile acid repression and induction of cholesterol 7[alpha]-hydroxylase and the ileal bile acid binding protein, respectively, did not occur in FXR/BAR null mice, establishing the regulatory role of FXR/BAR for the expression of these genes in vivo. These data demonstrate that FXR/BAR is critical for bile acid and lipid homeostasis by virtue of its role as an intracellular bile acid sensor.

-glutamate, the principal excitatory transmitter in the brain, gates ion channels mediating fast neurotransmission. Subunit components of two related classes of glutamate receptor channels have been characterized by cDNA cloning and shown to carry either an arginine or a glutamine residue in a defined position of their putative channel-forming segment. The arginine residue in this segment profoundly alters, and dominates, the properties of ion flow, as demonstrated for one channel class. We now show that the genomic DNA sequences encoding the particular channel segment of all subunits harbor a glutamine codon (CAG), even though an arginine codon (CGG) is found in mRNAs of three subunits. Multiple genes and alternative exons were excluded as sources for the arginine codon; hence, we propose that transcripts for three subunits are altered by RNA editing. This process apparently edits subunit transcripts of the two glutamate receptor classes with different efficiency and selectivity.


Functional maturation of B lymphocytes correlates with expression of the B lineage-specific cell surface glycoprotein CD22. Two CD22 polypeptides have been characterized and suggested to play a role in B cell-B cell interaction as well as in B cell adhesion to monocytes. In this work we provide evidence that CD22 is directly involved in the cognate interaction between B and T cells. One of the two CD22 polypeptides, CD22[beta], interacts with a specific ligand on a subpopulation of CD4+ T cells. Our results suggest that the T cell ligand of CD22 is CD45RO, an isoform of the leukocyte common antigen class of phosphotyrosine phosphatases associated with the helper T cell phenotype. We further demonstrate that CD22 recognizes a second ligand, CD75, expressed predominantly on activated B cells and shown to be a cell surface [alpha]2-6 sialyltransferase.


Hereditary major histocompatibility complex (MHC) class II deficiency (or bare lymphocyte syndrome) is a form of severe primary immunodeficiency with a total lack of MHC class II expression. It is due to a defect in the regulation of MHC class II genes. A novel gene was isolated by complementation cloning, using an MHC class II-negative mutant cell line. This gene (CIITA) functions as a transactivator of MHC class II gene expression and restores expression of all MHC class II isotypes in mutant cells. In addition, CIITA fully corrects the MHC class II regulatory defect of cells from patients with bare lymphocyte syndrome. In this disease we have identified a splicing mutation that results in a 24 amino acid deletion in CIITA, resulting in loss of function of the transactivator. Hence, the CIITA gene is essential for MHC class II gene expression and has been shown to be responsible for hereditary MHC class II deficiency.
mRNA capping requires the sequential action of three enzymatic activities: RNA triphosphatase, guanylyltransferase, and methyltransferase. Here we characterize a gene (CEL-1) believed to encode the C. elegans capping enzyme. CEL-1 has a C-terminal domain containing motifs found in yeast and vaccinia virus capping enzyme guanylyltransferases. The N-terminal domain of CEL-1 has RNA triphosphatase activity. Surprisingly, this domain does not resemble the vaccinia virus capping enzyme but does have significant sequence similarity to the protein tyrosine phosphatase (PTP) enzyme family. However, CEL-1 has no detectable PTP activity. The mechanism of the RNA triphosphatase is similar to that of PTPs: the active site contains a conserved nucleophilic cysteine required for activity. These results broaden the superfamily of PTP-like phosphatases to include enzymes with RNA substrates.

Mouse kif5B gene was disrupted by homologous recombination. kif5B-/- mice were embryonic lethal with a severe growth retardation at 9.5-11.5 days postcoitum. To analyze the significance of this conventional kinesin heavy chain in organelle transport, we studied the distribution of major organelles in the extraembryonic cells. The null mutant cells impaired lysosomal dispersion, while brefeldin A could normally induce the breakdown of their Golgi apparatus. More prominently, their mitochondria abnormally clustered in the perinuclear region. This mitochondrial phenotype was reversed by an exogeneous expression of KIF5B, and a subcellular fractionation revealed that KIF5B is associated with mitochondria. These data collectively indicate that kinesin is essential for mitochondrial and lysosomal dispersion rather than for the Golgi-to-ER traffic in these cells.

The ob gene product, leptin, is an important circulating signal for the regulation of body weight. To identify high affinity leptin-binding sites, we generated a series of leptin-alkaline phosphatase (AP) fusion proteins as well as [125I]leptin. After a binding survey of cell lines and tissues, we identified leptin-binding sites in the mouse choroid plexus. A cDNA expression library was prepared from mouse choroid plexus and screened with a leptin-AP fusion protein to identify a leptin receptor (OB-R). OB-R is a single membrane-spanning receptor most related to the gp130 signal-transducing component of the IL-6 receptor, the G-CSF receptor, and the LIF receptor. OB-R mRNA is expressed not only in choroid plexus, but also in several other tissues, including hypothalamus. Genetic mapping of the gene encoding OB-R shows that it is within the 5.1 cM interval of mouse chromosome 4 that contains the db locus.
Familial Mediterranean fever (FMF) is a recessively inherited disorder characterized by dramatic episodes of fever and serosal inflammation. This report describes the cloning of the gene likely to cause FMF from a 115-kb candidate interval on chromosome 16p. Three different missense mutations were identified in affected individuals, but not in normals. Haplotype and mutational analyses disclosed ancestral relationships among carrier chromosomes in populations that have been separated for centuries. The novel gene encodes a 3.7-kb transcript that is almost exclusively expressed in granulocytes. The predicted protein, pyrin, is a member of a family of nuclear factors homologous to the Ro52 autoantigen. The cloning of the FMF gene promises to shed light on the regulation of acute inflammatory responses.

The expression of H. influenzae fimbriae is subject to reversible phase variation between three expression levels. This phenomenon is controlled at the transcriptional level of two divergently orientated genes, hifA and hifB, encoding the major fimbrial subunit and the fimbrial chaperone, respectively. The hifA and hifB promoter regions were found to be clustered through an almost complete divergent overlap with a variable DNA backbone of repetitive TA units. Variation in the number of units changes the normally strictly constrained spacing between the -35 and -10 sequences and controls the bidirectional transcription initiation, thus forming a novel mechanism directing multiple gene transcription.

Mo-MLV infection of E[mu]-myc transgenic mice results in a dramatic acceleration of pre-B cell lymphomagenesis. We have used provirus tagging to identify genes that cooperate with the E[mu]-myc transgene in B cell transformation. Here we report on the identification of four loci, pim-1, bmi-1, pal-1, and bla-1, which are occupied by proviruses in 35%, 35%, 28%, and 14% of the tumors, respectively. bmi-1, pal-1, and bla-1 represent novel common proviral insertion sites. The bmi-1 gene encodes a 324 amino acid protein with a predominantly nuclear localization. bmi-1 is highly conserved in evolution and contains several motifs frequently found in transcriptional regulators, including a new putative zinc finger motif. No genes have yet been assigned to pal-1 and bla-1. The distribution of proviruses over the four common insertion sites suggests that provirus tagging can be used not only to identify the cooperating oncogenes but also to assign these genes to distinct complementation groups in tumorigenesis.
Smad proteins transmit TGF[beta] signals from the cell surface to the nucleus. Here we analyze Smad2 mutant embryos created using ES cell technology. Smad2 function is not required for mesoderm production per se, but, rather unexpectedly, in the absence of Smad2 the entire epiblast adopts a mesodermal fate giving rise to a normal yolk sac and fetal blood cells. In contrast, Smad2 mutants entirely lack tissues of the embryonic germ layers. Smad2 signals serve to restrict the site of primitive streak formation and establish anterior-posterior identity within the epiblast. Chimera experiments demonstrate these essential activities are contributed by the extraembryonic tissues. Thus, the extraembryonic tissues play critical roles in establishing the body plan during early mouse development.

Protozoan parasites of the genus Giardia are one of the earliest lineages of eukaryotic cells. To initiate infection, trophozoites emerge from a cyst in the host. Excystation is blocked by specific cysteine protease inhibitors. Using a biotinylated inhibitor, the target protease was identified and its corresponding gene cloned. The protease was localized to vesicles that release their contents just prior to excystation. The Giardia protease is the earliest known branch of the cathepsin B family. Its phylogeny confirms that the cathepsin B lineage evolved in primitive eukaryotic cells, prior to the divergence of plant and animal kingdoms, and underscores the diversity of cellular functions that this enzyme family facilitates.

We have completed a long-range restriction map of the terminal region of the short arm of human chromosome 16 (16p13.3) by physically linking a distal genetic locus ([alpha]-globin) with two recently isolated probes to telomere-associated repeats (TelBam3.4 and TelBam11). Comparison of 47 chromosomes has revealed major polymorphic length variation in this region: we have identified three alleles in which the [alpha]-globin genes lie 170 kb, 350 kb, or 430 kb from the telomere. The two most common alleles contain different terminal segments, starting 145 kb distal to the [alpha]-globin genes. Beyond this boundary these alleles are non-homologous, yet each contains sequences related to other (different) chromosome termini. This chromosome size polymorphism has probably arisen by occasional exchanges between the subtelomeric regions of nonhomologous chromosomes; analogous length variation is likely to be present at other human telomeres.

http://www.sciencedirect.com/science/article/B6WSN-41GP6VJ-6/2/b39a3b1a0bd33a5d927bff934da0a3ed

MALT B cell lymphomas with t(1;14)(p22;q32) showed a recurrent breakpoint upstream of the promoter of a novel gene, Bcl10. Bcl10 is a cellular homolog of the equine herpesvirus-2 E10 gene: both contain an amino-terminal caspase recruitment domain (CARD) homologous to that found in several apoptotic molecules. Bcl10 and E10 activated NF-\([\text{kappa}]\) B but caused apoptosis of 293 cells. Bcl10 expressed in a MALT lymphoma exhibited a frameshift mutation resulting in truncation distal to the CARD. Truncated Bcl10 activated NF-\([\text{kappa}]\) B but did not induce apoptosis. Wild-type Bcl10 suppressed transformation, whereas mutant forms had lost this activity and displayed gain-of-function transforming activity. Similar mutations were detected in other tumor types, indicating that Bcl10 may be commonly involved in the pathogenesis of human malignancy.


http://www.sciencedirect.com/science/article/B6WSN-41BD8T6-B/2/48d64b85589a7c51c5dd3cc50bf5ca43

A 62 kDa protein is highly phosphorylated in many cells containing activated tyrosine kinases. This protein, characterized mainly by its avid association with rasGAP, has proved elusive. Anti-phosphotyrosine antibody was used to purify p62. From peptide sequence, molecular cloning revealed a cDNA encoding a novel protein, p62dok, with little homology to others but with a prominent set of tyrosines and nearby sequences suggestive of SH2 binding sites. In cells, v-Abl tyrosine kinase binds and strongly phosphorylates p62dok, which then binds rasGAP. A monoclonal antibody, 2C4, to the rasGAP-associated p62 reacts with p62dok. Thus, p62dok appears to be the long-sought major substrate of many tyrosine kinases.

Cell Calcium  (5)


http://www.sciencedirect.com/science/article/B6WCC-487KHN4-7/2/077e00bd317f0c045b854bf18d27090

Ca2+ homeostasis mechanisms, in which the Ca2+ entry pathways play a key role, are critically involved in both normal function and cancerous transformation of prostate epithelial cells. Here, using the lymph node carcinoma of the prostate (LNCaP) cell line as a major experimental model, we characterize prostate-specific store-operated Ca2+ channels (SOCs)--a primary Ca2+ entry pathway for non-excitable cells--for the first time. We show that prostate-specific SOCs share major store-dependent, kinetic, permeation, inwardly rectifying, and pharmacological (including dual, potentiation/inhibition concentration-dependent sensitivity to 2-APB) properties with
"classical" Ca2+ release-activated Ca2+ channels (CRAC), but have a higher single channel conductance (3.2 and 12 pS in Ca2+- and Na+-permeable modes, respectively). They are subject to feedback inhibition via Ca2+-dependent PKC, CaMK-II and CaM regulatory pathways and are functionally dependent on caveolae integrity. Caveolae also provide a scaffold for spatial co-localization of SOCs with volume-regulated anion channels (VRAC) and their Ca2+-mediated interaction. The TRPC1 and TRPV6 members of the transient receptor potential (TRP) channel family are the most likely molecular candidates for the formation of prostate-specific endogenous SOCs. Differentiation of LNCaP cells to an androgen-insensitive, apoptotic-resistant neuroendocrine phenotype downregulates SOC current. We conclude that prostate-specific SOCs are important determinants in the transition to androgen-independent prostate cancer.


http://www.sciencedirect.com/science/article/B6WCC-4CPDG1S-3/2/b268108754a5fa2499243f6f9d5cb

In blood vessels, the ability to control vascular tone depends on extracellular calcium entry and the release of calcium from inositol 1,4,5-trisphosphate receptor (IP3R)-gated stores located in both the endothelial and smooth muscle cells of the vascular wall. Therefore, we examined mRNA expression and protein distribution of IP3R subtypes in intact aorta, basilar and mesenteric arteries of the rat. IP3R1 mRNA was predominantly expressed in all three arteries. Immunohistochemistry showed that IP3R1 was present in both the muscle and endothelial cell layers, while IP3R2 and IP3R3 were largely restricted to the endothelium. Weak expression of IP3R2 was observed in the smooth muscle of the basilar artery. Co-localisation studies of IP3R subtypes with known cellular elements showed no association of any of the three subtypes with the endothelial cell plasma membrane, but a close association between the subtypes and actin filaments was observed in all cell layers. IP3R2 was found to be present near the endothelial cell nucleus. We are the first to demonstrate differential IP3R subtype distribution between the cell layers of the intact vascular wall and hypothesise that this may underlie the diversity of IP3R-dependent responses, such as vasoconstriction, vasodilation and vasomotion, displayed by arteries.


http://www.sciencedirect.com/science/article/B6WCC-468TDB1-3/2/5c5bcf3ed3ca0c3ec9484c274fbcf1a590

Calsequestrin (CSQ) is the major Ca2+ binding protein of the cardiac sarcoplasmic reticulum (SR). Transgenic mice overexpressing CSQ at the age of 7 weeks exhibit concentric cardiac hypertrophy, and by 13 weeks the condition progresses to dilated cardiomyopathy. The present study used a differential display analysis to identify genes whose expressions are modulated in the CSQ-overexpressing mouse hearts to provide information on the mechanism of transition from concentric cardiac hypertrophy to failure. Cardiac ankyrin repeat protein (CARP), glutathione peroxidase (Gpx1), and genes which participate in the formation of extracellular matrix including decorin, TSC-36, Magp2, Osf2, and SPARC are upregulated in CSQ mouse hearts at 7 and 13 weeks of age compared to those of non-transgenic littermates. In addition, two novel genes without sequence similarities to any known genes are upregulated in CSQ-overexpressing mouse hearts. Several genes are downregulated at 13 weeks, including SR Ca2+-ATPase (SERCA2) and adenine nucleotide translocase 1 (Ant1) genes. Further, a functionally yet unknown gene
(NM_026586) previously identified in the mouse wolffian duct is dramatically downregulated in CSQ mice with dilated hearts. Thus, CARP, Gpx1, and genes encoding extracellular matrix proteins may participate in the development of cardiac hypertrophy and fibrosis, and changes in SERCA2, Ant1, and NM_026586 mRNA expression may be involved in transition from concentric to dilated cardiac hypertrophy.


http://www.sciencedirect.com/science/article/B6WCC-4C83495-4J/2/87f594ac240575957befe0e1f26be9be

It has been long known that neoplastic transformation is accompanied by a lowered requirement for extracellular Ca2+ for growth. The studies presented here demonstrate that human fibroblastic cell lines produce the two commonly found 'housekeeping' isoforms of the plasma membrane Ca2+-ATPase (PMCA), PMCA1 b and 4b, and that the expression of both is demonstrably lower in cell lines neoplastically transformed by SV40 than in the corresponding parental cell lines. Western blot analyses of lysates from control (GM00037) and SV40-transformed (GM00637) skin fibroblasts revealed a 138 kDa PMCA whose level was significantly lower in the SV40-transformed cells relative to either total cellular protein or [alpha]-tubulin. Similar analyses of plasma membrane preparations from control (WI-38) and SV40-transformed (WI-38VA13) lung fibroblasts revealed 3-4-fold lower levels of PMCA in the SV40-transformed cells. Competitive ELISAs performed on detergent solubilized plasma membrane preparations indicated at least 3-4-fold lower levels of PMCA in the SV40-transformed cell lines compared to controls. Reverse transcriptase coupled-PCR analyses showed that PMCA1 b and PMCA4b were the only isoforms expressed in all four cell lines. The PMCA4b mRNA level detected by Northern analysis was also substantially lower in SV40 transformed skin fibroblasts than in non-transformed fibroblasts. Quantitative RT-PCR analyses showed levels of PMCA1 b and 4b mRNAs to be 5 and 10-fold lower, respectively, in GM00637 than in GM00037 when the levels of PCR products were normalized to glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA. These results demonstrate that the expression of these distinct PMCA genes is substantially lower in SV40 transformed human skin and lung fibroblasts and may be coordinately regulated in these cells.


http://www.sciencedirect.com/science/article/B6WCC-4C834C3-5K/2/a813c84524cb8ff4f121b7f4af07171

Microfluorometric measurements in Fura-2-loaded single cultured human vascular endothelial cells were used to characterize the intracellular calcium [Ca2+]i responses triggered by extracellular application of adenosine 5'-triphosphate (ATP) and other nucleotides. Application of ATP or uridine 5'-triphosphate (UTP) gave rise to dose-dependent elevations of [Ca22+]i in all the cells tested. At saturating concentrations of agonist, the [Ca2+]i response was biphasic, with an early peak and a sustained plateau. Unlike peak responses, the sustained Ca2+ plateau was sensitive to removal of Ca2+ from the external medium. Mn2+ quenching revealed the presence of Ca2+ influx during the agonist-induced calcium plateau. The agonist-evoked calcium plateau was inhibited in a dose-dependent manner by the Cl-channel blocker NPPB, by the divalent cation Ni2+ and by the imidazole antmycotic econazole. Previously, these compounds have been shown to block store-operated Ca2+ entry. The two phases of the agonist-evoked [Ca2+]i response were blocked by the specific phospholipase C inhibitor U-73122 and by intracellular injection of low molecular weight heparin, suggesting the involvement of IP3 sensitive intracellular
Ca2+ stores. The pharmacological profile of the response, using different nucleotides and analogues, ATP = UTP > ADP = UDP, and no responses to P2x1 and P2Y1 agonists, suggested the involvement of P2Y2 receptors. The expression of mRNA for the P2Y2 receptor was detected by RT-PCR analysis. These results indicate that P2Y2 receptors linked to intracellular Ca2+ mobilization are present in human vascular endothelial cells. The initial [Ca2+]i mobilization is followed by a phase of elevated [Ca2+]i influx.

Cell Transplantation  (2)


Expression of a fluorescent reporter gene has been studied using two alternate promoters to transcribe the green fluorescent protein gfp from Aequorea victoria. The human cytomegalovirus (CMV) enhancer/promoter or the human muscle-specific creatine kinase promoter (CKM) were inserted along with the gfp cDNA into a plasmid expression vector based on a modified adenoassociated virus genome. Naked plasmid DNA was injected into the hamstring muscle of mdx mice and gfp gene expression determined from frozen muscle sections taken at 4, 14, and 42 days postinjection. Fluorescence patterns obtained by photomicroscopy and quantitative fluorescence measurements indicated a near-linear increase in the accumulation of the gfp in skeletal muscle during the length of the study, with gfp expression at 42 days being roughly four times the values obtained at 4 days. The levels of expression of gfp from the CKM construct were consistently higher than for the CMV construct. The CKM promoter/expression vector combination demonstrates significant potential for simple, direct delivery and long-term, high-level expression of genes in skeletal muscle.


A future possibility for treatment of genetic diseases may be gene therapy using autologous cord blood (CB) stem/progenitor cells. This might require cryopreservation of CB stem/progenitor cells prior to purification, gene transduction, and ex vivo expansion of cells. To address this possibility, nonadherent low density T-lymphocyte depleted (NALT-) cells from fresh or cryopreserved cord blood were sorted for CD34+++ phenotype, transduced with a recombinant retroviral vector encoding Fanconi anemia complementation C (FACC) gene, and cells expanded ex vivo in suspension culture for 7 days with growth factors. The results demonstrate: 1) high recovery of viable cells after thawing; 2) high efficiency purification of CD34+++ cells from NALT- cells prior to and after cryopreservation; 3) high degree of expansion of nucleated cells and immature
progenitors from CD34+++ cells before and after cryopreservation; 4) efficient transduction with stable integration and expression of newly introduced genes in cryopreserved and then sorted stem/progenitor cells, as detected prior to and after ex vivo expansion; and 5) high efficiency transduction of single isolated CD34+++ cells obtained from cryopreserved NALT-CB. This information should be of value for future studies evaluating the use of cryopreserved cord blood for gene transfer/gene therapy.

**Cellular Immunology** (8)


Coccidioides posadasii is a soil fungus that causes coccidioidomycosis or Valley Fever in the endemic regions of the southwestern US and Central America. Persons with decreased T cell reactivity and immune deficiency are at increased risk of developing severe disseminated infection. Among different mouse strains, DBA/2 mice are relatively resistant to C. posadasii whereas BALB/c mice are highly susceptible, and this discrepancy has been attributed to the difference in the development and expression of their Th1 cellular response. Dendritic cells (DC) are the most potent antigen-presenting cells that are activated after taking up pathogens or pathogens-derived antigens and regulate the immune response in the host, including Th1 cellular response. However, the DC responses against C. posadasii are not characterized. In the present study, we cultured bone-marrow derived DC (BMDC) from BALB/c and DBA/2 mice and infected with C. posadasii arthroconidia. The activation of BMDC was characterized by studying expression of cell surface co-stimulatory molecules (CD11c, MHC class II, CD40, CD80, and CD86), expression of genes encoding Toll-like receptors and release of IL-12. We found that the BMDC from DBA/2 mice showed significant upregulation of Toll-like receptor-2 and 4 genes expression, secretion of IL-12 (p C. posadasii.


http://www.sciencedirect.com/science/article/B6WCF-49WMTYD-1/2/c8537d4e00f19e85b8547847caf2e5d3

Investigation into the mechanism of action of vaccine adjuvants provides opportunities to define basic immune principles underlying the induction of strong immune responses and insights useful for the rational development of subunit vaccines. A novel HIV vaccine composed of plasmid DNA-encoding p55 gag formulated with poly-lactide-co-glycolide microparticles (PLG) and cetyl trimethyl ammonium bromide (CTAB) elicits both serum antibody titers and cytotoxic lymphocyte activity in mice at doses two orders of magnitude lower than those required for comparable response to plasmid DNA in saline. Using this model, we demonstrated the increase in potency requires the DNA to be complexed to the PLG-CTAB microparticles. Furthermore, the PLG-CTAB-DNA formulation increased the persistence of DNA at the injection site, recruited mononuclear phagocytes to the site of injection, and activated a population of antigen presenting
cells. Intramuscular immunization with the PLG-CTAB-DNA complex induced antigen expression at both the injection site and the draining lymph node. These findings demonstrate that the PLG-CTAB-DNA formulation exhibits multiple mechanisms of immunopotentiation.


http://www.sciencedirect.com/science/article/B6WCF-473FVF8-2/2/b2085c9be8bc9f18f9d0be1458e6aae5f

In a search for novel early T cell activation transcripts, we identified expressed sequence tags (ESTs) more abundantly expressed in normal human CD4+ T lymphocytes fully activated by a 5 h exposure to CD3 plus CD28 mAbs, compared to the same cells stimulated with either CD3 mAb or CD28 mAb alone. An EST was identified that hybridized with a 1.7 kb transcript expressed in activated T cells but was undetectable by Northern blot analysis in resting T cells or other normal tissues. The T cell transcript was maximally induced within 6 h and remained elevated for at least 47 h. Induction of the transcript was blocked by cyclosporin A, FK506, and dexamethasone but not by rapamycin. The transcript was polyadenylated but lacked an open reading. A BLAST search of the NCBI database revealed that the transcript shared identity with the recently reported human BIC proto-oncogene that encodes a noncoding mRNA (W. Tam, Gene 274 (2001) 157). Our data demonstrate that transcriptional activation of the BIC proto-oncogene is an early and sustained T cell activation event and suggest an important role for noncoding mRNA in T cell function.


http://www.sciencedirect.com/science/article/B6WCF-48063T1-2/2/52ca6a9dc906daa20236a90c7c35b51510

Lactoferrin, a glycoprotein present in milk, mucosal secretions and neutrophils contributes to host defense. We have previously shown that orally given milk lactoferrin (LF) mediates anti-infectious and anti-inflammatory activities in vivo. Moreover, we have shown that LF could inhibit the LPS-induced IL-6 secretion in a human monocyctic cell line, THP-1. This observation was expanded in the present study investigating the capacity of LF to inhibit cytokine mRNA expression and the involvement of nuclear transcription factor kappa B (NF-[kappa]B). Cells (THP-1 and Mono Mac 6 monocyctic cell lines) were stimulated with Escherichia coli LPS (5-10 ng/106 cells) and LF was added (50-500 [mu]g/106 cells) 30 min before, or after the LPS addition. By a semiquantitative RT-PCR lower levels of TNF-[alpha], IL-1[beta], IL-6, and IL-8 mRNA expression were detected at the peak of the expression in THP-1 cells treated with LF. The reduction in the cytokine expression was followed by a similar reduction in the secreted cytokines as analyzed by ELISA. LF down-regulated also the IL-10 secretion (detected only in LPS-stimulated Mono Mac 6 cells). A similar level of inhibition of these cytokines was detected regardless of the time at which LF was added to the cells in relation to LPS. In addition, LF was internalized into cells and detected in the nucleoli as determined by immunostaining and immunofluorescence. Moreover, by electrophoretic mobility shift assay (EMSA) analysis LF decreased the LPS-induced binding of NF-[kappa]B to the TNF-[alpha] promoter. The results show that LF down-regulates the LPS-induced cytokine production in monocyctic cells. The inhibitory mechanism is suggested to involve the interference of LF with NF-[kappa]B activation.
Iwamoto, S., M. Ishida, et al. "A human Langerhans cell-like cell line, ELD-1, promotes CD8 T cells to produce IFN-\(\gamma\) through CD70-dependent alternative pathway." Cellular Immunology In Press, Corrected Proof http://www.sciencedirect.com/science/article/B6WCF-4FRHD9N-1/2/7e61a6280b56bcaffad180e6f80753dc

A novel pathway of CD8+ T-cell activation by a previously established human Langerhans cell (LC)-like cell line, ELD-1 [Dendritic Cells 9 (1999) 41] is reported. ELD-1 cells possess LC-specific and dendritic cell (DC) lineage-specific markers including Birbeck granules. Intriguingly, ELD-1 cells stimulated interferon (IFN)-\(\gamma\) production by purified allogeneic CD8+ T cells in an IL-2- but not IL-12-dependent manner, but failed to stimulate CD4+ T cells due to their lack of HLA-DR, CD40, CD80, and CD86 expression. Comparing active and inactive subclones of ELD-1 cells revealed that CD70 was a key molecule determining stimulatory ability. This was confirmed by the ability of transfected CD70-encoding cDNA to confer stimulatory capacity on inactive subclones of ELD-1. Therefore, it is concluded that CD70 expressed on ELD-1 cells has a crucial role in stimulating IFN-\(\gamma\) production by CD8+ T cells through an alternative pathway which does not require CD4+ T-cell help or CD28-B7 interactions.


http://www.sciencedirect.com/science/article/B6WCF-4DKSWY1-C7/2/260cd5411728e0a060fcdf31ba6b0b49

Although murine peritoneal B cells were homogenously positive for an epitope Lp-2, coded for by the alternative exon 4 of the CD45 gene, they were heterogenous with respect to the expression of another CD45R epitope, 6B2, of unknown exon dependency. While the majority of 6B2-high peritoneal B cells was composed of CD5- B cells, those with low or negative 6B2 were CD5+ B cells. Both 6B2+ and 6B2- peritoneal B cells expressed mainly the same largest CD45R transcripts, with all three alternative exon (4, 5, and 6) sequences. Further, a CD5+ B lymphoma cell line, BCL-1, which was found to be Lp-2+6B2- also had the largest isoform of CD45R molecules with all three alternate structures. Although enzyme digestion studies suggested that the 6B2 epitope resides in protein, not in sugar structures, it is likely that a post-translational modification of CD45R molecules is responsible for the presence or absence of 6B2 epitope expression on peritoneal CD5+ B cells. This event may be related to the differential role of CD45R molecules in regulating lymphocyte function.


http://www.sciencedirect.com/science/article/B6WCF-4F0KJ94-2/2/16a796b06388f7a13ea2f2dad3187cd27

The chemokine receptor CX3CR1 is thought to regulate inflammation in part by modulating NK cell adhesion, migration, and killing in response to its ligand CX3CL1 (fractalkine). Recent reports indicate that IL-15, which is essential for development and survival of NK cells, may negatively regulate CX3CR1 expression, however, the effects of the cytokine on human NK cell CX3CR1 expression and function have not been fully delineated. Here, we demonstrate that short term culture in IL-15 decreases surface expression of CX3CR1 on cultured CD56+ cells from human
blood resulting in diminished chemotaxis and calcium flux in response to CX3CL1. Cells cultured long term in IL-15 (more than five days) completely lost surface expression as well as mRNA and protein for CX3CR1. The effect was specific since mRNA for CCR5 was increased and mRNA for CXCR4 was unchanged in these cells by IL-15. Thus, exogenous IL-15 is a negative regulator of CX3CR1 expression and function in human CD56+ NK cells. The data imply that the use of IL-15 alone to expand NK cells ex vivo for immunotherapy may produce cells impaired in their ability to traffic to sites of inflammation.


http://www.sciencedirect.com/science/article/B6WCF-471VF7J-12/2/699d2488c4608f5c1dffd217e9afff1f

In vitro 5-day cultures of naive spleen cells with viable Listeria monocytogenes (VLM), but not heat-killed *L. monocytogenes*, induced CD4+ T cells that produced IFN-[gamma] upon secondary antigen stimulation. The VLM-induced Listeria-specific T cells produced IFN-[gamma] but lacked expression of IL-2 and IL-4. To study the role of IFN-[gamma] in the induction of the IFN-[gamma]-producing T cells, we added anti-IFN-[gamma] mAb to the primary culture and analyzed IFN-[gamma] production upon secondary antigen stimulation. Addition of anti-IFN-[gamma] mAb to the culture suppressed generation of IFN-[gamma]-producing CD4+ T cells, suggesting that IFN-[gamma] is important in the induction of IFN-[gamma]-producing CD4+ T cells. Furthermore, our results showed that depletion of NK cells from spleen cells by anti-asialo GM1 antibody plus complement before culture enhanced induction of IFN-[gamma]-producing CD4+ T cells. Although NK cells are known to produce IFN-[gamma], the results indicate that NK cell-derived IFN-[gamma] may not be important in induction of the Listeria-specific IFN-[gamma]-producing CD4+ T cells in the culture system. In addition, we demonstrated that IFN-[gamma] expression was high in CD4+ T cells from cultures of spleen cells with VLM at the primary culture level. These results suggest that IFN-[gamma] derived from T cells may enhance production of IFN-[gamma] by CD4+ T cells, while NK cells rather suppress the induction of IFN-[gamma]-producing CD4+ T cells.

**Cellular Signalling** (8)


http://www.sciencedirect.com/science/article/B6T2M-4F8TKK-3/2/e67a784a06fe21d076165f6c914235db

Sphingosine-1-phosphate, a lipid mediator produced by sphingosine kinases, regulates diverse cellular processes, ranging from cell growth and survival to effector functions, such as proinflammatory mediator synthesis. Using human A549 epithelial lung carcinoma cells as a model system, we observed transient upregulation of sphingosine kinase type 1 (SPHK1) enzyme activity upon stimulation with both TNF-[alpha] or IL-1[beta]. This transient activation of SPHK1 was found to be required for cytokine-induced COX-2 transcription and PGE2 production, since
not only specific siRNA (abolishing both basal and induced SPHK1 enzyme activity), but also a dominant-negative SPHK1 mutant (suppressing induced SPHK1 activity only) both reduced COX-2 and PGE2. Furthermore, TNF-[alpha]- or IL-1[beta]-induced transcription of selected cytokines, chemokines, and adhesion molecules (IL-6, RANTES, MCP-1, and VCAM-1) was found to require SPHK1 activation. Suppression of SPHK1 activation led to reduction of cytokine-induced \([\kappa]\text{B}[\alpha]\) phosphorylation and consequently diminished NF[\kappa][\gamma]B activity due to reduced nuclear translocation of RelA (p65), explaining the dependence of inflammatory mediator production on SPHK1 activation. Inhibition of basal SPHK1 activity by N,N-dimethylsphingosine or by downregulation of its expression using siRNA induced spontaneous apoptosis in A549 cells, an effect that can be explained through interference with constitutive NF[\kappa][\gamma]B activity in this cell type. In contrast, expression of the dominant-negative mutant did not induce apoptosis. Taken together, these findings demonstrate a role of SPHK1 activation in proinflammatory signalling and of SPHK1 basal activity in survival of A549 lung carcinoma cells.


http://www.sciencedirect.com/science/article/B6T2M-3RD0TT72-8/2/b8307f117d577eefc46f17367340fa1a5

S49 cells expressed type 2 somatostatin receptors (sstr2) by immunoblotting. Analysis by reverse transcription and polymerase chain reaction (RT-PCR) methodologies showed that S49 cells express predominantly sstr2A and sstr2B mRNAs; other subtypes were either not detected, in the case of sstr1, sstr3, sstr4, or variably detected, in the case of sstr5. No mutations were present in S49 cells at codon 12, 13, or 61 of the N-, K-, or H-ras genes. Nevertheless, randomly growing S49 cells contained Raf-1 activity by specific immune complex kinase assays. Treatment of S49 cells with somatostatin transiently inactivated the basal activity of Raf-1, but not that of B-Raf. Addition of somatostatin plus guanyl-5'-yl imidodiphosphate (GMPPNP) to S49 membranes stimulated PTPase activity. The concentration dependence for stimulation of PTPase activity correlated with high affinity binding of [125I-Tyr11]somatostatin-14. Both the effect of somatostatin to stimulate PTPase activity and to inactivate Raf-1 were abrogated by PTx. PTPase activity stimulated by somatostatin plus GMPPNP was recovered in a peak of high apparent Mr (670,000) after solubilisation with Triton X-100 and Superose 6 chromatography. Furthermore, addition of activated, brain G[alpha]i/o subunits to fractions from control membranes stimulated PTPase activity in the high Mr peak. Thus, S49 membranes contain a G-protein regulated PTPase (PTPase-G), and PTPase-G in these cells may reside in a high molecular weight complex.


http://www.sciencedirect.com/science/article/B6T2M-44YNJNN-3/2/47d4ccf2013aa7a89e5250aa5dfb5d5

Lipopolysaccharide (LPS) induces a delayed release (lag phase of 2-4 h) of arachidonic acid (AA) and prostaglandin (PG) D2 in rat liver macrophages. Group IV cytosolic phospholipase A2 (cPLA2) becomes phosphorylated within minutes after the addition of LPS. The phosphorylated form of cPLA2 shows an enhanced in vitro activity. The Ca2+ dependence of cPLA2 activity is not affected by phosphorylation of the enzyme. In addition, LPS induces an enhanced expression of cPLA2 mRNA (after 2-4 h) and an enhanced expression of cPLA2 protein (after 8 h). The cellular
cPLA2 activity is enhanced about twofold 24 h after LPS treatment. Liver macrophages constitutively express mRNAs encoding Groups V and IIA secretory PLA2 (sPLA2). LPS has no effect on the levels of Groups V and IIA sPLA2 mRNA expression. Despite mRNA expression, Groups V and IIA sPLA2 protein and sPLA2 activity are not detectable in unstimulated or LPS-stimulated liver macrophages. Collectively, these and earlier [Mediators Inflammation 8 (1999) 295.] results suggest that in liver macrophages the LPS-induced delayed release of AA and prostanoids is mediated by phosphorylation and an enhanced expression of cPLA2, a de novo expression of cyclooxygenase (COX)-2, but not by the actions of Group V or Group IIA sPLA2.


http://www.sciencedirect.com/science/article/B6T2M-45TS5Y3-4/2/97d2301fd3edc1036bad353186cc3c2d

Activation regulates the responsiveness of G-protein-coupled receptors (GPCRs) on T cells, and modifications in the activity of GPCRs characterize lymphocytes from some immune disorders such as multiple sclerosis (MS) and rheumatoid arthritis (RA). Some lines of evidence suggest that such an effect is connected with the altered expression of some GPCRs regulatory proteins. Herein we demonstrate that phytoeagglutinin (PHA)-induced activation leads to differential expression of G-protein-coupled receptor kinase (GRK) 2, GRK3, [beta]-arrestin-1, regulators of G-protein signalling (RGS) 2, and RGS16 and decreases responsiveness of mononuclear leukocytes (MNL) to the [beta]-adrenergic agonist isoproterenol. Interferon beta-1a (IFN[beta]-1a), which is known to ameliorate the course of MS, counteracts the activation-induced effects on the expression of these GPCR regulatory proteins in MNL. Furthermore, IFN[beta]-1a quenches the effects of PHA on the isoproterenol-induced accumulation of cyclic AMP (cAMP). We suggest that regulation of GPCRs responsiveness may be a relevant property of IFN[beta]-1a in MS.


http://www.sciencedirect.com/science/article/B6T2M-3X6SDJX-7/2/0dd29e9a57f5729118054de8194b0f2a

67% of total cAMP phosphodiesterase activity (PDE) in cultured rat hepatocytes could be detected in the cytosol, 15% in plasma membrane, 15% in 'dense vesicle,' and 3% in endoplasmatic reticulum fractions. Up to 84% of the PDE activity of the cytosol is represented by the rolipram-sensitive PDE 4. ICI 118233-inhibited PDE 3 was found predominantly in membranes. We were able to show that dexamethasone acts on the PDE 4 in cytosolic and plasma membrane fractions whereas glucagon effected the PDE 4 of the cytosol and the PDE 3 in 'dense vesicle' membranes. Primary culture of hepatocytes was used to study long-term effects of dexamethasone and glucagon on PDE 4 activity. Addition of dexamethasone (0.1 [mu]M) at the beginning of cultivation leads to a decrease of total PDE 4 activity whereas after 24 h precultivation no dexamethasone effect could be observed. Glucagon effects on PDE 4 were investigated in 20 h precultured hepatocytes. Maximal stimulation was achieved after 2 h of exposure. PDE 4 subtypes A, B, D and, to a lesser degree, subtype C could be detected by RT-PCR analysis. The results of semiquantitative RT-PCR show that the presence of dexamethasone during the first 24 h of cultivation reduced selectively the transcription of PDE 4D, whereas glucagon was without any effect. Also the translation of PDE 4D was reduced as shown in the Western blot. We would like to discuss the way that dexamethasone influences PDE
4D expression--most likely in combination with other factors such as cytokines--during the time of cell plating, whereas glucagon actions are part of metabolic regulations via phosphorylation reactions.


http://www.sciencedirect.com/science/article/B6T2M-47PGCMJ-8/2/bee22a6a229f3f7d93107470aed975da

Five high affinity G-protein-coupled receptors for sphingosine 1-phosphate (S1P) have been characterised so far (S1P1,2,3,4,5 formerly referred to as edg1,5,3,6,8). In this study, we show that S1P, dihydro-sphingosine 1-phosphate (dihydro-S1P) and dioleoylphosphatidic acid (doPA) are agonists for the orphan receptor GPR63. All three phospholipids mobilise intracellular calcium in CHO cells transiently transfected with GPR63. Calcium signals required cotransfection of a chimeric G[alpha]q/i protein in a fluorometric imaging plate reader (FLIPR(TM)) assay but did not require overexpressed G proteins in an aequorin assay, using a green fluorescent protein (GFP)-aequorin fusion protein as a bioluminescent Ca2+ reporter. GPR63 expression in CHO cells confers proliferative responses to S1P in a pertussis toxin (PTX)-insensitive manner. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) indicated highest expression in brain, especially in the thalamus and the nucleus caudatus. In peripheral tissues, highest expression was observed in thymus, stomach and small intestine; lower abundance of transcripts was detected in kidney, spleen, pancreas and heart. The discovery that S1P, dihydro-S1P and dioleoylphosphatidic acid activate GPR63 will facilitate the identification of agonists and antagonists, and help to unravel the biological function of this receptor.


http://www.sciencedirect.com/science/article/B6T2M-3WHKSRF-G/2/ceeb21c87f8c2aeae04404e1fe3849be

Members of the type 2 phosphatidic acid phosphatase (PAP2) family catalyse the dephosphorylation of phosphatidic acid (PA), lysophosphatidate and sphingosine 1-phosphate. Here, we demonstrate the presence of a Mg2+-independent and N-ethymaleimide-insensitive PAP2 activity in cultured guinea-pig airway smooth muscle (ASM) cells. Two PAP2 cDNAs of 923 and 926 base pairs were identified and subsequently cloned from these cells. The ORF of the 923 base pair cDNA encoded a protein of 285 amino acids (Mr = 32.1 kDa), which had 94% homology with human PAP2a (hPAP2a) and which probably represents a guinea-pig specific PAP2a (gpPAP2a1). The ORF of the 926 base pair cDNA encoded a protein of 286 amino acids (Mr = 32.1 kDa) which had 84% and 91% homology with hPAP2a and gpPAP2a1, respectively. This protein, termed gpPAP2a2, has two regions (aa 21-33 and 51-74) of marked divergence and altered hydrophobicity compared with hPAP2a and gpPAP2a1. This occurs in the predicted first and second transmembrane domains and at the extremes of the first outer loop. Other significant differences between gpPAP2a1/2 and hPAP2a, hPAP2b and hPAP2c occur at the cytoplasmic C-terminal. Transient expression of gpPAP2a2 in Cos-7 cells resulted in an approx. 4-fold increase in Mg2+-independent PAP activity, thereby confirming that gpPAP2a2 is another catalytically active member of an extended PAP2 family.
The cytoplasmic domain of the platelet-derived growth factor (PDGF) [beta]-receptor was expressed in insect cells by using a baculovirus system. The resulting protein was a constitutively active tyrosine kinase that could phosphorylate both protein and peptide substrates. A recently identified potent and selective inhibitor of intact PDGF receptor autophosphorylation, 3744W, inhibited the autophosphorylation of the cytoplasmic domain both in vitro (50 1.8 +/- 0.12 [mu]M) and within intact insect cells (50 2.0 [mu]M). However, under identical assay conditions, 3744W did not inhibit the phosphorylation of the synthetic polymeric peptide poly(Glu4Tyr1) even at concentrations as high as 100 [mu]M. These results suggest that, although 3744W inhibits PDGF receptor autophosphorylation directly, it can discriminate between phosphate acceptor substrates.

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**Chest (5)**


http://www.chestjournal.org/cgi/content/abstract/125/5/1843

Study objective: We investigated the gene expression profiles of malignant pleural mesothelioma (MPM) specimens to identify novel genes that are potentially involved in the oncogenic transformation of human pleural cells. Design: Complementary DNA (cDNA) microarray transcriptional profiling studies of 10 MPM cell lines and 4 MPM primary tumor specimens were performed using hierarchic clustering. To confirm microarray data, we used real-time polymerase chain reaction and immunoblotting. Results: Cluster analysis differentiated among epithelial (E), sarcomatoid, and biphasic MPM variants. Expression profiling identified common overexpressed or underexpressed genes in MPM. Notably, matriptase messenger RNA was found to be overexpressed by 826-fold in E MPM, with protein expression subsequently confirmed by immunoblot analysis. This recently characterized trypsin-like serine protease has been implicated in tumor invasion and metastasis of E-derived cancers, but has not been described until now in MPM. We also identified other novel genes, such as insulin-like growth factor binding protein 5 and a cDNA clone similar to proteolipid MAL2. Conclusions: Thus, further large-scale profiling of MPM may elucidate previously unrecognized molecular mechanisms by identifying novel genes that are involved in malignant transformation. Our study has now found matriptase to be one of these mesothelioma-associated genes, with potential pathogenic and therapeutic significance.

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http://www.chestjournal.org/cgi/content/abstract/125/1/63
Background: The finding that only 15 to 20% of cigarette smokers acquire COPD suggests that there is a genetic predisposition to the disease. Genetic polymorphism of the group-specific component of serum globulin (Gc-globulin), also known as vitamin-D-binding protein, is considered one of the candidates for the susceptibility to COPD. However, the role of Gc-globulin polymorphism in the development of COPD remains inconclusive. Study objectives: To determine whether Gc-globulin gene polymorphism plays a role in the development of COPD in the Japanese population, and whether it is associated with the physiologic deterioration in COPD, and its radiologically detectable correlates. Design: Association study. Subjects and methods: One hundred three patients with COPD and 88 healthy smokers sampled from the Japanese population were genotyped for Gc-globulin by the restriction fragment-length polymorphism method. Based on the results of the genotyping, we investigated the relationship between Gc-globulin polymorphism and a physiologic/radiologic indicator of lung function, namely, the annual decline of FEV1 (dFEV1) in 86 patients with COPD and 21 healthy smokers. Additionally, high-resolution CT parameters such as low-attenuation area percentage (LAA%) and average CT number (mean CT score) were measured in 85 patients with COPD. Results: There was an increased proportion of Gc*1F homozygotes in the patients with COPD (32%) compared with the healthy smokers (17%) \( p = 0.01; \) odds ratio, 2.3; 95% confidence interval, 1.2 to 4.6. Patients with COPD and the Gc*1F allele showed a larger dFEV1 \( (p = 0.01), \) higher frequency with LAA\% > 60% \( (p = 0.01), \) and lower mean CT score than patients without this allele \( (p = 0.03). \) Conclusion: Gc-globulin polymorphism is significantly associated with susceptibility to COPD, and also with the severity of the disease.


http://www.chestjournal.org/cgi/content/abstract/126/1/66

Study objective: To investigate the relationship of common single nucleotide polymorphisms (SNPs) of the {beta}2-adrenergic receptor (AR) gene at codons 16 and 27, and the intermediate phenotype of airways hyperresponsiveness. Design: A case-control study in 543 white men (152 case patients and 391 control subjects), who were nested in an ongoing longitudinal cohort. Setting: Subjects were selected from the Normative Aging Study, an ongoing longitudinal cohort of healthy aging. Participants: Case patients were defined as those having a positive response to methacholine challenge testing. Control subjects were selected among those who did not have a diagnosis of asthma and who had no response to methacholine. Results: There was a trend for an association of the Arg16 SNP genotype with airways hyperresponsiveness (odds ratio, 1.25; 95% confidence interval, 0.96 to 1.64 [in an additive model]). In stratified analyses, the effect of the Arg16 variant was seen mainly among nonsmokers. Smokers had increased risks for airway hyperresponsiveness regardless of genotype at either SNP. Using a program to estimate haplotype frequencies, three common haplotypes were identified. Adjusting for age, baseline FEV1, serum IgE level, and smoking status, the Gly16/Gln27 haplotype was negatively associated with airways hyperresponsiveness in the full complement of case patients and control subjects (score statistic, -2.43; \( p = 0.02 \)). The effect of the {beta}2-AR haplotypes was much stronger among lifelong nonsmokers, among whom the Gly16/Gln27 haplotype remained negatively associated with airways hyperresponsiveness in the full complement of case patients and control subjects (score statistic, -3.114; \( p = 0.002 \)), whereas the Arg16/Gln27 haplotype was positively associated with airways hyperresponsiveness (score statistic, 3.142; \( p = 0.002 \)). No effects were seen among ever-smokers. Conclusions: In this cohort of middle-aged to older white men, {beta}2-AR polymorphisms were associated with airways hyperresponsiveness, particularly among lifelong nonsmokers. Our results illustrate an instance in which greater power is obtained by performing haplotype analyses as opposed to single SNP analysis.
Study objectives: Severe acute respiratory syndrome (SARS) is a rapidly progressive disease caused by a novel coronavirus (CoV) infection. However, the disease presentation is nonspecific. The aim of this study was to define clearly the presentation, clinical progression, and laboratory data in a group of patients who had SARS. Design: Retrospective observational study. Setting: A tertiary care medical center with 51 negative-pressure isolation rooms in Taipei, Taiwan. Patients: Fifty-three patients with SARS seen between April 27 and June 16, 2003. Results: Fever (ie, temperature > 38(degrees)C) was the most common symptom (98%) and the earliest. When admitted to the isolation unit of the hospital for observation, most patients reported nonspecific symptoms associated with their fever. Only two patients with preexisting illnesses had cough on the same day the fever began. Eventually, 39 patients (74%) developed cough, beginning at a mean (+/- SD) time of 4.5 (+/-) 1.9 days after fever onset, and 35 patients (66%) had diarrhea beginning at a mean time of 6.0 (+/-) 3.3 days after fever onset. Thirty-one patients (59%) had abnormal findings on chest radiographs on hospital admission, and all but 1 patient (98%) eventually developed lung infiltrates that were consistent with pneumonia. The majority of patients (63%) first developed unifocal infiltrates at a mean time of 4.5 (+/-) 2.1 days after fever onset, while in 37% of patients the initial infiltrates were multifocal, appearing at a mean time of 5.8 (+/-) 1.3 days after fever onset. Common laboratory findings included lymphopenia (on hospital admission, 70%; during hospitalization, 95%), thrombocytopenia (on hospital admission, 28%; during hospitalization, 40%), elevated lactate dehydrogenase (on hospital admission, 58%; during hospitalization, 88%), creatine kinase (on hospital admission, 18%; during hospitalization, 32%), and aspartate aminotransferase or alanine aminotransferase levels (on hospital admission, 27%; during hospitalization, 62%). Throat or nasopharyngeal swab for SARS-CoV by reverse transcriptase polymerase chain reaction (PCR) and real-time PCR was positive in 40 of the 47 patients (85%) in whom the test was performed. Conclusions: None of the presenting symptoms or laboratory findings are pathognomonic for SARS. Even though cough developed in a majority of patients, it did not occur until later in the disease course, suggesting that a cough preceding or concurrent with the onset of fever is less likely to indicate SARS. While PCR for SARS-CoV appears to be the best early diagnostic test currently available, it is clear that better methods are needed to differentiate between SARS and non-SARS illness on initial presentation.
used to quantitate the expression of six lung cancer-associated genes (i.e., CEA, CK19, KS1/4, lunx, muc1, and PDEF) relative to the expression of an internal control gene (β2-microglobulin). Results: Clinical thresholds of marker positivity were set at 100% specificity, as determined by the receiver operating characteristic curve analysis. Of the cytology-positive lymph nodes (27 lymph nodes), the expression of the KS1/4 gene was above its respective clinical threshold in 25 of 27 samples (93%), making this the most sensitive marker for the detection of metastatic NSCLC. At least one of the six lung cancer-associated genes was overexpressed in 18 of 61 cytology-negative patients (30%), of which KS1/4 was overexpressed in 15 of 61 patients (25%). Conclusions: Based on the high accuracy of EUS-guided FNA/RT-PCR, we predict that some of the patients in the cytology-negative/marker-positive category will have high NSCLC recurrence rates. Among the genes used in our marker panel, KS1/4 appears particularly useful for the detection of overt or occult metastatic disease.


http://circres.ahajournals.org/cgi/content/abstract/90/9/951

We studied a Syrian family with 3 children who had low-density lipoprotein cholesterol (LDL) concentrations of 13.3, 12.2, and 8.6 mmol/L, respectively. Three other siblings and the parents all had LDL values <4.52 mmol/L, suggesting an autosomal-recessive mode of inheritance. The extended pedigree had 66 additional persons with normal LDL values. A genome-wide scan in the core family with 427 markers showed support for linkage on both chromosomes 1 and 13. Markers on chromosome 1 revealed a 3.07 multipoint LOD score between 1p36.1-p35, an 18-cM interval. Surprisingly, we also found linkage to 13q22-q32, a 14-cM interval, with a 3.08 LOD score. We had identified this locus earlier as containing a gene strongly influencing LDL in another Arab family with autosomal-dominant familial hypercholesterolemia and in normal dizygotic twins. We found evidence for an interaction between these loci. We next genotyped our twin panel and confirmed linkage of the 1p36.1-p35 locus to LDL (P<0.002) in this normal population. Elucidation of ARH, the LDL receptor adaptor protein at chromosome 1p35, caused us to sequence that gene. We first identified the genomic structure of ARH gene and then sequenced the gene in our family. We found an intron 1 acceptor splice-site mutation. This mutation was not found in any other family members, in 31 nonrelated Syrian persons, or in 30 Germans. Our results underscore the importance of ARH on chromosome 1 and the chromosome 13q locus to LDL, not only in families with unusual illnesses, but also to the general population.


http://circres.ahajournals.org/cgi/content/abstract/92/3/293

Heat shock protein 72 (HSP72) is a stress-inducible protein capable of protecting a variety of cells from toxins, thermal stress, and ischemic injury. The cytoprotective role and mechanism of
action of HSP72 in renal cell ischemic injury remain unclear. To study this, HSP72 was introduced (liposomal transfer) or induced (thermal stress, 43°C×1 hour) in renal tubular cells (LLC-PK1) with Western blot confirmation. Cells were subjected to simulated ischemia 24 hours after liposomal HSP72 transfer or thermal stress, and the effect of HSP72 on nuclear factor-κB (NF-κB) activation (electrophoretic mobility shift assay and immunohistochemistry), IκBα production (Western blot), posts ischemic tumor necrosis factor-α (TNF-α) production (RT-PCR), and apoptosis (TUNEL assay) were determined. In separate experiments, the role of TNF-α in apoptosis was determined (anti-TNF-α neutralizing antibody). Results demonstrated that both liposomal transfer of HSP72 and thermal induction of HSP72 prevented NF-κB activation and translocation, TNF-α gene transcription, and subsequent ischemia-induced renal tubular cell apoptosis. Furthermore, TNF-α neutralization also inhibited ischemia-induced renal tubular cell apoptosis. These results indicate that liposomal delivery of HSP72 inhibits ischemia-induced renal tubular cell apoptosis by preventing NF-κB activation and subsequent TNF-α production. Further elucidation of the mechanisms of HSP-induced cytoprotection may result in therapeutic strategies that limit or prevent ischemia-induced renal damage.


http://circres.ahajournals.org/cgi/content/abstract/92/1/41

Thrombomodulin (TM), a key component of the anticoagulant protein C pathway, is a major contributor to vascular thromboresistance. We previously found that TM protein expression is dramatically reduced in autologous vein grafts during the first two weeks after implantation, coincident to a local inflammatory response, and remains suppressed for at least 6 weeks. To determine the proximate cause of TM loss, in vivo gene expression was quantified by real-time PCR. TM gene expression in vein grafts declined >85% during the first postoperative week and remained suppressed >55% at 6 weeks, accounting for the observed changes in protein expression. The effects of vein graft inflammation were evaluated in animals rendered leukopenic with vinblastine before graft implantation. Abrogating the local inflammatory response affected neither TM protein nor gene expression. To determine how hemodynamic forces might modulate TM expression, the surgical protocol was modified to alter blood flow and pressure-induced vessel distension. TM protein and gene expression did not correlate to changes in shear stress but highly correlated to changes in wall tension, both acutely and over time. We conclude that the primary stimulus for altered TM expression in vein grafts is the exposure to arterial pressure. Furthermore, these data identify strain as a novel and important pathway for in vivo TM gene regulation.


http://circres.ahajournals.org/cgi/content/abstract/94/11/1515

Subject-- Peroxisome proliferator-activated receptor (PPAR)-γ agonists are emerging as potential protectors against inflammatory cardiovascular diseases including atherosclerosis and diabetic complications. However, their molecular mechanism of action within vasculature remains unclear. We report here that PPAR(γ) agonists, thiazolidinedione class drugs (TZDs), or 15-deoxy-(Δ12,14)-prostaglandin J2 (15d-PGJ2) were capable of activating diacylglycerol (DAG) kinase (DGK), resulting in attenuation of DAG levels and inhibition of protein kinase C (PKC) activation. The PPAR(γ) agonist-induced DGK was completely blocked by a
dominant-negative mutant of PPAR{gamma}, indicating an essential receptor-dependent action. Importantly, the suppression of DAG-PKC signaling pathway was functional linkage to the anti-inflammatory properties of PPAR{gamma} agonists in endothelial cells (EC), characterized by the inhibition of proinflammatory adhesion molecule expression and adherence of monocytes to the activated EC induced by high glucose. These findings thus demonstrate a novel molecular action of PPAR{gamma} agonists to suppress the DAG-PKC signaling pathway via upregulation of an endogenous attenuator, DGK.


http://circ.ahajournals.org/cgi/content/abstract/106/24/3104

Background-- Low-density lipoprotein (LDL) receptor-related protein (LRP) is highly expressed in vascular smooth muscle cells (VSMCs) of both normal and atherosclerotic lesions. However, little is known about LRP regulation in the vascular wall. Methods and Results-- We analyzed the regulation of LRP expression in vitro in human VSMCs cultured with native LDL (nLDL) or aggregated LDL (agLDL) by semiquantitative reverse transcriptase-polymerase chain reaction, real-time polymerase chain reaction, and Western blot and in vivo during diet-induced hypercholesterolemia by in situ hybridization. LRP expression in human VSMCs is increased by nLDL and agLDL in a time- and dose-dependent manner. Maximal induction of LRP mRNA expression was observed after 24 hours of exposure to LDL. However, agLDL induced higher LRP mRNA expression (3.0-fold) than nLDL (1.76-fold). LRP mRNA upregulation was associated with an increase on LRP protein expression with the greatest induction by agLDL. VSMC-LRP upregulation induced by nLDL or agLDL was reduced by an inhibitor of sterol regulatory element binding protein (SREBP) catabolism (N-acetyl-leucyl-leucyl-norleucinal). In situ hybridization analysis indicates that there is a higher VSMC-LRP expression in hypercholesterolemic than in normocholesterolemic pig aortas. Conclusions-- These results indicate that LRP expression in VSMCs is upregulated by intravascular and systemic LDL.


http://circ.ahajournals.org/cgi/content/abstract/105/20/2423

Background-- Reactive oxygen species, such as superoxide (O2-), are involved in the abnormal growth of various cell types. Angiotensin II (Ang II) is one of the most potent inducers of oxidative stress in the vasculature. The molecular events involved in Ang II-induced proliferation of vascular smooth muscle cells (VSMCs) are only partially understood. Methods and Results-- Ang II as well as xanthine/xanthine oxidase (X/XO) led to enhanced DNA synthesis and proliferation of VSMCs. The effect of Ang II was abolished by diphenylene iodonium. Consequently, VSMCs were incubated with X/XO, and modulation of gene expression was monitored by differential display, leading to the identification of a novel redox-sensitive gene, the dominant-negative helix-
loop-helix protein Id3, which was upregulated within 30 minutes by X/XO and Ang II. Superoxide dismutase but not catalase inhibited this effect. Overexpression of antisense Id3 via transfection in VSMCs completely abolished Ang II- and X/XO-induced cell proliferation. Ang II, X/XO, and overexpression of sense Id3 downregulated protein expression of p21WAF1/Cip1, p27Kip1, and p53. Overexpression of antisense Id3 abrogated the effect of Ang II on the expression of p21WAF1/Cip1, p27Kip1, and p53. Ang II and overexpression of sense Id3 caused hyperphosphorylation of the retinoblastoma protein. Ang II-induced phosphorylation of the retinoblastoma protein was decreased by overexpression of antisense Id3. Conclusions-- Ang II induces proliferation of VSMCs via production of superoxide, which enhances the expression of Id3. Id3 governs the downstream mitogenic processing via depression of p21WAF1/Cip1, p27Kip1, and p53. These findings reveal a novel redox-sensitive pathway involved in growth control.


http://circ.ahajournals.org/cgi/content/abstract/109/10/1292

Background-- The delivery of autologous cells to increase angiogenesis is emerging as a treatment option for patients with cardiovascular disease but may be limited by the accessibility of sufficient cell numbers. The beneficial effects of delivered cells appear to be related to their pluripotency and ability to secrete growth factors. We examined nonadipocyte stromal cells from human subcutaneous fat as a novel source of therapeutic cells. Methods and Results-- Adipose stromal cells (ASCs) were isolated from human subcutaneous adipose tissue and characterized by flow cytometry. ASCs secreted 1203{\pm}254 pg of vascular endothelial growth factor (VEGF) per 106 cells, 12 280{\pm}2944 pg of hepatocyte growth factor per 106 cells, and 1247{\pm}346 pg of transforming growth factor-beta per 106 cells. When ASCs were cultured in hypoxic conditions, VEGF secretion increased 5-fold to 5980{\pm}1066 pg/106 cells (P=0.0016). The secretion of VEGF could also be augmented 200-fold by transfection of ASCs with a plasmid encoding VEGF (P<0.05). Conditioned media obtained from hypoxic ASCs significantly increased endothelial cell growth (P<0.001) and reduced endothelial cell apoptosis (P<0.05). Nude mice with ischemic hindlimbs demonstrated marked perfusion improvement when treated with human ASCs (P<0.05). Conclusions-- Our experiments delineate the angiogenic and antiapoptotic potential of easily accessible subcutaneous adipose stromal cells by demonstrating the secretion of multiple potentially synergistic proangiogenic growth factors. These findings suggest that autologous delivery of either native or transduced subcutaneous ASCs, which are regulated by hypoxia, may be a novel therapeutic option to enhance angiogenesis or achieve cardiovascular protection.


http://circ.ahajournals.org/cgi/content/abstract/111/5/633

Background-- Drugs that simultaneously decrease platelet function and inflammation may improve the treatment of cardiovascular disorders. Here, we determined whether dipyridamole and aspirin, a combination therapy used to prevent recurrent stroke, regulates gene expression in platelet-monocyte inflammatory model systems. Methods and Results-- Human platelets and monocytes were pretreated with dipyridamole, aspirin, or both inhibitors. The cells were stimulated with thrombin or activated by adhesion to collagen, and gene expression was measured in the target monocytes. Thrombin-stimulated platelets increased monocyte chemotactic protein-1 (MCP-1) expression by monocytes. Dipyridamole but not aspirin attenuated
nuclear translocation of NF-{kappa}B and blocked the synthesis of MCP-1 at the transcriptional level. Dipyridamole delayed maximal synthesis of interleukin-8 but did not alter cyclooxygenase-2 accumulation. Adherence to collagen and platelets also increased the expression of matrix metalloproteinase-9 (MMP-9) in monocytes, a response that was inhibited by dipyridamole. In this case, however, dipyridamole did not block transcription or distribution of MMP-9 mRNA to actively translating polysomes, indicating that it regulates the expression of MMP-9 protein at a postinitiation stage of translation. Dipyridamole also blocked MCP-1 and MMP-9 generated by lipopolysaccharide-treated monocytes, indicating that at least part of its inhibitory action is unrelated to its antiplatelet properties. Conclusions-- These results indicate that dipyridamole has selective antiinflammatory properties that may contribute to its actions in the secondary prevention of stroke.


http://circ.ahajournals.org/cgi/content/abstract/110/24/3727

Background-- Genetic factors have an important role in the pathogenesis of intracranial aneurysm (IA). The results of previous studies have suggested several loci. Methods and Results-- From 29 IA families with [\geq]3 individuals affected by IA, we used nonparametric (model-free) methods for linkage analyses, using GENEHUNTER and Merlin software. Genome-wide linkage analyses revealed 3 regions on chromosomes 17cen (maximum nonparametric logarithm of the odds score [MNS] = 3.00, nominal P=0.001), 19q13 (MNS=2.15, nominal P=0.020), and Xp22 (MNS=2.16, nominal P=0.019). We tested 4 candidate genes in these regions: the microfibril-associated protein 4 gene (MFAP4) and the promoter polymorphism of the inducible nitric oxide synthase gene (NOS2A) on chromosome 17cen, the epsilon genotypes of the apolipoprotein E gene (APOE) on chromosome 19q13, and the angiotensin I converting enzyme 2 gene (ACE2) on chromosome Xp22. Associations of their polymorphisms with IA were evaluated by a case-control study (100 cases: 29 probands from IA families and 71 unrelated subjects with IAs, 100 unrelated control subjects [unaffected members with IAs and absence of family history of IAs]). However, the case-control study showed that none of the polymorphisms of the examined genes had associations with IA. Conclusions-- A genome-wide scan in 29 Japanese families with a high degree of familial clustering revealed 1 suggestive linkage region on chromosome 17cen and 2 potentially interesting regions on chromosomes 19q13 and Xp22. These regions were consistent with previous findings in various populations.


http://circ.ahajournals.org/cgi/content/abstract/108/14/1724

Background-- Matrix metalloproteinase (MMP)-2 and MMP-9 have been shown to play a role in the progression of hemorrhagic stroke. We hypothesized that donor intracerebral hemorrhage (ICH) is associated with activation of the metalloproteinases before transplantation that play a key role in the subsequent development of transplant vasculopathy. Methods and Results-- We evaluated mRNA expressions of MMP-2 and MMP-9 in donor spleen lymphocytes (before transplantation) and in heart biopsies at 1 week after transplantation in 20 recipients from ICH donors and 20 recipients from trauma donors. Patients underwent serial coronary intravascular ultrasound, and interstitial myocardial fibrosis was quantified at 1 year. The baseline characteristics were similar except for increased donor age in the ICH group. Heart biopsies from
the ICH group showed significant increased expression of MMP-2 (17-fold, P<0.0001) and MMP-9 (20-fold, P<0.0001) compared with the trauma group. Furthermore, the ICH group showed 1.8-fold (P=0.016) increased mRNA expression of MMP-2 and 1.7-fold (P=0.015) increased mRNA expression of MMP-9 in the donor spleen lymphocytes, suggesting the presence of systemic activation of metalloproteinases before transplantation. At 1 year, the ICH group showed increased myocardial fibrosis and accelerated coronary vasculopathy. Using multivariate regression analysis, MMP-9 was found to be associated with increased risk for vasculopathy independent of donor age (OR, 2.41; P=0.01; 95% CI, 1.24 to 4.69). Conclusions-- This is the first report to describe systemic activation of MMP-2 and MMP-9 in donors with intracerebral hemorrhage and subsequent development of allograft vasculopathy.


http://circ.ahajournals.org/cgi/content/abstract/109/10/1230

Background-- CD4+CD28null T cells are present in increased numbers in the peripheral blood of patients with acute coronary syndrome (ACS) compared with patients with chronic stable angina (CSA). The triggers of activation and expansion of these cells to date remain unclear. Methods and Results-- Twenty-one patients with ACS and 12 CSA patients with angiographically confirmed coronary artery disease and 9 healthy volunteers were investigated. Peripheral blood leukocytes were stimulated with human cytomegalovirus (HCMV), Chlamydia pneumoniae, human heat-shock protein 60 (hHSP60), or oxidized LDL (ox-LDL). CD4+CD28null cells were separated by flow cytometry and assessed for antigen recognition using upregulation of interferon-γ and perforin mRNA transcription as criteria for activation. CD4+CD28null cells from 12 of 21 patients with ACS reacted with hHSP60. No response was detected to HCMV, C. pneumoniae, or ox-LDL. Incubation of the cells with anti-MHC class II and anti-CD4 antibodies but not anti-class I antibodies blocked antigen presentation, confirming recognition of the hHSP60 to be via the MHC class II pathway. Patients with CSA had low numbers of CD4+CD28null cells. These cells were nonreactive to any of the antigens used. Circulating CD4+CD28null cells were present in 5 of the 9 healthy controls. None reacted with hHSP60. Conclusions-- We have shown that hHSP60 is an antigen recognized by CD4+CD28null T cells of ACS patients. Endothelial cells express hHSP60 either constitutively or under stress conditions. Circulating hHSP60-specific CD4+CD28null cells may, along other inflammatory mechanisms, contribute to vascular damage in these patients.

Cladistics (1)


Purpose: Clear cell renal carcinoma (ccRCC) is strongly associated with loss of the von Hippel-Lindau (VHL) tumor suppressor gene. The VHL gene is functionally lost through hypermethylation in up to 19% of sporadic ccRCC cases. We theorized that re-expressing VHL silenced by methylation in ccRCC cells, using a hypo-methylating agent, may be an approach to treatment in patients with this type of cancer. We tested the ability of two hypo-methylating agents to re-express VHL in cell culture and in mice bearing human ccRCC and evaluated the effects of re-expressed VHL in these models.

Experimental Design: Real-time reverse transcription-PCR was used to evaluate the ability of zebularine and 5-aza-2'-deoxycytidine (5-aza-dCyd) to re-express VHL in four ccRCC cell lines with documented VHL gene silencing through hypermethylation. We evaluated if the VHL re-expressed after hypo-methylating agent treatment could recreate similar phenotypic changes in ccRCC cells observed when the VHL gene is re-expressed via transfection in cell culture and in a xenograft mouse model. Finally we evaluated global gene expression changes occurring in our cells, using microarray analysis.

Results: 5-Aza-dCyd was able to re-express VHL in our cell lines both in culture and in xenografted murine tumors. Well described phenotypic changes of VHL expression including decreased invasiveness into Matrigel, and decreased vascular endothelial growth factor and glucose transporter-1 expression were observed in the treated lines. VHL methylated ccRCC xenografted tumors were significantly reduced in size in mice treated with 5-aza-dCyd. Mice bearing nonmethylated but VHL-mutated tumors showed no tumor shrinkage with 5-aza-dCyd treatment. Conclusion: Hypo-methylating agents may be useful in the treatment of patients having ccRCC tumors consisting of cells with methylated VHL.


Purpose: WW domain-containing oxidoreductase (WWOX) is a tumor suppressor gene that maps to the common fragile site FRA16D on chromosome 16q23.3-24.1. To investigate the role of the WWOX gene in the development of gastric carcinoma, we examined a large series of primary adenocarcinomas and nine gastric cancer cell lines for the expression of Wwox. Experimental Design: Loss of heterozygosity, reverse-transcription-PCR, and immunohistochemistry were used to assess the role of WWOX in stomach cancer. A total of 81 primary gastric adenocarcinoma were analyzed. Results: Loss of heterozygosity was observed in 31% of the cases and loss of Wwox protein expression was found in 65% of gastric adenocarcinoma primary specimens and 33% of gastric cancer cell lines. In addition, we found a high correlation between Wwox and Fhit protein expression. Conclusions: Our results indicate that alterations of the WWOX gene may be involved quite frequently in gastric tumorigenesis. Our data could be used in future studies to develop diagnostic and targeted therapy of stomach cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/13/4865

Purpose: The goal of this study was to develop a molecular diagnostic assay to detect circulating breast cancer cells in the peripheral blood for the purpose of staging breast cancer. Our aim was to make available an assay that was not limited by the low concentration of circulating breast cancer cells and the background gene expression that is typically found in peripheral blood.

Experimental Design: In this study, we investigated the ability of two new technologies to significantly enhance the quantification of gene expression in the peripheral blood: enrichment by a novel porous barrier density gradient centrifugation technology; and multimarker real-time reverse transcription-PCR (RT-PCR).

Results: Using fluorescence-labeled breast cancer cells and flow cytometry, we show that processing peripheral blood by porous barrier density gradient centrifugation results in a 300-fold enrichment of breast cancer cells. Real-time RT-PCR analysis confirmed a concomitant reduction in background expression of the CK19 and MUC1 genes after enrichment. In a pilot study, porous barrier density gradient centrifugation and multimarker real-time RT-PCR enabled our laboratory to detect breast cancer-associated gene overexpression in 13 of 20 (65%) stage IV breast cancer patients. Nine of these 14 patients overexpressed three or more markers. Conclusions: These results confirm the promise of such a molecular diagnostic assay and suggest that additional studies are needed to precisely define the clinical relevance.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/11/3678

Purpose: G3139 is an antisense bcl-2 phosphorothioate oligodeoxyribonucleotide that is currently being evaluated in Phase III clinical trials in several human cancers. The aim of the present work was to further identify the apparent non-bcl-2-dependent mechanism of this action of this compound in PC3 prostate cancer cells. Experimental Design: We performed Affymetrix U95A oligonucleotide microarray studies on mRNA isolated from cells treated with G3139 and related oligonucleotides. Results: Hierarchical clustering revealed the presence of a set of genes of which the expression was elevated on both 1 and 3 days after oligonucleotide treatment. Significantly, the persistence of expression of the up-regulation of these genes, many of which are members of the IFN cascade, was greater for G3139 than for any other oligomer evaluated. Furthermore, many of the genes with the greatest up-regulation of expression are also those of which the expression is up-regulated after treatment of cells with IFNs. Treatment of PC3 cells with either IFN-{beta} or -{gamma} recapitulated some of the aspects of the molecular and phenotypic changes observed after treatment with a G3139/Lipofectin complex. These include down-regulation of bcl-2 protein expression itself, down-regulation of protein kinase C {alpha} protein expression (but not that of other protein kinase C isoforms), alteration in p21/Waf1/Cip1 protein expression, up-regulation of MHC-I cell surface expression, and profound suppression of cell growth in the absence of a notable increase in cellular apoptosis. However, G3139 (when complexed with Lipofectin) did not induce the up-regulation of expression of either type I or type II IFNs, nor could IFNs be found in conditioned media from treated cells. Conclusions: Oligonucleotide microarray experiments demonstrated that G3139 could induce elements of the IFN cascade in PC3 cells in vitro. In addition, the cellular phenotype obtained after treatment with exogenous IFN could, at least in part, recapitulate that obtained after G3139 treatment. Nevertheless, the oligonucleotide microarray experiments we performed also demonstrated that there are extremely large qualitative and quantitative differences between the two treatments.

http://clincancerres.aacrjournals.org/cgi/content/abstract/9/1/235

Purpose: 9p21 is a major target in the pathogenesis of human urinary bladder cancer. The locus harbors the CDKN2A/ARF tumor suppressor gene, which encodes two cell cycle regulatory proteins cyclin dependent kinase 2A (p16INK4a) and alternate reading frame (p14ARF). We have designed a real-time quantitative PCR (QPCR) application to study homozygous deletion (HD) of CDKN2A/ARF in 186 urinary bladder cancer patients. Experimental Design: Real-time QPCR, based on simultaneous amplification of ARF and a reference gene, GAPDH, was developed and evaluated in three melanoma cell lines with HDs at the CDKN2A/ARF locus (IGR-1, SK-MEL-5, and WM-266-4). In addition, loss of heterozygosity was analyzed at the D9S942, D9S1748, and D12S99 markers. Mutation analysis of the CDKN2A/ARF gene was performed using single-strand conformational polymorphism and sequencing. Results from the present investigation were combined with previous p53 analysis of the same urinary bladder neoplasms. Results: Real-time QPCR analysis showed 26 (14%) HDs, 22 (12%) hemizygous deletions, and 3 (2%) multiple duplications. Loss of heterozygosity was determined in 30 (22%) cases at the D9S942 locus, which is located between E1{alpha} and E1{beta} of the CDKN2A/ARF gene. No association was established between occurrence of genetic aberrations at 9p21 and tumor stage or grade, supporting previous suggestions that CDKN2A/ARF inactivation is an early event in bladder carcinogenesis. Conclusions: We have established a fast and efficient method for detection of HDs. Our data support the notion that inactivation, including HDs, of CDKN2A/ARF is an early event in transitional cell carcinoma. We observed separate and specific targeting of the CDKN2A and ARF genes, respectively, and that simultaneous inactivation of ARF and p53 occurs.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/8/2862

Purpose: Infection with human papillomaviruses (HPV) is associated with the development of cervical cancer, but whether HPVs have a role in colorectal cancer remains controversial. Experimental Designs: To determine the relationship between HPV and colorectal cancer, we did a retrospective, controlled study using tumor and tumor-adjacent colorectal tissues dissected from patients with colorectal cancer, as well as colorectal tissues from control individuals with no cancer. The samples were processed in a blinded fashion for nested PCR and in situ PCR detection of HPV DNAs. The PCR products were gel-purified and sequenced for HPV genotyping. Results: We found that colorectal tissues from 28 of 55 (51%) patients with colorectal cancer were positive for HPV DNA. Colorectal tissues from all 10 control individuals were negative for HPV DNA (P = 0.0034). Of the 107 usable (GAPDH+) samples collected as paired colorectal tissues (tumor and tumor-adjacent tissues) from the patients, 38 (36%) had HPV16 (n = 31), HPV18 (n = 5), or HPV45 (n = 2), with HPV DNA in both tumor and tumor-adjacent tissues of 10 paired samples, 13 in only the tumor, and 5 in only tumor-adjacent tissues. In situ PCR detection of the tumor tissues confirmed the presence of HPV DNA in tumor cells. Conclusion: Our results suggest that colorectal HPV infection is common in patients with colorectal cancer, albeit at a low DNA copy number, with HPV16 being the most prevalent type. HPV infection may play a role in colorectal carcinogenesis.

http://clincancerres.aacrjournals.org/cgi/content/abstract/10/12/4101

Purpose: The c-kit protein, also known as CD117, is a member of the type III receptor tyrosine kinase family. Kinase activity has been implicated in the pathophysiology of many tumors, including small-cell lung carcinoma (SCLC). Autocrine or paracrine activation of c-kit by its ligand has been postulated for lung cancer, but this receptor can also be activated by mutations of the c-kit gene. We examined c-kit expression and mutational status in SCLC to verify its putative expression and genetic alterations, as well as its eventual prognostic impact. Experimental Design: We studied 60 SCLC samples to determine the mutations of the coding region of the gene; the exons 9 and 11 were analyzed by PCR-single-strand conformational polymorphism and automated sequencing. Moreover, c-kit expression was evaluated in 55 samples by immunohistochemical method. Results: Expression of c-kit was demonstrated in about 40% of SCLC samples. Two mutations in exon 9 and three mutations in exon 11 were found. Kaplan-Meier analysis revealed no prognostic significance of c-kit expression for survival. Conclusions: In our series, the expression of c-kit and its mutational status failed to appear relevant or to have a significant impact on survival; this makes the therapeutic approach with an inhibitor of tyrosine kinase more difficult in SCLC until a sure demonstration of c-kit implication is obtained for this tumor.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/9/3243

Purpose: Prostate cancer is the most common male malignancy and the second leading cause of male cancer death; therefore, there is urgent necessity for noninvasive assays for early detection of prostate cancer. Obtaining prostate tumor samples surgically is problematic because the malignancy is heterogeneous and multifocal and early-stage tumors are nonpalpable. In contrast, exfoliated cells represent the cancer status of the entire gland better due to the general tendency of cancer cells to exfoliate into biological fluids. The purpose of this study was to clarify whether quantitative analysis of telomerase activity in exfoliated cells in urine could serve as a reliable molecular marker of prostate malignancy. Experimental Design: We analyzed prospectively post-prostatic examination-exfoliated cells from the urine of 56 patients undergoing routine prostate screening. Epithelial cells were isolated and enriched by immunomagnetic separation. Telomerase activity was analyzed by quantitative real-time PCR telomeric-repeat amplification protocol assay using Opticon MJ research instrument. Results: We report now that all prostate cancer patients revealed high levels of telomerase activity thereby showing 100% of the assay sensitivity. In contrast, the majority of patients with clinically confirmed benign prostatic hyperplasia (BPH) did not express any telomerase activity (70% of all BPH patients), most likely presenting cancer-free cases, or expressed low levels of activity (18%). However, about 12% of BPH patients revealed high levels of telomerase activity that potentially can reflect hidden prostate cancer. Conclusions: We suggest that the quantitative analysis of telomerase activity can be useful for the selection of prostate cancer and cancer-free cases.

Background: Retinoid X receptors (RXRs) have inhibitory effects on non-small cell lung cancer (NSCLC) cell growth, and RXR(β) expression is reduced in NSCLC specimens compared with normal lung tissue. We hypothesized that suppressed RXR expression might be a prognostic factor of worse clinical outcome in patients with NSCLC.

Experimental Design: Using a quantitative real-time reverse transcription-PCR (TaqMan) method, we analyzed RXR(α), RXR(β), and RXR(γ) mRNA expression in normal lung tissue and matching tumor samples from 88 patients with NSCLC. Results: The median mRNA expression levels of all three RXR subtypes were frequently decreased in tumor tissues compared with matching normal lung tissue (RXR(α), 67%; RXR(β), 55%; RXR(γ), 89%). The RXR(α) (P = 0.001) and RXR(γ) (P < 0.001) median expression levels were significantly lower in the tumors. Patients whose tumors exhibited low RXR(β) expression levels had a statistically significant worse overall survival (P = 0.0005), whereas a trend toward worse survival was observed for patients with low RXR(α) expression. Multivariate analysis indicated that low RXR(β) expression is an independent predictor of worse survival in patients with NSCLC (P = 0.017).

Conclusion: Suppressed mRNA expression of all three RXR subtypes is a frequent event in NSCLC. Reduced RXR(β) expression might be an important biomarker for more aggressive disease in patients with NSCLC.


Purpose: The aim of this study was to investigate whether expression of particular drug resistance genes in primary operable breast cancer correlates with response to first-line chemotherapy in advanced disease.

Experimental Design: We determined mRNA levels of BCRP, LRP, MRP1, MRP2, and MDR1 in 59 primary breast tumor specimens of patients who received chemotherapy as first-line systemic treatment after diagnosis of advanced disease. The relative expression levels were measured by quantitative real-time reverse transcription-PCR and subsequently analyzed in relation to the type of response to chemotherapy, the length of progression-free survival (PFS), and post-relapse overall survival. Results: For each of these drug resistance genes, a large variation in expression level was observed among the tumors of the different patients. When analyzing mRNA expression in relation to overall response, it was found that the median expression level of these five drug resistance genes in the responding tumors, as compared with nonresponding tumors, was markedly lower. Classification of tumors as high versus low with respect to the expression level of these genes showed that the overall response in the MDR1-high subset (17%), as compared with the MDR1-low subset (68%), was significantly lower (P = 0.005). Although similar differences in response rate were found for subsets of tumors stratified by the expression level of the other drug resistance genes, none of the observed differences were statistically significant. However, in the subgroup of patients treated with anthracycline-based chemotherapy (5-fluorouracil, Adriamycin/epirubicin, and cyclophosphamide), a correlation between response and the expression of BCRP and MRP1 (only PFS) was found, whereas such an association was not present in the cyclophosphamide, methotrexate, and 5-fluorouracil-treated group of patients. Furthermore, high expression of LRP as well as MDR1 was found to be significantly associated with a poor PFS (P = 0.04 and P < 0.001, respectively). For lung resistance-related protein, this association was limited to 5-fluorouracil, Adriamycin/epirubicin, and cyclophosphamide. Expression levels of BCRP, MRP1, or MRP2 were not related with the length of PFS. Furthermore, no correlation between the expression level of these drug resistance genes and post-relapse overall survival was found.

Conclusions: In this pilot study, MDR1 expression in primary breast tumors was inversely related
with the efficacy of first-line chemotherapy, and high expression level was a significant predictor of poor prognosis for patients with advanced disease. Apart from MDR1, the expression levels of BCRP, LRP, and MRP1 might have some additional predictive value for clinical outcome.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/9/3198

Purpose: The Int6 gene was originally identified as a common insertion site for the mouse mammary tumor virus in virally induced mouse mammary tumors. Recent studies indicate that Int6 is a multifaceted protein involved in the regulation of protein translation and degradation through binding with three complexes: the eukaryotic translation initiation factor 3, the proteasome regulatory lid, and the constitutive photomorphogenesis 9 signalosome. This study aimed to investigate the prognostic role of Int6 in a large series of stage I non-small cell lung cancers (NSCLC) patients with long-term follow-up. Experimental Design: We determined the methylation status of Int6 DNA by methylation-specific PCR and the steady-state levels of Int6 RNA by quantitative real-time reverse transcription-PCR in 101 NSCLCs and matched normal lung tissues. Results: In 27% of the tumors, Int6 RNA levels were reduced relative to normal tissue. In 85% of the tumors with reduced Int6 expression, the transcription promoter and first exon were hypermethylated, whereas only 4% of the tumors with elevated Int6 RNA levels were hypermethylated (P < 0.000001). Low levels of Int6 RNA were found a significant predictor of overall and disease-free survival (P = 0.0004 and P = 0.0020, respectively). A multivariate analysis confirmed that low Int6 expression was the only independent factor to predict poor prognosis, for both overall (P = 0.0006) and disease-free (P = 0.024) survival. Conclusions: Our results suggest that Int6 expression, evaluated by quantitative real-time PCR, may represent a new prognostic factor in patients with stage I NSCLC.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/6/2015

Purpose: Helicobacter pylori causes gastric damage and is involved in gastric carcinogenesis. Vascular endothelial growth factor (VEGF) plays a major role in gastric mucosa repair and is overexpressed in gastric cancer. We investigated: (a) whether H. pylori, and in particular H. pylori VacA toxin, affected VEGF expression in gastric epithelial cells in culture; and (b) the signal transduction pathway involved in any effect exerted by H. pylori. Experimental Design: MKN-28 cells were incubated with uninoculated BCF (control) or with BCF obtained from VacA-producing wild-type H. pylori 60190 strain or from its isogenic mutant 60190:v1, specifically lacking vacA gene in the presence or absence of ZD 1839, a selective inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase, PD098059, a selective inhibitor of mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase, the kinase responsible for ERK phosphorylation, or SC-236, a selective inhibitor of cyclooxygenase (COX)-2 for 24-48 h. Results: (a) Toxigenic H. pylori up-regulated VEGF mRNA and protein expression and caused a 2.5-fold increase in VEGF release compared with control, whereas nontoxigenic H. pylori did not; (b) H. pylori VacA toxin-induced up-regulation of VEGF was counteracted by selective inhibition of EGFR tyrosine kinase; (c) toxigenic H. pylori activated the ERK/MAP kinase cascade, and inhibition of MAP kinase activation counteracted H. pylori-induced VEGF up-regulation; (d) toxigenic H. pylori up-regulated COX-2 expression, and this effect was counteracted by blockade of EGFR tyrosine kinase; and (e) COX-2 selective inhibition counteracted H. pylori-induced up-
regulation of VEGF. Conclusion: (a) H. pylori up-regulates VEGF expression in gastric epithelial cells; and (b) this effect is specifically related to VacA toxin and seems to depend on the activation of an EGFR-, MAP kinase-, and COX-2-mediated pathway.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/9/3338

Purpose: The suitability of neuroblastoma patients for therapy using radiolabeled meta-iodobenzylguanidine (MIBG) is determined by scintigraphy after the administration of a tracer dose of radiiodinated MIBG whose uptake is dependent upon the cellular expression of the noradrenaline transporter (NAT). As a possible alternative to gamma camera imaging, we developed a novel molecular assay of NAT expression. mRNA extracted from neuroblastoma biopsy samples, obtained retrospectively, was reverse transcribed, and NAT-specific cDNA was quantified by real-time PCR, referenced against the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. Experimental Design: Tumor specimens from 54 neuroblastoma patients were analyzed using real-time PCR, and NAT expression was compared with the corresponding diagnostic scintigrams. Results: Forty-eight of 54 (89%) of tumors showed MIBG uptake by scintigraphy. NAT expression was found to be significantly associated with MIBG uptake (P < 0.0001, Fisher's exact test). None of the samples from the six tumors that failed to concentrate MIBG expressed detectable levels of the NAT (specificity = 1.0). However, of the 48 MIBG uptake-positive tumors, only 43 (90%) expressed NAT (sensitivity = 0.9). The real-time PCR test has a positive predictive value of 1.0 but a negative predictive value of 0.55. Conclusions: The results indicate that whereas this method has substantial ability to predict the capacity of neuroblastoma tumors to accumulate MIBG, confirmation is required in prospective studies to determine more accurately the predictive strength of the test and its role in the management of patients with neuroblastoma.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/12/3741

This study was aimed at defining patterns of aberrant gene methylation in non-small cell lung cancer (NSCLC) in Chinese patients and its use in detecting cancer cells in bronchoalveolar lavage (BAL). The methylation-specific PCR (MSP) was used to study methylation of the p16, retinoic acid receptor-(beta) (RAR(beta)), death-associated protein (DAP) kinase, and O6-methylguanine-DNA-methyltransferase (MGMT) genes in 75 NSCLCs [44 adenocarcinomas and 31 squamous cell carcinomas (SCCs)] and 68 BALs from suspected lung cancers. More females had adenocarcinoma than SCC (11 of 44 versus 2 of 31, P = 0.04). Aberrant methylation in at least one gene was found in 63 of 75 (84%) NSCLCs. p16, RAR(beta), DAP kinase, and MGMT methylation was similar in adenocarcinoma and SCC. However, females with NSCLC showed more frequent p16 methylation than males (12 of 13 versus 36 of 62, P = 0.02), because of more frequent p16 methylation in female adenocarcinomas (10 of 11 versus 17 of 33, P = 0.02). This sexual difference was not observed in RAR(beta), DAP kinase, and MGMT. At 92%, the frequency of p16 methylation in Chinese female NSCLC is one of the highest known. For BAL, MSP and cytological analysis showed concordant and discordant results in 25 of 68 and 43 of 68 samples. Of 41 MSP+/cytology- cases, 35 were eventually shown to have malignant lung lesions, 4 were at high risk but had no evidence of lung cancer, and 2 were lost to follow-up. There were
two MSP-/cytology+ cases. Frequent gene methylations were seen in Chinese NSCLC patients. More frequent p16 methylation was seen in female patients. MSP is a useful molecular adjunct for cancer cell detection in BAL samples.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/2/464

Purpose: We aimed to investigate the methylation pattern in bladder cancer and assess the diagnostic potential of such epigenetic changes in urine. Experimental Design: The methylation status of 7 genes (RAR{beta}, DAPK, E-cadherin, p16, p15, GSTP1, and MGMT) in 98 cases of bladder transitional cell carcinoma and 4 cases of carcinoma in situ was analyzed by methylation-specific PCR. Twenty-two cases had paired voided urine samples for analysis. Results: In transitional cell carcinoma tumor tissues, aberrant methylation was frequently detected in RAR{beta} (87.8%), DAPK (58.2%), E-cadherin (63.3%), and p16 (26.5%), whereas methylation of p15 (13.3%), GSTP1 (5.1%), and MGMT (5.1%) is not common. No association between methylation status and grading or muscle invasiveness was demonstrated. In 22 paired voided urine samples of bladder cancer, methylation of DAPK, RAR{beta}, E-cadherin, and p16 could be detected in 45.5%, 68.2%, 59.1%, and 13.6% of the cases, respectively. The sensitivity of methylation analysis (90.9%) was higher than that of urine cytology (45.5%) for cancer detection. Methylation of RAR{beta} (50%), DAPK (75%), and E-cadherin (50%) was also detected in carcinoma in situ. In 7 normal urothelium samples and 17 normal urine controls, no aberrant methylation was detected except for RAR{beta} methylation in 3 normal urothelium samples (42.9%) and 4 normal urine samples (23.5%), respectively. Conclusions: Our results demonstrated a distinct methylation pattern in bladder cancer with frequent methylation of RAR{beta}, DAPK, E-cadherin, and p16. Detection of gene methylation in routine voided urine using selected markers appeared to be more sensitive than conventional urine cytology.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/6/2364

Purpose: We have reported previously that intratumoral microvessel density (MVD) is a significant prognostic indicator of event-free survival in the Ewing's sarcoma family of tumors (ESFT). Here, the angiogenic growth factor expression profile and its relationship with MVD has been investigated in ESFT. Experimental Design and Results: Using ESFT model systems, the potential of these factors as therapeutic targets has been evaluated. A significant correlation (P = 0.02) was observed between vascular endothelial growth factor (VEGF) expression and MVD, consistent with the hypothesis that VEGF regulates the development of microvessels in ESFT. There was no correlation between MVD and any of the other growth factors studied. All six ESFT cell lines studied produced and secreted VEGF; five of six cell lines also secreted placental growth factor, one cell line (A673) at high levels. Tumor conditioned medium induced proliferation of human umbilical vein endothelial cells. Expression of VEGF receptors Flt-1 and Flk-1/KDR was heterogeneous across the cell lines. Both receptor tyrosine kinase inhibitors SU6668 (targets Flk-1/KDR, platelet-derived growth factor receptor-{beta}, and fibroblast growth factor receptor 1) and SU5416 (targets Flk-1/KDR) as well as anti-VEGF agents rhuMAB-VEGF (bevacizumab) and VEGF Trap delayed s.c. growth of ESFT in mice compared with untreated groups: SU6668 (100 mg/kg/d), SU5416 (25 mg/kg/d), rhuMAB-VEGF (10 mg/kg twice weekly), and VEGF Trap (2.5 or 25 mg/kg twice weekly). Conclusions: These data suggest that VEGF is the single most important regulator of angiogenesis in ESFT and may be exploited for therapeutic advantage.

http://clincancerres.aacrjournals.org/cgi/content/abstract/8/7/2061

Purpose: Recently, familial paraganglioma (PGL) was shown to be caused by mutations in the gene encoding succinate dehydrogenase subunit D (SDHD). However, the prevalence of SDHD mutations in apparently sporadic PGL is unknown. We studied the frequency and spectrum of germ-line and somatic SDHD mutations in patients with parasympathetic PGL. Experimental Design: We studied 57 unselected patients who developed parasympathetic PGLs (n = 105 tumors) and who were treated between 1987 and 1999 at the Erasmus MC (Rotterdam, the Netherlands). Thirty-eight (67%) of these patients (n = 51 tumors) lacked a family history of parasympathetic PGL. We used conformation-dependent gel electrophoresis and sequence determination analysis of germ-line and tumor DNA to identify SDHD mutations. We compared the clinical and molecular characteristics of sporadic and hereditary PGLs. Results: Three different SDHD germ-line mutations were identified in 32 of the 57 (56%) patients. These included 19 of 19 (100%) patients with familial PGL and also 13 of 38 (34%) patients with apparently sporadic PGL. All three mutations were characterized as missense mutations (D92Y, L95P, and L139P) in highly conserved regions of the SDHD gene and were not observed in 200 control alleles. No somatic mutations were found. Conclusions: Germ-line mutations of the SDHD gene appear in a significant number of patients with apparently sporadic parasympathetic PGL. Somatic SDHD mutations do not play a significant role in the sporadic form of this tumor. Genetic testing for SDHD germ-line mutations should be considered for every patient presenting with this tumor, even if a personal or family history of PGL is absent, to allow appropriate clinical management.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/17/5889

Purpose: The ATP-binding cassette transporter ABCG2 (breast cancer resistance protein) is an efflux protein that plays a role in host detoxification of various xenobiotic substrates, including the irinotecan metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). The ABCG2 421C>A polymorphism has been associated with reduced protein expression and altered function in vitro. The aim of this study was to evaluate the ethnic distribution and potential functional consequence of the ABCG2 421C>A genotype in cancer patients treated with irinotecan. Experimental Design: ABCG2 genotyping was performed using Pyrosequencing on DNA from 88 American Caucasians, 94 African Americans, 938 Africans, and 95 Han Chinese, as well as in 84 European Caucasian patients treated with irinotecan undergoing additional blood sampling for pharmacokinetic studies. Results: Significant differences in allele frequencies were observed between the given world populations (P < 0.001), the variant allele being most common in the Han Chinese population with a frequency as high as 34%. The mean area under the curve of irinotecan and SN-38 were 19,851 and 639 ng x hour/mL, respectively. The frequency of the variant allele (10.7%) was in line with results in American Caucasians. No significant changes in irinotecan pharmacokinetics were observed in relation to the ABCG2 421C>A genotype, although one of two homozygous variant allele carriers showed extensive accumulation of SN-38 and SN-38 glucuronide. Conclusions: The ABCG2 421C>A polymorphism appears to play a limited role in the disposition of irinotecan in European Caucasians. It is likely that the contribution of this genetic variant is obscured by a functional role of other polymorphic proteins.
Purpose: The purpose is to document the clinical, pathological, and genetic features of pancreatic carcinoma (PC) in carriers of a specific p16-Leiden mutation (a 19-bp deletion in exon 2 of the CDKN2A gene). Experimental Design: Clinical data and paraffin embedded tissue were obtained from 12 patients of p16-Leiden-positive families with PC. Because of the known 19-bp germ-line deletion, we could specifically analyze the genotype of the wild-type allele for loss of heterozygosity. K-ras codon 12 mutations were determined and immunohistochemical testing for p16, Tp53, Smad4, and cyclooxygenase 2 was performed. Results: The average age of subjects that developed PC (8 males) was 58 years (range, 43-74 years). Histology was considered as conventional ductal adenocarcinoma in 11 of 12 and neuroendocrine carcinoma (1 of 12). The carcinomas were located in the head (10 of 12), corpus (1 of 12), and tail (1 of 12) of the pancreas. The specific p16-Leiden mutation was confirmed in the tissue of all subjects. Loss of heterozygosity of the wild-type allele was present in 2 of 7 tumors analyzed. Immunostaining for p16 was negative in 10 of 10. Tp53 mutations were detected in 5 of 12. Smad4 was negative in 5 of 12 and cyclooxygenase 2 was overexpressed in 11 of 12. K-ras codon 12 mutations were present in 9 of 10 and in three precursor lesions even before abrogation of p16 protein expression was seen (one of three). Conclusions: The p16-Leiden deletion was associated with progression toward conventional ductal adenocarcinomas in all cases but one. Our observations might support the feasibility of early diagnosis of PC in p16-Leiden mutation carriers and might also indicate that chemoprevention needs consideration.

Purpose: Over the last several years, donor lymphocyte infusions have become the standard approach for patients with chronic myelogenous leukemia (CML) who relapse after allogeneic stem cell transplantation (SCT). Recent reports indicate that imatinib mesylate (Gleevec) can induce remissions in these patients as well. Less is known about the extent and durability of these responses. Experimental Design: We studied 15 patients treated with imatinib for recurrent CML after SCT, 10 patients with stable phase CML (SP-CML), 1 with accelerated phase (AP-CML), and 4 with blast phase (BP-CML). The dose of imatinib was 600 mg (n = 10) or 400 mg (n = 5) daily. Patients were followed for hematological, cytogenetic, and molecular response. A subset of responders was followed for changes in donor-derived hematopoietic chimerism. Results: Of the 10 patients with SP-CML, all achieved a hematological response. Within 3 months, five of these patients had achieved a complete cytogenetic response (CCR). By six months, 9 of 10 patients had achieved CCR. The BCR-ABL transcript could not be detected by reverse transcription-PCR in 7 of these 9 patients. Patients who achieved CCR showed evidence of conversion to complete donor chimerism. No patient developed graft-versus-host disease. With a median follow up of 25 months, all patients are alive and 9 of 10 patients remain in CCR. Only one of the 5 patients with AP/BP-CML achieved a complete cytogenetic response. Conclusions: We find that imatinib is well tolerated in patients with SP-CML who relapse after SCT. Responses were rapid, durable, and associated with conversion to full donor chimerism without graft-versus-host disease. Imatinib was far less effective in patients who relapsed with AP/BP-CML. Imatinib
should be evaluated as either an alternative or as an adjunct to donor lymphocyte infusions for patients with SP-CML who relapse after SCT.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/16/5537

Purpose: Productive infection of the human neurotropic polyomavirus JCPyV in oligodendrocytes leads to the development of progressive multifocal leukoencephalopathy, a fatal demyelinating disorder of the central nervous system. In addition to its role in viral infection, JCPyV T-antigen can transform cells in vitro and induce tumors in experimental animals in the absence of viral DNA replication and late gene expression. The goal of this study is to examine the presence of JCPyV DNA sequences and viral antigens in a series of human oligodendrogliomas.

Experimental Design: A total of 20 well-characterized oligodendrogliomas were examined for detection of the JCPyV genome by PCR and immunohistochemistry for expression of viral proteins. Results: Gene amplification has revealed the presence of JCPyV DNA sequences corresponding to the NH2-terminal of T-antigen in 15 of 20 samples. DNA sequences corresponding to late regions, which are responsible for production of the capsid protein, VP1, were detected in 14 of 20 samples. Sequencing of the viral control region determined the presence of JCPyV Mad-4 or JCPyVCY in these tumors. By immunohistochemistry, T-antigen expression was detected in the nuclei of tumor cells from 10 samples that also contained corresponding DNA sequences by PCR. Eleven of 20 tumors exhibited immunoreactivity for the late auxiliary gene product, agnoprotein. None of the samples showed immunoreactivity for the capsid proteins, ruling out productive infection of neoplastic cells by JCPyV. Conclusions: Collectively, these observations provide new evidence in support of the association of the oncogenic human neurotropic JCPyV and oligodendrogliomas.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/16/5537

Purpose: Granulosa cell tumors (GCTs) are relatively rare and are subtypes of the sex-cord stromal neoplasms. A better understanding of the molecular genetics underlying various steps in malignant transformation is critical to success in the battle against this disease. Changes in the status of methylation, known as epigenetic alterations, are one of the most common molecular alterations in human cancers, including GCTs. Chromosomal instability and microsatellite instability (MSI) are common in these GCTs. We tested the hypothesis that C[-&gt;T] transition polymorphism in the promoter region of cytosine DNA-methyltransferase-3B (DNMT3B) and its altered expression are also associated with hypermethylation of the genes. We also attempted to determine the relationship between MSI of ovarian carcinoma and hMLH1 hypermethylation in these tumors. Experimental Design: We studied chromosome instability in 25 GCTs by detecting gross chromosome rearrangements in cultured peripheral blood lymphocytes. MSI was assessed using six microsatellite markers (BAT25, BAT26, D2S123, D5S346, D11S1318, and D17S250). Using sensitive methylation-specific PCR, we searched for aberrant promoter hypermethylation in a panel of genes including p16, BRCA1, RASSF1A, ER-{alpha}, TMS1, TIMP3, Twist, GSTP1, AR, and hMLH1. Polymorphism in the DNMT3B gene was assessed by the PCR-RFLP method, and DNMT3B expression was studied by reverse transcription-PCR assay. Results: Chromosome instability was indicated by significantly higher frequencies of chromosome aberrations (6.24%; P < 0.001) compared with controls (2.12%). The most frequently observed changes include trisomy
14 and monosomy 22. MSI has been found in 19 of 25 tumors, and loss of heterozygosity has been found in 9 of 25 tumors. Frequencies of methylation in GCTs were 40% for p16 and ER-α; 36% for BRCA1 and RASSF1A; 28% for hMLH1; 24% for TIMP3, Twist, and GSTP1; and 20% in TMS1 and AR. TT genotype was found only in two cases; the remainder were either CC or CT type. There was no significant alteration in the expression of DNMT3B in these patients.

Conclusions: Coexistence of chromosome instability, MSI, and hypermethylation suggests that both genetic and epigenetic mechanisms may act in concert to inactivate the above-mentioned genes in these GCTs. These mechanisms can be an early event in the pathogenesis of these tumors, and it can be a critical step in the tumorigenic process. All these events might play an important role in early clinical diagnosis and in chemotherapeutic management and treatment of the disease. Larger studies may lend further understanding to the etiology and clinical behavior of these tumors.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/4/980

Purpose: Several genes are reported to be implicated in bladder carcinogenesis, including p53, p16INK4a, pRb, erbB-2, Cyclin D1, H-ras, EGFR, and c-myc. Gene alterations in plasma DNA identical to those observed within the tumor have been detected in various types of neoplasia. Experimental Design: We analyzed loss of heterozygosity in six microsatellite markers (D17S695, D17S654, D13S310, TH2, D9S747, and D9S161), p53 and K-ras mutations, and the promoter status of p14ARF and p16INK4a in the mononuclear normal blood cells, tumor, and plasma DNA of 27 bladder cancer patients. We also studied the distribution of several clinicopathological parameters in these patients in regard to molecular alterations. Results: Seventeen (63%) cases displayed the same alteration in plasma and tumor DNA (some patients showed more than one alteration simultaneously). Plasma p14ARF promoter hypermethylation was associated with the presence of multicentric foci (P = 0.03), larger tumors (P = 0.01), and relapse of the disease (P = 0.03). Plasma loss of heterozygosity was also linked to disease recurrence (P = 0.02).

Conclusions: The results indicate that p14ARF aberrant promoter methylation could be involved in bladder carcinogenesis and that plasma DNA is a potential prognostic marker in urinary bladder cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/9/2812

Purpose: The purpose of the study is to investigate the tolerability of interleukin 2 (IL-2) after intensive chemotherapy in elderly acute myeloid leukemia (AML) patients in first complete remission (CR). Experimental Design: AML patients [≥60 years in CR after induction and consolidation chemotherapy on Cancer and Leukemia Group B study 9420 were eligible if they had neutrophils [≥1 x 10^9/liters and platelets [≥75 x 10^9/liters. Patients received low-dose IL-2 (1 x 10^6 IU/m2/day s.c. for 90 days) or low-dose IL-2 with intermediate pulse doses (6-12 x 10^6 IU/m2/day s.c. for 3 days) every 14 days (maximum five pulses). In a subset of patients, we investigated the expression of NKG2D ligands by leukemic cells because they are likely important mediators of natural killer cytotoxicity. Results: Of 35 CR patients receiving IL-2, 34 were evaluable for toxicity. Median age was 67 (range, 60-76) years. Thirteen of 16 patients receiving low-dose IL-2 completed the planned therapy, and 11 of 18 who also received
intermediate pulse dose IL-2 therapy completed all five pulses. The spectrum of toxicity in both
groups was similar, with predominantly grade 1-2 fatigue, fever, injection site reactions, nausea,
anemia, and thrombocytopenia. Grade 3-4 hematological and nonhematological toxicity were
more frequent in patients also receiving intermediate pulse dose IL-2 therapy. Grade 3-4 fatigue
and hematological toxicity, although uncommon, were the major causes for discontinuing or
attenuating therapy. In 8 cases, mRNA for one or more NKG2D ligands was detected in leukemic
cells obtained at diagnosis before treatment. Conclusions: Low-dose IL-2, with or without
intermediate pulse dose therapy, given immediately after chemotherapy in first CR to elderly AML
patients is well tolerated. Expression of NKG2D ligands by leukemic cells was detected in the
majority of cases tested and should be assessed for correlation with response to IL-2 in future
studies.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/2/792

Purpose: The purpose of this research was to evaluate the predictive value of expression of
thymidylate synthase (TS) and other genes for response to raltitrexed (RTX). Experimental
Design: Twenty-five patients with metastatic colorectal cancer received RTX 3 mg/m2 3-weekly.
Pretreatment tumor biopsies were analyzed for TS, dihydropyrimidine dehydrogenase (DPD),
thymidine phosphorylase (TP), folypolyglutamate synthetase, and reduced folate carrier mRNA
expression by real-time reverse transcription-PCR. TS protein expression was evaluated by
immunohistochemistry using a polyclonal TS antibody. Results: Twenty patients were evaluable
for response and gene expression. Six of 20 (30%) achieved a partial response. Median
TS/{beta}-actin was 5.7 x 10³ (range, 2.2-42 x 10³). Median TS/{beta}-actin was 3.7 x 10³ in
responding patients and 6.1 x 10³ in nonresponders (P = 0.048). Five of 6 patients with
TS/{beta}-actin [\leq]4.1 x 10³ and 1 of 14 with higher values responded (P = 0.002). Overall
survival was 21.7 months in patients with TS/{beta}-actin [\leq]4.1 x 10³ and 5.7 months in
patients with higher values (P = 0.013). No correlations were seen between expression of TP,
DPD, reduced folate carrier, or folypolyglutamate synthetase mRNA and response or survival.
Weak TS staining was seen in 10 of 21 tumors evaluable for immunohistochemistry, including 5
responders All 4 of the patients with both weak staining and TS/{beta}-actin [\leq]4.1 x 10³
responded. Conclusions: High TS mRNA expression predicts nonresponse to RTX. By contrast
with 5-fluorouracil, high levels of TP and DPD mRNA are not associated with RTX resistance.
Limited genomic fingerprinting could optimize single-agent therapy, allowing combinations to be
reserved for high TS-expressing patients or for treatment failures, with potential reductions in
toxicity and cost.

Carcinoma: No Mutations in the Class I {beta}-Tubulin Gene but Changes in Tubulin Isotype

http://clincancerres.aacrjournals.org/cgi/content/abstract/11/9/3439

Purpose: The primary purpose of this study was to determine whether mutations of the class I
{beta}-tubulin gene may be implicated in the inherent resistance to tubulin-binding agents (TBA)
in renal cancer, with a small number of samples and cell lines also being examined for class I and
III {beta}-tubulin isotype protein expression. Experimental Design: DNA was extracted from 90
renal tumors and the class I {beta}-tubulin gene analyzed for mutations. For each sample, eight
PCRs were used to cover the complete coding sequence with intronic primers ensuring highly
homologous pseudogenes were not coamplified. Additionally, expression levels of class I and III
{beta}-tubulin isotypes in 17 matched normal and malignant renal samples and a panel of renal cell carcinoma cell lines with differing intrinsic resistance to the TBAs was examined by Western blotting. Results: Four polymorphic sequence changes of the class I {beta}-tubulin gene were identified with no mutations. Class I protein expression levels were higher in tumor tissue versus normal tissue, whereas class III expression showed no consistent change. In renal cancer cell lines, a significant correlation between class III isotype expression and vinblastine sensitivity was observed. Conclusions: These results do not support a role for mutations in the class I {beta}-tubulin gene in the intrinsic resistance of renal cancer to TBAs. Class III isotype expression may be implicated in resistance in vitro but in vivo, changes in class I isotype expression in renal cell carcinoma tissue may support a role in resistance to the TBAs and warrants further investigation.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/21/7329

Purpose: Increased levels of matrix metalloproteinase 1 (MMP-1) expression have been associated with poor outcome in chondrosarcoma. The existence of a single nucleotide polymorphism creating an Ets-binding site in the MMP-1 promoter may be one mechanism for elevated MMP-1 transcription. The aim of our study was to identify the prevalence of this single nucleotide polymorphism (SNP) in chondrosarcoma patients, to determine its correlation with disease outcome, and to discern whether it could serve as a prognostic marker in patients with chondrosarcoma. Experimental Design: Sixty-seven chondrosarcoma specimens were selected sequentially from an established tumor bank with a median duration of 47 months follow-up (range, 24 to 179 months). DNA was extracted, amplified with PCR, and sequenced to determine presence (GG) or absence of the Ets-binding site created by the SNP. Results: Eighteen (27%) samples were homozygous for the absence of the Ets site, 34 (51%) were heterozygous for the SNP, and 15 (22%) were homozygous for the SNP. The 5-year overall survival rate for patients was 78, 80, and 84%, respectively (P = 0.5527). The disease-free survival rate was 16, 63, and 76%, respectively (P = 0.0801). The 5-year disease-free survival rate for patients with the homozygous G/G genotype was 16%, compared with 71% for patients who were either homozygous or heterozygous for the GG allele (P = 0.0444). Conclusions: Despite a statistical correlation between MMP-1 gene expression and outcome in chondrosarcoma, this study demonstrates an absence of a correlation between the presence of the SNP and prognosis in patients with chondrosarcoma.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/10/3479

Purpose: Molecular profiling of alterations associated with lung cancer holds the promise to define clinical parameters such as response to treatment or survival. Because <5% of small cell lung cancers and <30% of non-small cell lung cancers are surgically resectable, molecular analysis will perforce rely on routinely available clinical samples such as biopsies. Identifying tumor mutations in such samples will require a sensitive and robust technology to overcome signal from excess amounts of normal DNA. Experimental Design: p53 mutation status was assessed from the DNA and RNA of biopsies collected prospectively from 83 patients with lung cancer. Biopsies were obtained either by conventional bronchoscopy or computed tomography-guided percutaneous biopsy. Matched surgical specimens were available for 22 patients. Three
assays were used: direct sequencing; a functional assay in yeast; and a newly developed PCR/ligase detection reaction/Universal DNA array assay. Results: Using the functional assay, p53 mutation was found in 62% of biopsies and 64% of surgical specimens with a concordance of 80%. The sensitivity of the functional assay was determined to be 5%. Direct sequencing confirmed mutations in 92% of surgical specimens but in only 78% of biopsies. The DNA array confirmed 100% of mutations in both biopsies and surgical specimens. Using this newly developed DNA array, we demonstrate the feasibility of directly identifying p53 mutations in clinical samples containing <5% of tumor cells. Conclusions: The versatility and sensitivity of this new array assay should allow additional development of mutation profiling arrays that could be applied to biological samples with a low tumor cell content such as bronchial aspirates, bronchoalveolar lavage fluid, or serum.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/12/4015

Purpose: The issue of whether colon and rectal cancer should be considered as a single entity or two distinct entities is still debated, and there is a need to improve studies addressing the heterogeneity of the pathogenetic pathway leading to sporadic colorectal cancers (SCRCs) as well as to identify biological and/or molecular differences between colon and rectal cancers. Experimental Design: Specimens of SCRCs were analyzed for somatic mutations in APC, K-ras, and TP53 genes and loss-of-heterozygosity of chromosome 18. Results: Eleven SCRCs showed microsatellite instability. APC mutation frequency was significantly lower in microsatellite instability (MIN+) than in MIN- SCRCs. All MIN- SCRCs showed (beta)-catenin overexpression. A combined analysis of the biomarkers revealed two pathways mainly represented by MIN- SCRCs and differently followed on the basis of tumor location, APC-K-ras-TP53-Ch18q and APC-TP53-Ch18q. Conclusions: The APC-(beta)-catenin pathway is inactivated in MIN- SCRCs and represents the first hit of SCRC development. Two preferential pathways followed by SCRCs occur, one K-ras dependent, in agreement with the Fearon and Vogelstein model, and the other K-ras independent. Significant differences between colon and rectal tumors occur in our series of MIN- SCRCs. The different pathways observed and their distribution can be summarized as follows: (a) K-ras mutations were more commonly detected in colon than in rectum; (b) the number of mutations detected was significantly higher in colon than in rectal tumors; and (c) a mutational pattern restricted to the APC gene was more common in rectal than in colon tumors. This molecular characterization can be translated into a clinical setting to improve diagnosis and to direct a rationale pharmacological treatment.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/19/6551

Purpose: Although NY-ESO-1 was isolated from an esophageal carcinoma patient, its expression in this type of cancer and its immunogenicity in esophageal cancer patients have not yet been fully elucidated. We report here the frequency of NY-ESO-1 mRNA and protein expression in esophageal cancer and the presence of NY-ESO-1-specific immune response in patients. Experimental Design: One hundred twenty three esophageal squamous cell carcinoma specimens were analyzed for the expression of NY-ESO-1 mRNA by conventional and real-time reverse transcription-PCR and the expression of protein by immunohistochemistry and Western blot. Sera and peripheral blood lymphocytes from 51 patients were analyzed for the NY-ESO-1 antibody production by enzyme-linked immunosorbent assay and NY-ESO-1 T cell response by
enzyme-linked immunospot assay. Survival analyses were also performed. Results: NY-ESO-1 mRNA was expressed in 41 of 123 (33%) esophageal squamous cell carcinoma specimens, and its expression was found at higher frequency in well-differentiated and moderately differentiated type of cancer. No mRNA copy was detected in any of the adjacent normal tissues. Twenty-one of 24 (87.5%) NY-ESO-1 mRNA-positive tumors were stained positively by immunohistochemistry. Correlation between the level of NY-ESO-1 mRNA expression and the degree of immunohistochemistry positivity was observed. Antibody production was observed in 2 patients with tumors that showed protein expression. Furthermore, a CD8 T-cell response against NY-ESO-1 was observed in 1 of the 2 seropositive patients. Conclusions: The high expression frequency of NY-ESO-1 mRNA and protein indicates NY-ESO-1 as a feasible vaccine target in esophageal cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/9/3233

Purpose: Growth factors, particularly insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR) in some nonendocrine and a few endocrine tumors, are thought important in recurrence, growth, and aggressiveness. Whether this is true of neuroendocrine tumors such as gastrinomas is unclear. The aim of this study was to address this question in gastrinomas. Experimental Design: IGF-I and IGF-IR expression in gastrinomas from 54 patients with Zollinger-Ellison syndrome were analyzed and correlated with clinical/tumor characteristics. IGF-I and IGF-IR mRNA levels were determined by competitive reverse transcription-PCR. IGF-IR expression, assessed by immunohistochemistry, was done on a subset. Results: IGF-IR mRNA was found in 100% and IGF-I in 89%. IGF-I mRNA expression varied by >254-fold, IGF-IR by 2,670-fold, and the levels correlated in a given tumor. The IGF-IR level was lower in gastrinomas of patients who were rendered disease free and increased levels correlated with tumor growth, aggressiveness, extent, and with liver metastases. Increased IGF-I levels correlated with increased growth, tumor extent, and aggressiveness. Neither IGF-IR nor IGF-I levels correlated with tumor location, size, or its clinical/functional features. The IGF-IR level was found in 31 of 32 tumors (97%) by immunohistochemistry. Conclusions: These results indicate that IGF-I and IGF-IR are expressed in almost all gastrinomas. Furthermore, assessment of IGF-I/IGF-IR expression in gastrinomas may be clinically useful in identifying those patients with more aggressive tumors who might benefit from more aggressive treatment.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/7/2545

Purpose: Prostate-specific antigen (PSA) test has become a widely used screening test in prostate cancer (CaP). However, low specificity of serum PSA leads to many false-positive and false-negative results and clinical uncertainty. Development of CaP-specific diagnostic and prognostic markers is needed. Detection of circulating PSA-expressing cells (CPECs) in blood and bone marrow of CaP patients has potential in molecular diagnosis and prognosis. Our novel observations of the frequent presence of CPECs in CaP patients with organ-confined disease by reverse transcription (RT)-PCR-PSA assay in epithelial cells enriched from peripheral blood (ERT-PCR/PSA) have led us to test the hypothesis that CPECs have diagnostic potential for CaP. Experimental Design: Epithelial cells from peripheral blood of radical prostatectomy patients or prostate biopsy patients were isolated using antiepithelial cell antibody, Ber-EP4-coated
magnetic beads, and total RNA specimens from these cells were analyzed for PSA expression by RT-PCR. Results: Peripheral blood specimens of 108 of 135 (80.0%) CaP patients were positive in ERT-PCR/PSA assay. Peripheral blood specimens from 45 control men were virtually negative (97.8%). In the blinded investigation, 84 patients who had biopsy for suspicion of CaP were evaluated by ERT-PCR/PSA assay. Eighteen of 22 (81.8%) patients with biopsy-proven CaP were positive, and 54 of 62 (87.1%) patients with biopsy negative for CaP were negative in this assay (P < 0.001). Conclusions: Our study provides intriguing novel results showing that the majority of patients with clinically organ-confined CaP contain CPECs. Strong concordance between the biopsy results and ERT-PCR/PSA assay (sensitivity 81.8%; specificity 87.1%) suggests a potentially new diagnostic application of this type of assay in CaP diagnosis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/15/5582

Purpose: Recent studies have found an association between certain TP53 mutations and resistance to anthracycline-based primary medical therapy in breast cancer. The purpose of this study was to investigate whether TP53 mutational status also might influence the response to a non-anthracycline-containing regimen in primary breast cancer. Experimental Design: Thirty-five patients with locally advanced breast cancer were investigated for TP53 mutations before receiving combination chemotherapy with 5-fluorouracil (1000 mg/m2 on days 1 and 2) and mitomycin (6 mg/m2 on day 2), administered every 3 weeks for 2-10 cycles in the neoadjuvant setting. Results: Mutations in the TP53 gene, in particular those affecting loop domains L2 or L3 of the p53 protein, were associated with lack of response to chemotherapy (i.e., increase in the diameter product of tumor lesion by >25%; P = 0.177 for all mutations and P = 0.006 for those affecting L2/L3 domains, respectively). No statistically significant correlation between TP53 LOH and response to therapy was seen. Conclusion: This study revealed a significant association between lack of response to 5-fluorouracil and mitomycin and mutations affecting the L2/L3 domains of the p53 protein. Together with our previous finding that such mutations predict resistance to weekly doxorubicin, our data suggest that mutations affecting this particular domain of the p53 protein may cause resistance to several different cytotoxic compounds applied in breast cancer treatment.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/4/1259

Purpose: Dysregulated expression of steroid receptor transcriptional coactivators and corepressors has been implicated in tamoxifen resistance, especially in estrogen receptor (ER) {alpha}-positive breast cancer patients. Therefore, expression analysis of these ER{alpha} coregulators may identify new predictors of the response to tamoxifen treatment. Experimental Design: We measured mRNA levels of 16 coactivator and 11 corepressor genes with a real-time quantitative reverse transcription-PCR method in 14 ER{alpha}-positive breast tumors. Three selected coactivator genes (TIF2, AIB1, and GCN5L2) and two corepressor genes (NCOR1 and MTA1L1) were additionally investigated in a well-characterized series of ER{alpha}-positive unilateral invasive primary breast tumors from 99 postmenopausal patients who only received tamoxifen as adjuvant hormone therapy after primary surgery. We sought relationships between mRNA levels of the coregulators and those of molecular markers, including ER{alpha}, ER{beta},


CCND1, and ERBB2. Results: ER{alpha} coregulator expression was unrelated to age, histological grade, lymph node status, and macroscopic tumor size. The relationship between mRNA expression of the coregulators, and ER{alpha} and {beta} only showed a significant positive correlation between GCN5L2 and ER{alpha} (P = 0.015). mRNA levels of CCND1 correlated with those of all of the coregulators studied (P < 0.05 or trend), whereas ERBB2 mRNA levels only correlated with AIB1 mRNA levels (P = 0.011). Low NCOR1 expression (versus intermediate and high) was associated with significantly shorter relapse-free survival (log-rank test; P = 0.0076). The prognostic significance of low NCOR1 expression persisted in Cox multivariate regression analysis (P = 0.043). Conclusions: These findings point to NCOR1 as a promising independent predictor of tamoxifen resistance in patients with ER{alpha}-positive breast tumors.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/12/4415

Purpose: Three genes, namely DNA methyltransferase (DNMT) 1, DNMT3A, and DNMT3B, coding for DNMTs that affect promoter methylation status are thought to play an important role in the development of cancers. Little is known of the biological and clinical significance of these genes in human breast cancer. Experimental Design: We used real-time reverse transcription-PCR assays to quantify the mRNA expression of the three DNMT genes in a series of 130 breast cancer patients. We also sought relationships between mRNA levels of the DNMTs and those of 20 target genes involved in the DNMT pathway (subgroup of 46 breast tumors). Results: The DNMT3B gene showed the highest range of expression (81.8 compared with 16.6 and 14 for DNMT1 and DNMT3A, respectively). DNMT3B was overexpressed in 30% of the patients (5.4 and 3.1% for DNMT1 and DNMT3A, respectively). DNMT3B overexpression was significantly related to Scarff, Bloom, and Richardson histopathological grade III (P = 0.002), ER{alpha} negativity (P = 0.0015), and strong MKI67 expression (P = 3 x 10-6). In univariate analysis, DNMT3B overexpression was associated with poor relapse-free survival in the subgroup of patients who received adjuvant hormone therapy (with or without chemotherapy; P = 0.0064). Although the poor prognosis associated with DNMT3B overexpression was confirmed by univariate analysis in an independent series of 98 postmenopausal women exclusively treated with adjuvant tamoxifen therapy (P = 0.0036), DNMT3B expression status did not persist as an independent prognostic factor in multivariate analysis. Conclusions: Although we failed to identify underexpression of specific target genes associated with DNMT increasing expression, the frequent overexpression of DNMT3B in this breast tumor series points to DNMT3B as a potential new therapeutic target in breast cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/7/2673

Purpose: We assess the feasibility of a urinary test for prostate cancer detection in a high-risk patient cohort based on methylation-specific PCR analysis of the π class glutathione S-transferase (GSTP1) gene promoter. Experimental Design: A total of 45 men underwent transrectal ultrasound-guided biopsy of the prostate for suspected malignancy. Clean-catch voided urine specimens were prospectively collected from each patient immediately after biopsy. Genomic DNA was isolated from urine specimens and subjected to sodium bisulfite modification. Methylation of the GSTP1 promoter was examined in a blinded manner by methylation-specific PCR analysis and correlated with pathology results, and clinical information was obtained from
Results: Methylation of GSTP1 in the urine was detected in a total of 18 of 36 (50%) informative cases. A total of 7 of 18 (39%) patients with prostate adenocarcinoma identified on their initial biopsy had detectable urinary GSTP1 methylation (58% sensitivity among informative cases). Abnormal urinary GSTP1 methylation was also detected in 7 of 21 (33%) patients without evidence of cancer on biopsy and in 4 of 6 (67%) patients diagnosed with atypia or high-grade prostatic intraepithelial neoplasia. Conclusions: We have demonstrated the feasibility of a novel, noninvasive molecular approach for the detection of epigenetic changes associated with prostate cancer. A screening test based on GSTP1 methylation in the urine specimens of patients with suspected prostate malignancy may be a useful adjunct to serum screening tests and digital rectal examination findings for identification of men at increased risk of harboring cancer despite a negative biopsy. This molecular assay has potential application for stratification of patients into low- and high-risk groups for surveillance versus repeat biopsy.


methods were rather complicated. We have improved the RT-PCR assay combining three prostate-associated molecules, prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA) to reveal patients with poor prognosis.

Experimental Design: Peripheral blood samples were obtained from 129 patients including 58 cases of PCa and 71 cases of nonmalignant disorders. Total RNA was extracted from 1 ml of whole blood using a commercially available kit. Results: The sensitivity of PSA-, PSMA-, and PSCA-nested RT-PCR was verified with positive signals of a single LNCaP cell in 1 ml of female blood sample. PSA-, PSMA-, and PSCA-mRNA were detected in 7 (12.1%), 12 (20.7%), and 8 (13.8%) PCa, and in 1, 2, and 0 samples in nonmalignant disorders, respectively. Among 58 PCa patients, each PCR indicated the prognostic value in the hierarchy of PSCA>PSA>PSMA RT-PCR, and extraprostatic cases with positive PSCA PCR indicated lower disease-progression-free survival than those with negative PSCA PCR. Conclusions: The present findings suggest that PSCA PCR would be most promising for the molecular staging of PCa. The present RT-PCR is a highly cost-effective and rapid procedure, enabling the molecular staging of PCa with RT-PCR as a diagnostic routine.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/17/5777

Purpose: Reverse-transcriptase PCR (RT-PCR) assays for carcinoembryonic antigen (CEA) have been described to identify lymph node micrometastases. These assays are not quantitative and can be confounded by false-positive results. The purpose of this study was to determine whether quantification of CEA in lymph nodes could more readily identify clinically relevant groups.

Experimental Design: Specimens included 400 lymph nodes from 64 patients undergoing colon resections. Specimens were tested by immunohistochemistry and by RT-PCR using nested primers for CEA. Specimens from 59 patients that were positive by nested RT-PCR were further quantified by detection of CEA mRNA fluorescence increase at a threshold PCR cycle. Results: CEA was detected by nested RT-PCR analysis in 4 of 34 (12%) nodes of nonneoplastic disease, 2 of 13 (15%) nodes from T1N0 patients, 32 of 81 (40%) nodes of T2N0 patients, 49 of 109 (45%) nodes from T3N0 patients, and 92 of 163 (56%) nodes from T1-4N1-2 patients. The overall presence of any RT-PCR-detectable CEA in nodes did not differentiate patient groups. Immunohistochemistry was positive in nodes from 7% of T3N0 patients and 100% of T1-3N1-2 patients. CEA quantification revealed that 0 of 7 patients with nonneoplastic disease and 2 of 17 (12%) patients with stage I T1-2N0 cancers had one or more lymph nodes with \( [\geq] 1.0 \times 10^2 \) CEA transcripts per sample. In contrast, 4 of 13 (31%) patients with stage II T3N0 cancer and 10 of 22 (45%) stage III patients with known metastases had lymph nodes with \( [\geq] 1.0 \times 10^2 \) CEA transcripts. Conclusions: These data suggest that quantification of CEA levels in lymph nodes may more accurately identify patients at risk for cancer recurrence than does routine nested RT-PCR or immunohistochemistry.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/14/4734

Purpose and Experimental Design: The molecular mechanisms by which the p14ARF gene is altered in non-small cell lung cancer (NSCLC) are complex and unclear. Using genetic and epigenetic analyses, we examined various molecular alterations including the loss of protein and
mRNA expression, and 5'CpG hypermethylation, allelic imbalance, and mutation of the p14ARF gene in a series of 102 NSCLC samples, in parallel with clinicopathological and prognostic analyses. To clarify the biological significance of p14ARF alterations, its relationship with p16INK4a and p53 alterations was also examined. Results: We found that 34% of NSCLC patients had aberrant P14ARF protein expression, which was more frequent in adenocarcinomas (AD; 44%) than in squamous cell carcinomas (22%; P = 0.024). A high concordance was observed between alterations in protein and mRNA expression and 5'CpG hypermethylation (P ≤ 0.001). The p14ARF hypermethylation inversely correlated with P53 overexpression (P = 0.001). This mutually exclusive relationship for alteration between p14ARF and p53 was also supported by a worse prognosis of AD patients with positive P14ARF expression (P = 0.01) and of AD patients with P53 overexpression (P = 0.006). Our data also indicated that hemizygous/homozygous deletion and mutation in the p14ARF gene occurred at 26%, 9%, and 0%, respectively, of microdissected NSCLCs. Conclusions: Our data suggest that p14ARF 5'CpG hypermethylation is the predominant mechanism involved in the aberrant expression of the p14ARF gene. In addition, p14ARF 5'CpG hypermethylation occurs inversely to P53 overexpression.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/18/6013

Purpose: The purpose is to characterize alterations of the annexin I gene, its mRNA, and protein expression in esophageal squamous cell carcinoma. Experimental Design: Fifty-six cases of esophageal squamous cell carcinoma were analyzed using four microsatellite markers flanking the annexin I gene (9q11-q21) to identify loss of heterozygosity. In addition, we performed (a) single-strand conformation polymorphism and DNA sequencing along the entire promoter sequence and coding region to identify mutations, (b) real-time quantitative reverse transcription-PCR of RNA from frozen esophageal squamous cell carcinoma tissue (n = 37) and in situ hybridization (n = 5) on selected cases to assess mRNA expression, and (c) immunohistochemistry (n = 44) to evaluate protein expression. The prevalence of the allelic variants identified in the first 56 patients was refined in 80 additional esophageal squamous cell carcinoma patients and 232 healthy individuals. Results: Forty-six of 56 (82%) esophageal squamous cell carcinoma patients showed loss of an allele at one or more of the four microsatellite markers; however, only one (silent) mutation was seen. Two intragenic variants were identified with high frequency of allelic loss (A58G, 64%; L109L, 69%). Thirty of 37 (81%) esophageal squamous cell carcinoma patients showed reduced annexin I mRNA expression, which was confirmed by in situ hybridization, whereas annexin I protein expression was reduced in 79% of poorly differentiated tumor cell foci but in only 5% of well-differentiated tumor foci, although allelic loss on chromosome 9 was found in both tumor grades. Conclusions: Allelic loss of annexin I occurs frequently, whereas somatic mutations are rare, suggesting that annexin I is not inactivated in esophageal squamous cell carcinoma via a two-hit mechanism. A decrease in annexin I protein expression was confirmed, consistent with a quantitative decrease in mRNA expression, and appeared to be related to tumor cell differentiation. We conclude that annexin I is not the tumor suppressor gene corresponding to the high levels of loss of heterozygosity observed on chromosome 9 in esophageal squamous cell carcinoma; however, dysregulation of mRNA and protein levels is associated with this tumor type.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/17/5845
Purpose: Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant syndrome of familial malignancies. Colorectal and endometrial cancers are most frequently observed. The syndrome results mainly from germ-line mutations in DNA mismatch repair genes. A common G-to-C polymorphism at codon 72 in the p53 gene has been associated with increased risk for lung, nasopharyngeal, oral, prostate, and breast cancers and may be a marker for genetic susceptibility to colorectal cancer. We studied the influence of this p53 polymorphism on HNPCC age of onset.

Experimental Design: We determined the p53 genotype of 92 Caucasian mismatch repair mutation carriers, of which, 47 had colorectal cancer. The subjects were genotyped by single-strand conformational polymorphism analysis. We tested the association between age of onset and the p53 genotypes by comparing Kaplan-Meier survival curves, evaluating the homogeneity of the curves using the log-rank test and Wilcoxon’s test, and estimating the association using the Cox proportional hazards regression model to adjust for potential demographic confounding factors.

Results: The HNPCC patients who were heterozygous developed their colorectal cancer 13 years earlier than HNPCC patients who were homozygous for the wild-type allele. Conclusions: Combining knowledge of an individual's p53 genotype with information on other genetic and environmental risk factors may improve risk estimates and help to identify individuals who are genetically susceptible to developing HNPCC at an earlier age.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/1/44

Purpose: The role of growth factors in ovarian cancer development and progression is complex and multifactorial. We hypothesized that new growth factors may be identified through the molecular analysis of ovarian tumors as they exist in their native environment. Experimental Design: RNA extracted from microdissected serous low malignant potential (LMP) and invasive ovarian tumors was used to construct cDNA libraries. A total of 7300 transcripts were randomly chosen for sequencing, and those transcripts were statistically evaluated. Reverse transcription-PCR and immunohistochemistry were used to validate the findings in tumor tissue samples. Ovarian cancer cell lines were used to test gene effects on monolayer growth, proliferative capacity, and density-independent growth. Results: Analysis of the pooled library transcripts revealed 26 genes differentially expressed between LMP and invasive ovarian cancers. The granulin-epithelin precursor [GEP/PC-cell derived growth factor (PCDGF)] was expressed only in the invasive ovarian cancer libraries (P < 0.028) and was absent in the LMP libraries (0 of 2872 clones). All of the invasive tumor epithelia, 20% of the LMP tumor epithelia, and all of the stroma from both subsets expressed GEP by reverse transcription-PCR. Immunohistochemical staining for GEP was diffuse and cytosolic in invasive ovarian cancer tumor cells compared with occasional, punctate, and apical staining in LMP tumor epithelia. Antisense transfection of GEP into ovarian cancer cell lines resulted in down-regulation of GEP production, reduction in cell growth (P < 0.002), decrease in the S-phase fraction (P < 0.04), and loss of density-independent growth potential (P < 0.01). Conclusion: cDNA library preparation from microdissected tumor epithelium provided a selective advantage for the identification of growth factors for epithelial ovarian cancer. Differential granulin expression in tumor samples and the antiproliferative effects of its antisense down-regulation suggest that GEP may be a new autocrine growth factor and molecular target for epithelial ovarian cancer.

Purpose: Although an important risk factor for oral cancer is the presence of epithelial dysplasia, many lesions will not progress to malignancy. Matrix metalloproteinases (MMPs) are zinc-dependent proteinases capable of digesting various structural components of the extracellular matrix. Because MMPs are frequently overexpressed in oral squamous cell carcinoma (SCC), we hypothesized that they are also overexpressed in oral dysplasias; we also hypothesized that those dysplasias that progress to oral cancer express higher levels of MMPs than those lesions that do not progress. Experimental Design: In this retrospective study, we examined changes in MMP-1, -2, and -9 mRNA expression using quantitative TaqMan reverse transcription-polymerase chain reaction in 34 routinely processed oral dysplasias and 15 SCCs obtained from 34 patients. After several years of close follow-up, 19 dysplasias progressed to oral SCC and 15 did not. Results: Overall, MMP-1 mRNA was overexpressed (>2-fold) in 24 of 34 (71%) dysplasias and 13 of 15 (87%) oral SCCs. MMP-2 overexpression was seen in 11 of 34 (32%) dysplasias and 7 of 15 (47%) cancers; for MMP-9, overexpression was identified in 29 of 34 (85%) dysplasias and 15 of 15 (100%) cancers. MMP-1 and -9 levels were significantly higher in the SCCs compared with all oral dysplasias (P = 0.004 and P = 0.01, respectively). MMP-1 and -9 mRNA levels were significantly higher in the oral dysplasias that progressed to oral cancer compared with those that did not (P = 0.04 and P = 0.002, respectively). Conclusions: Levels of MMP-1 and -9 mRNA may be markers of malignant transformation of oral dysplasia to oral cancer.


Purpose: The functional expression of CXCR4, which plays roles in cell migration and proliferation in response to its unique ligand stromal cell-derived factor-1 (SDF-1), has been reported in variety of carcinomas. However, CXCR4 expression and its functional role in head and neck squamous cell carcinomas (HNSCC) remain unclear. In this study, we investigated CXCR4 expression and analyzed its functions in HNSCC cell lines. We also attempted to regulate CXCR4 expression using cytokines, such as interleukin-1\{beta\}, tumor necrosis factor-\{alpha\}, and IFN-\{gamma\}. Finally, we investigated correlation between CXCR4 expression and clinical features in patients with HNSCC. Experimental Design: Six HNSCC cell lines were used in this study. Reverse transcription-PCR and flow cytometry analysis were shown for CXCR4 expressions with or without stimulations of cytokines. SDF-1-mediated cell migration was assayed in Matrigel-coated chemotaxis chamber. The SDF-1-mediated cell proliferation was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The SDF-1-mediated signaling pathways were analyzed by Western blot analysis. Biopsy specimens from 56 patients with HNSCC were used for immunohistologic analysis. Results: The significant CXCR4 expression was found in HSQ-89, IMC-3, and Nakamura cells. The SDF-1-mediated cell migration and proliferation were observed in CXCR4-positive cells. SDF-1 also promoted rapid phosphorylation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways in CXCR4-positive cells. SDF-1-mediated cell migration and proliferation of CXCR4-positive cells were inhibited by neutralization of CXCR4. Among three cytokines tested, IFN-\{gamma\} significantly reduced CXCR4 expression and SDF-1-induced cell migration and proliferation of CXCR4-positive cells. Immunohistologic analysis revealed that patients with advanced neck status and patients who developed distant metastases showed significantly higher CXCR4 expression, and the cause-specific survival of patients with CXCR4-expression was significantly shorter. Furthermore, multivariate analysis confirmed that CXCR4 positive was the independent factor for cause-specific death. Conclusion: Our results may provide an insight into future therapeutic agent that inhibits tumor metastasis and progression via down-regulating CXCR4 expression in patients with HNSCC.

http://clincancerres.aacrjournals.org/cgi/content/abstract/8/1/258

Although the receptor for interleukin-4 (IL-4R) is highly expressed on solid human cancer cells, its significance and internalization function is still unclear. To address these issues, we reconstituted Chinese hamster ovarian (CHO-K1) cells with various components of the IL-4R by transient transfection and performed internalization assays using radiolabeled IL-4. Radiolabeled IL-4 internalized through the IL-4R(alpha) chain in a time-dependent manner. When the IL-4R(alpha) chain was cotransfected with the IL-13R(alpha)1 or -{gamma}c chain, the IL-4 internalization level was identical to IL-4R(alpha) transfectants, suggesting that the IL-4R(alpha) chain plays a major role in IL-4 internalization. These results were confirmed by determining the cytotoxicity of a chimeric protein composed of IL-4 and a mutated form of Pseudomonas exotoxin [IL4(38-37)-PE38KDEL] in CHO-K1 cells transfected with increasing concentrations of IL-4R(alpha) cDNA. To use the internalization property of the IL-4R(alpha) chain in the context of IL-4R-targeted cytotoxin therapy, we transiently transfected pancreatic and brain tumor cells with IL-4R(alpha) chain. Surprisingly, these tumor cells acquired 4-75-fold higher binding activity to IL-4 compared with control cells. Consequently, the cytotoxic activity of IL-4 toxin to these cancer cells was enhanced 5-13-fold compared with control cells as assessed by protein synthesis inhibition and clonogenic assays. Taken together, a combination approach that involves transfer of the IL-4R(alpha) gene and IL-4R-targeted cytotoxin therapy may serve as a novel approach for cancer therapy.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/11/3503

Previous studies have demonstrated that human lung tumor cell lines express interleukin 4 (IL-4) receptors, and IL-4 can mediate modest to moderate antiproliferative activity in vitro and in vivo in animal models of human lung tumors. On the basis of these studies, IL-4 was tested in clinical trials; however, it showed little antitumor activity in lung cancer patients. In the present study, we examined the expression of IL-4 receptors (IL-4Rs) in lung tumor samples and normal lung tissues and tested whether an IL-4R targeted agent will have better antitumor activity in vitro and in vivo compared with IL-4. IL-4R expression was tested by immunohistochemistry in 54 lung tumor samples and normal lung tissues in a tissue array, by reverse-transcription PCR and Northern blot analyses in lung tumor cell lines. Cytotoxic activity of IL-4 cytotoxin [IL-4(38-37)-PE38KDEL], composed of a circular permuted IL-4 and a mutated form of Pseudomonas exotoxin (PE38KDEL) was tested by protein synthesis inhibition and clonogenic assays in seven lung tumor cell lines. Antitumor activity of IL-4 cytotoxin was tested in vitro and in immunodeficient animal models of human lung tumors. We observed that IL-4Rs are expressed at higher levels in situ in lung tumor samples compared with normal lung tissues and IL-4 cytotoxin is highly and specifically cytotoxic to lung tumor cell lines in vitro. Intratumoral and i.p. administration of IL-4 cytotoxin to immunodeficient mice with s.c. established human lung H358 non-small cell lung cancer tumors mediated considerable antitumor activity in a dose-dependent manner with the higher dose producing durable complete responses. On the other hand, H460 non-small cell lung cancer tumors expressing low levels of IL-4R did not respond to IL-4 cytotoxin therapy. Because IL-4 cytotoxin mediates its antitumor activity through IL-4R, and a variety of lung tumors expressed high levels of IL-4R, we propose testing the safety of this agent in patients with lung cancer.
The epithelial cell adhesion molecule (Ep-CAM) exhibited an ovarian cancer:normal human ovarian surface epithelium ratio of 444. For validation studies, real-time quantitative PCR analysis and immunohistochemistry were performed in normal and malignant ovarian epithelial cell lines and tissues. To evaluate the potential of the Ep-CAM autoantibody as a tumor marker, we examined the amount of Ep-CAM autoantibody in serum samples obtained from ovarian cancer patients and normal controls by an ELISA. Real-time quantitative PCR analysis revealed significant overexpression of Ep-CAM mRNA in cancer cell lines (P < 0.001) and microdissected cancer tissues (P < 0.05), compared with that in cultured normal human ovarian surface epithelium and microdissected germinal epithelium, respectively. Immunolocalization of the Ep-CAM autoantibody showed that the sera of ovarian cancer patients expressed higher levels of Ep-CAM autoantibody than benign tumor patients and normal controls (P < 0.05). The levels of Ep-CAM autoantibody found were as follows: 0.132 in 52 patients with ovarian cancer, 0.098 in 26 cases with benign gynecologic disease, and 0.090 in 26 normal women. This investigation has shown that the Ep-CAM autoantibody was found to be associated with ovarian cancer and suggested that future research assessing its clinical usefulness would be worthwhile.

The extent of lymph node metastasis is a major determinant in the prognosis of oral squamous cell carcinoma (OSCC). Abnormalities of cell adhesion molecules are known to play an important role in invasion and metastasis of cancer cells through the loss of cell-to-cell adhesion. In this study, we isolated highly invasive clones from an OSCC cell line established from a lymph node metastasis by using an in vitro invasion assay method and compared the abnormalities of cell adhesion molecule E-cadherin and {beta}-catenin in these cells. The isolated, highly invasive clones showed significant invasive capacity and reduction of E-cadherin and membranous {beta}-catenin protein in comparison with parent cells. We found that reduced expression of E-cadherin was due to methylation of its promoter region. In fact, most invasive and metastatic area of OSCCs showed reduced expression and methylation of E-cadherin. Moreover, we found that reduced expression of membranous {beta}-catenin was also found frequently in invasive and metastatic areas of OSCCs. In summary, invasion and metastasis of OSCC cells require methylation of E-cadherin and/or degradation of membranous {beta}-catenin. In addition, we suggest that the method of isolation of highly invasive clones may be useful for studies aimed at discovering novel genes involved in invasion and metastasis.
Purpose: The RAS association domain family 1A (RASSF1A) gene, a candidate tumor suppressor gene, is frequently inactivated by hypermethylation of its promoter region in several human cancers. The aim of this study was to evaluate the promoter methylation status of the RASSF1A in esophageal squamous cell carcinoma. Experimental Design: We analyzed the methylation status of RASSF1A promoter by methylation-specific PCR in 23 esophageal squamous cell carcinoma cell lines and 48 primary tumors. Results: Hypermethylation of RASSF1A was found in 74% of cell lines and 52% of primary tumors. The presence of hypermethylation was statistically associated with loss of RASSF1A mRNA expression in both cell lines (P = 0.007) and primary tumors (P = 0.003). There was a statistically significant correlation between the presence of hypermethylation and tumor stage (P = 0.009). Conclusions: Our findings suggest that epigenetic silencing of RASSF1A gene expression by promoter hypermethylation could play an important role in primary esophageal squamous cell carcinogenesis.


Purpose: WWOX (WW domain containing oxidoreductase) is a tumor suppressor gene that maps to the common fragile site FRA16D. We showed previously that WWOX is frequently altered in human lung and esophageal cancers. The purpose of this study was to delineate more precisely the role of WWOX in pancreatic carcinogenesis. Experimental Design: We analyzed 15 paired pancreatic adenocarcinoma samples and 9 pancreatic cancer cell lines for WWOX alterations. Colony assay and cell cycle analysis were also performed to evaluate the role of the WWOX as a tumor suppressor gene. Results: Loss of heterozygosity at the WWOX locus was observed in 4 primary tumors (27%). Methylation analysis showed that site-specific promoter hypermethylation was detected in 2 cell lines (22%) and treatment with the demethylating agent 5-aza-2'-deoxycytidine demonstrated an increase in the expression of WWOX. In addition, 2 primary tumor samples (13%) showed promoter hypermethylation including the position of site-specific methylation. Transcripts missing WWOX exons were detected in 4 cell lines (44%) and in 2 tumor samples (13%). Real-time reverse transcription PCR revealed a significant reduction of WWOX expression in all of the cell lines and in 6 primary tumors (40%). Western blot analysis showed a significant reduction of the WWOX protein in all of the cell lines. Furthermore, transfection with WWOX inhibited colony formation of pancreatic cancer cell lines by triggering apoptosis. Conclusion: These results indicate that the WWOX gene may play an important role in pancreatic tumor development.


Purpose: The purpose of this study was to establish a sensitive and semiquantitative method for the detection of minimal residual disease of neuroblastoma, the most common solid tumor in childhood. Experimental Design: Analysis was performed on a molecular level by reverse transcription-PCR using a new, real-time detection method. We measured two genes simultaneously, tyrosine hydroxylase (TH) as the target gene and glyceraldehyde-3-phosphate dehydrogenase as a reference gene, in blood and bone marrow samples at diagnosis and after follow-up from six patients with neuroblastoma, one patient with ganglioneuroma, and one patient with ganglioneuroblastoma. Results: The sensitivity of the assay was 1:106 peripheral WBCs.
Four patients with stage IV neuroblastoma and one patient with stage III neuroblastoma were scored positive. The other stage III patient and the other two patients with ganglioneuroma and ganglioneuroblastoma followed by acute lymphoblastic leukemia, respectively, were scored negative. Control bone marrow aspirates were also negative. The TH assay is more sensitive than immunohistochemical detection, and the results of the TH assay corresponded with the results of MYCN amplification. Conclusions: The described TH assay is specific, sensitive, and semiquantitative and can be used for the detection of neuroblastoma cell involvement in bone marrow and blood at diagnosis and during therapy. Furthermore, the TH assay is a possible prognostic marker for neuroblastoma.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/6/1761

Purpose: Aberrant promoter methylation, an alternative mechanism for gene silencing, is frequently detected in gastric cancer. We studied the feasibility of detecting aberrant methylation in serum of gastric cancer patients. Experimental Design: Patients (54) with gastric adenocarcinoma were studied. The tumor and the paired serum were examined for aberrant methylation in DAP-kinase, E-cadherin, GSTP1, p15, and p16 by methylation-specific PCR. Serum from 30 age-matched noncancer patients was used as control. Results: Promoter methylation in DAP-kinase, E-cadherin, GSTP1, p15, and p16 were detected in 70.3, 75.9, 18.5, 68.5, and 66.7% of primary tumor. In serum of gastric cancer patients, methylation in DAP-kinase, E-cadherin, GSTP1, p15, and p16 were detected in 48.1, 57.4, 14.8, 55.6, and 51.9%, respectively. None of the control serum showed aberrant methylation. Aberrant methylation in serum DNA was all accompanied with methylation in the corresponding tumor samples. In general, >60% of serum from cancers with aberrant methylation demonstrated these epigenetic alterations. Conclusion: Our findings suggest that aberrant promoter methylation in serum can be detected in a substantial proportion of gastric cancer patients, and this strategy should be evaluated in the screening and surveillance of gastric cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/5/973

Purpose: Although p53 mutations occur in alkylating agent-related leukemias, their frequency and spectrum in leukemias after ovarian cancer have not been addressed. The purpose of this study was to examine p53 mutations in leukemias after ovarian cancer, for which treatment with platinum analogues was widely used. Experimental Design: Adequate leukemic or dysplastic cells were available in 17 of 82 cases of leukemia or myelodysplastic syndrome that occurred in a multicenter, population-based cohort of 23,170 women with ovarian cancer. Eleven of the 17 received platinum compounds and other alkylating agents with or without DNA topoisomerase II inhibitors and/or radiation. Six received other alkylating agents, in one case, with radiation. Genomic DNA was extracted and p53 exons 5, 6, 7, and 8 were amplified by PCR. Mutations and loss of heterozygosity were analyzed on the WAVE instrument (Transgenomic) followed by selected analysis by sequencing. Results: Eleven p53 mutations involving all four exons studied and one polymorphism were identified. Genomic DNA analyses were consistent with loss of heterozygosity for four of the mutations. The 11 mutations occurred in 9 cases, such that 6 of 11 leukemias after platinum-based regimens (55%) and 3 of 6 leukemias after other treatments (50%) contained p53 mutations. Two leukemias that occurred after treatment with platinum analogues contained two mutations. Among eight mutations in leukemias after treatment with
platinum analogues, there were four G-to-A transitions and one G-to-C transversion. Conclusions: p53 mutations are common in leukemia and myelodysplastic syndrome after multiagent therapy for ovarian cancer. The propensity for G-to-A transitions may reflect specific DNA damage in leukemias after treatment with platinum analogues.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/10/3667

Purpose: Epidemiological evidence implicates a heightened androgenic state in women with epithelial ovarian cancer. Androgen activity may be modulated by altered expression or activity of the androgen receptor (AR) or AR polymorphisms. Exon 1 of the AR gene contains a polymorphic (CAG)n sequence whose length is inversely correlated with transcriptional activity. Experimental Design: Differential expression of AR mRNA and protein was examined in 46 primary cultures of normal human ovarian surface epithelium (HOSE) and malignant Cedars-Sinai ovarian cancer (CSOC) ovarian epithelial cells. AR allele length was characterized by genotyping in 77 ovarian cancer specimens. Results: AR mRNA expression was higher in CSOC primary cultures (1.58 +/- 0.17) when compared with HOSE (1 +/- 0.09, P = 0.005), but protein expression was not statistically different. CAG repeat lengths were shorter in CSOC (20.6 +/- 1.2) than in HOSE (23.4 +/- 0.9, P = 0.04). Patients with an AR allele containing [<=]19 CAG repeats had a shorter time to recurrence (5.5 versus 19.4 months, P < 0.0001) and overall survival (9 versus 32.6 months, P = 0.0007). There was no correlation between AR allelotype and age of diagnosis, stage, or grade; however, a short CAG length [<=]19 repeats was associated with decreased surgical cytoreducibility (44.4 versus 10.3%, P = 0.035). Multivariate analyses confirmed a short AR allele as an independent prognostic factor (P = 0.02). Conclusions: These data support epidemiological evidence linking heightened androgenicity to the pathogenesis and tumor biology of epithelial ovarian cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/5/1809

Purpose: Among tumor antigens identified to date, cancer-testis (CT) antigens, which are coded by CT genes, are identified as a group of highly attractive targets for cancer vaccines. This study is the first to analyze the mRNA expression and possible correlation with pathologic characteristics of multiple CT genes in a large cohort of colorectal cancer (CRC) patients. Experimental Design: The expression of 10 individual CT genes in 121 CRC and adjacent tissues were analyzed by RT-PCR method. The presence of autologous antibodies against NY-ESO-1 was examined in serum samples by ELISA. To confirm the protein expression, immunohistochemistry was done for detecting the NY-ESO-1 antigen in mRNA-positive CRC tissues. Results: The CT genes were detected with various frequencies in CRC tissue, SCP-1, 1.7%; SSX-2, 2.5%; SSX-4, 2.5%; SSX-1, 5.0%; CT10, 6.6%; NY-ESO-1, 9.9%; MAGE-1, 11.6%; LAGE-1, 15.7%; MAGE-4, 22.3%; and MAGE-3, 27.3%. In 56.2% of tumor tissues examined in this study, at least one CT gene was detected. In contrast, no CT gene expression was found in cancer adjacent tissues. Among 10 CT genes investigated, NY-ESO-1 and LAGE-1 are of particular interest because their mRNA expression in CRC was rarely reported before. In our study, NY-ESO-1 mRNA was found to express in 9.9% of the samples, and also correlated significantly with stages (P = 0.041) and local lymph node metastasis (P = 0.002). In addition, we also identified one NY-ESO-1 antibody-positive serum sample. MAGE-4 mRNA was expressed at a high frequency in tumor tissues with vessel emboli samples (P = 0.025). Conclusions: These
results suggested that CT genes, especially NY-ESO-1 and LAGE-1, do express in CRC. More than 50% of the CRC patients in this study express at least one CT gene, making them eligible for CT vaccination. NY-ESO-1 gene may serve as a marker for local metastasis and advanced disease. MAGE-4 gene is significantly associated with the vessel emboli.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/4/1372

Purpose: The transcription factor CCAAT/enhancer binding protein (alpha), encoded by the CEBPA, is crucial for the differentiation of immature granulocytes. Mutation of the CEBPA may play an important role in leukemogenesis and prognosis. We sought to characterize the CEBPA mutation in acute myeloid leukemia (AML) and to clarify if there is a distinct immunophenotype for leukemic cells with the mutation. Experiment Design: One hundred and four patients with de novo AML were evaluated for the CEBPA mutation and immunophenotype of the leukemic cells. Results: Twenty-two distinct mutations were identified in 16 (15%) of 104 AML patients. Fourteen patients had biallelic mutations, mostly involving both the NH2-terminal TAD1 region and the COOH-terminal basic leucine zipper domain (bZIP). The mutations in the bZIP region were always tandem duplications and were located at hot-spot regions for topoisomerase II sites. Sequential study of the CEBPA mutations showed that the mutations disappeared at complete remission but the same mutations reappeared at relapse. None of the patients developed novel mutations during the follow-up period. Patients with CEBPA mutations had significantly higher incidences of CD7 (73%), CD15 (100%), CD34 (93%), and HLA-DR (93%) expression on the leukemic cells. Conclusion: These data revealed that most AML with CEBPA mutations were associated with an immunophenotype of HLA-DR+CD7+CD13+CD14-CD15+CD33+CD34+. The close relationship of CEBPA mutations with the leukemia status of the patients and the concordance of mutation in presenting and relapse samples implicate the CEBPA mutation as a potential marker for monitoring minimal residue disease.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/7/2286

Purpose: Overexpression of the excision repair cross-complementing 1 (ERCC1) gene, which is crucial in the repair of cisplatin (CDDP)-DNA adducts, is reported to negatively influence the effectiveness of CDDP-based therapy for gastric and ovarian cancers. Recent evidence indicates that Gemcitabine (Gem) may modulate ERCC1 nucleotide excision repair activity, and down-regulation of DNA repair activity by ERCC1 antisense RNA reportedly inhibits synergism of CDDP/Gem. We investigated whether ERCC1 mRNA expression levels were associated with clinical outcomes after treatment with a combination Gem/CDDP regimen for patients with advanced stage non-small cell lung cancer (NSCLC). Experimental Design: Response and survival were correlated with the level of ERCC1 expression in 56 patients with advanced (stage IIIb or IV) NSCLC treated as part of a multicenter randomized trial with Gem 1250 mg/m2 days 1 and 8 plus CDDP 100 mg/m2 on day 1 every 3 weeks. mRNA was isolated from paraffin-embedded pretreatment primary tumor specimens, and relative expression levels of ERCC1/β-actin were measured using a quantitative reverse transcription-PCR (Taqman) system. Results: ERCC1 expression was detectable in all tumors. There were no significant differences in ERCC1 levels by gender, age, performance status, weight loss, or tumor stage.
The overall response rate was 44.7%. There were no significant associations between ERCC1 expression and response. Median overall survival was significantly longer in patients with low ERCC1 expression tumors (61.6 weeks; 95% confidence interval, 42.4-80.7 weeks) compared to patients with high expression tumors (20.4 weeks, 95% confidence interval, 6.9-33.9 weeks). ERCC1 expression, Eastern Cooperative Oncology Group performance status, and presence of weight loss were significant prognostic factors for survival in a Cox proportional hazards multivariable analysis. Conclusions: These data suggest that ERCC1 expression is a predictive factor for survival after CDDP/Gem therapy in advanced NSCLC. Although there was a trend toward decreased response with high ERCC1 mRNA levels, this difference failed to reach statistical significance. This result may reflect the impact of Gem and the requirement for ERCC1 expression for CDDP/Gem synergism or may be attributable to the relatively small patient sample size in this study. Prospective studies of ERCC1 as a predictive marker for activity of CDDP-based regimens in NSCLC are warranted.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/2/693

Purpose: ONYX-015 is a genetically modified adenovirus with a deletion of the E1B early gene and is therefore designed to replicate preferentially in p53-mutated cells. A Phase II trial of intralesional ONYX-015 was conducted in patients with hepatobiliary tumors to determine the safety and efficacy of such a treatment. Experimental Design: All patients had biopsy-proven, measurable tumors of the liver, gall bladder, or bile ducts that were beyond the scope of surgical resection. Patients received intrallesional injections of ONYX-015 at either 6 x 10^9 or 1 x 10^10 plaque-forming units/lesion up to a total dose of 3 x 10^10 plaque-forming units, and i.p. injections were allowed in patients with malignant ascites. The status of p53 was assessed by immunohistochemistry or Affymetrix GeneChip microarray analysis. Studies were conducted for viral shedding and for the presence of antiadenoviral antibodies before and after the injection of ONYX-015. Patients were assessed for response and toxicity. Results: Twenty patients were enrolled, and 19 patients were eligible. Half of the patients had primary bile duct carcinomas. Serious toxicities (> grade 2) were uncommon and included hepatic toxicity (three patients), anemia (one patient), infection (one patient), and cardiac toxicity (one patient, atrial fibrillation). Sixteen patients were evaluable for response. Among these evaluable patients, 1 of 16 (6.3%) had a partial response, 1 of 16 (6.3%) had prolonged disease stabilization (49 weeks), and 8 of 16 (50%) had a >50% reduction in tumor markers. Of the 19 eligible patients, 18 (94.7%) had specimens available for p53 analysis. Fifteen of these 18 patients (83.3%) had evidence of p53 mutation by one or both methods, although the methods correlated poorly. Viral shedding was confined to bile (two of two patients) and ascites (four of four patients). Pretreatment adenoviral antibodies were present in 14 of 14 patients and increased by 33.2% after ONYX-015 treatment. Conclusions: Intrallesional treatment with ONYX-015 in patients with hepatobiliary tumors is safe and well tolerated, and some patients had evidence of an anticancer effect. The high incidence of p53 mutations in these tumors makes this a logical population in which to test this therapy but precludes definitive evaluation about the necessity of a p53 mutation for ONYX-015 clinical activity.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/4/1338
Purpose: The aim of this study was to evaluate the prevalence and the clinical significance of HIN-1 mRNA expression in early stage non-small cell lung carcinomas (NSCLCs). Experimental Design: A series of 91 NSCLC patients with stage I neoplastic disease was studied. HIN-1 expression was investigated by quantitative real-time reverse transcription-PCR on tumor specimens and matching normal lung tissues. Variables were analyzed by \( \chi^2 \) test and Fisher's exact tests. Survival was evaluated with the method of Kaplan-Meier. Multivariate analysis was performed with Cox's proportional hazards model. Results: Seventy one (78%) tumors showed a reduction of HIN-1 mRNA compared with the normal counterpart. The range of reduction varied greatly, from -2-fold to -3350-fold. Setting a cutoff at -46-fold (median value of HIN-1 mRNA reduction), 46 cases (51%) had a markedly reduced expression, and 45 cases (49%) showed a normal or slightly reduced expression. A statistically significant association between low HIN-1 mRNA levels and T status was observed \((P = 0.036)\). Univariate survival curves, estimated using the method of Kaplan-Meier, defined a significant association between HIN-1 expression and both overall survival \((P = 0.0095)\) and disease-free survival \((P = 0.0122)\). A multivariate analysis, performed by Cox's proportional hazards regression model, confirmed that a low HIN-1 expression was the only significant factor to predict poor prognosis. Conclusions: Our data indicate that HIN-1 expression, measured by real-time reverse transcription-PCR, is a possible prognostic factor in patients with stage I NSCLC. Additional studies are required to further validate this potential prognostic marker.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/11/3825

Purpose: Promoter hypermethylation is one of the major mechanisms in the transcriptional inactivation of certain carcinoma-associated genes. Concurrent methylation analysis of multiple, functionally distinct genes may provide important information on their differential alterations and potential association in head and neck squamous carcinogenesis. Experimental Design: Methylation-specific PCR analysis of the CpG islands of 8 cancer-related genes was performed on 19 cell lines and 32 primary head and neck squamous cell carcinoma (HNSC) specimens with matched histologically normal mucosa and 6 dysplastic lesions. The methylation status and histological features of the specimens were investigated. Results: In histologically normal squamous mucosa, no to low-level methylation (0-22%) was noted in some specimens at all genes except RAR\( \beta \)2 (50%). Considerable variation in the incidence of methylation of these genes within and between cell lines and tumor specimens was noted. The highest incidences of methylation in the cell lines and primary tumors were noted in RAR\( \beta \)2 (53%), MGMT (37%), p16 (33%), and DAP-K (25%); low incidence of methylations were noted in E-cadherin (2%), p73 (2%) RASSF1A (10%), and p14 (20%) genes. The incidences of methylation of each gene were almost similar between the HNSC cell lines and primary cancer specimens, although methylation of RASSF1A was observed in cell line (26%), but not in dysplasia and primary tumor. RAR\( \beta \), p16, and MGMT genes showed the highest incidences of methylation in premalignant and invasive carcinomas. Conclusions: Methylation of p16, RAR\( \beta \), and MGMT may constitute early events in HNSC tumorigenesis. The infrequent methylation at certain genes suggests a minimal role for this feature in their functional assessment in HNSC. The variability within and between cell lines and tumor specimens supports a heterogeneous and dynamic state of methylation in genes associated with HNSC tumorigenesis.

Purpose: The purpose was to explore the relationships between irinotecan disposition and allelic variants of genes coding for adenosine triphosphate binding cassette transporters and enzymes of putative relevance for irinotecan. Experimental Design: Irinotecan was administered to 65 cancer patients as a 90-min infusion (dose, 200-350 mg/m²), and pharmacokinetic data were obtained during the first cycle. All patients were genotyped for variants in genes encoding MDR1 P-glycoprotein (ABCB1), multidrug resistance-associated proteins MRP-1 (ABCC1) and MRP-2 (canalicular multispecific organic anion transporter; ABCC2), breast cancer resistance protein (ABCG2), carboxylesterases (CES1, CES2), cytochrome P450 isozymes (CYP3A4, CYP3A5), UDP glucuronosyltransferase (UGT1A1), and a DNA-repair enzyme (XRCC1), which was included as a nonmechanistic control. Results: Eighteen genetic variants were found in nine genes of putative importance for irinotecan disposition. The homozygous T allele of the ABCB1 1236C>T polymorphism was associated with significantly increased exposure to irinotecan (P = 0.038) and its active metabolite SN-38 (P = 0.031). Pharmacokinetic parameters were not related to any of the other multiple variant genotypes, possibly because of the low allele frequency. The extent of SN-38 glucuronidation was slightly impaired in homozygous variants of UGT1A1*28, although differences were not statistically significant (P = 0.22). Conclusions: It is concluded that genotyping for ABCB1 1236C>T may be one of the factors assisting with dose optimization of irinotecan chemotherapy in cancer patients. Additional investigation is required to confirm these findings in a larger population and to assess relationships between irinotecan disposition and the rare variant genotypes, especially in other ethnic groups.


Purpose: Ascitic disease is a common occurrence in human ovarian cancer, but it is unclear how the cellular composition of ascitic fluid is determined. Because chemokines can determine host cell infiltration in solid ovarian cancer, we assessed CC chemokine protein and CC chemokine receptor expression in ovarian cancer ascites. Experimental Design: We used reverse transcription-PCR and RNase protection assay to determine CC chemokine and chemokine receptor mRNA expression and ELISA to measure CC chemokine protein levels. Flow cytometry was used to identify cell populations and their chemokine receptor protein expression. Results: mRNA for the CC chemokines CCL2, -3, -4, -5, -8, and -22 was expressed in cell isolates from ascites samples, and the corresponding proteins were detected in ascitic fluid. mRNA for CC chemokine receptors CCR1, -2a, -2b, -3, -4, -5, and -8 was detected in cells from ascites. Fluorescence-activated cell-sorting analysis showed variable numbers of macrophages and CD3+ T lymphocytes (predominantly CD4+) within ovarian cancer ascites. CD14+ macrophages within ascites consistently expressed protein for CCR1. -2, and -5. CCR1 was expressed by >60% of all T cells, but more CD4+ than CD8+ T cells expressed CCR2 and -5. A direct correlation was found between the CCL5 concentration and CD3+ T-cell infiltration. Conclusions: We conclude that there is a complex chemokine/chemokine receptor network in ovarian cancer ascites. However, associations between chemokine receptor expression, chemokine levels, and cell counts were limited.

The vast majority of women with advanced ovarian cancer will ultimately relapse and develop a drug-resistant disease with an overall 5-year survival of <50%. Unfortunately, the mechanisms of drug resistance actually operating in patients are still unknown. To address this issue, in 41 patients affected by advanced ovarian cancer the three main mechanisms of paclitaxel resistance were investigated: overexpression of MDR-1 gene, point mutations at prominently expressed (alpha) -tubulin and (beta)-tubulin genes and selective alterations in the expression of (beta)-tubulin isotypes. MDR-1 and the (beta)-tubulin isotypes expression were evaluated by semiquantitative and real-time PCR. On the same specimens, quantitative immunohistochemistry was also done in the tumor area. No statistically significant changes of MDR-1 expression were noticed between the sensitive and resistant patients either at the mRNA or protein level. The tubulin mutations for the ubiquitous (alpha) -tubulin and (beta)-tubulin genes were evaluated by automated DNA sequencing, and in all patients, no mutations were detected in both resistant and sensitive cases. With regard to the expression of tubulin isoforms, a statistically significant up-regulation of class III (beta)-tubulin was found in the resistant subset. It is worth noting that this statistically significant increase of the expression of class III (beta)-tubulin was detectable at the mRNA and protein level. By a direct comparison of the three main known mechanisms of paclitaxel resistance, this study indicates that overexpression of class III (beta)-tubulin is the most prominent mechanism of paclitaxel resistance in ovarian cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/8/2536

The significance of microsatellite instability (MSI) as a prognostic predictor for resectable pancreatic cancer patients was examined. Forty-six histologically confirmed pancreatic cancer patients who had undergone resection were studied. DNA was extracted from the paraffin block sections by means of the microdissection method. PCR was performed using eight microsatellite primer marker sets. The mixed PCR sample was analyzed by a genetic analyzer. The number of MSI-positive patients was eight (17.4%) as determined by assessment of microsatellite variations in three or more of the eight tested markers. Univariate analysis revealed that patients with MSI-positive tumors had significantly longer survival times than patients with MSI-negative tumors, although there were no significant differences in clinicopathological factors between the two groups (median survival term, 62 months versus 10 months, respectively; P = 0.011). According to univariate survival analysis, patients with T3/T4, N1, or M1 tumors, as classified by Union Internationale Contre le Cancer staging, had significantly shorter survival times than patients with less progressive tumors. Multivariate survival analysis indicated that MSI status had an independent predictive value (hazard ratio = 5.577; P = 0.007). The tumor-infiltrating leukocyte intensity in MSI-positive tumors was significantly larger than that in MSI-negative tumors, suggesting that MSI-positive tumors may induce stronger antitumor immunity. In conclusion, a patient with MSI-positive pancreatic cancer may have a comparatively better prognosis after resection, possibly due to intensive immunoreaction to the tumor.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/15/5620

Purpose: Currently, many forms of leukemia are considered potentially curable, with prognosis and clinical outcome strongly dependent on the underlying molecular pathophysiology. A substantial number of leukemia patients harbor nonrandom karyotypic abnormalities that define
subgroups with unique biological and clinical features. For detection of these types of gene rearrangements, a combination of multiplex RT-PCR with hybridization on oligonucleotide gel array was presented previously, which identified five chromosomal translocations with fusion variants. In the present study, additional clinically relevant translocations were included in our analysis using a second generation of microarrays. We also expanded significantly on the clinical correlation of our findings. Experimental Design: An oligonucleotide microarray was designed for hybridization with products of a multiplex RT-PCR to identify the following translocations: t(9;22)p190, t(4;11), t(12;21), t(1;19), typical for acute lymphoblastic leukemia; t(9;22)p210 for chronic myeloid leukemia; and t(8;21), t(15;17), inv16, typical for acute myeloblastic leukemia. Results: To demonstrate the potential clinical application of the method, 247 cases of childhood leukemia were screened, and the above-mentioned gene rearrangements were found in 30% of cases. The sensitivity and specificity of the assay is comparable with the RT-PCR technique, so that it can be used to follow minimal residual disease. The feasibility of an additional refinement of the method, on-chip-multiplex PCR, has been successfully demonstrated by identifying a common translocation, t(9;22), in chronic myeloid leukemia. Conclusions: Our data suggest that the microarray-based assay can be an effective and reliable tool in the clinical screening of leukemia patients for the presence of specific gene rearrangements with important diagnostic and prognostic implications. The method is amenable for automation and high-throughput analysis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/4/986

Purpose: The purpose of this work was to study the sera of patients with lymphoepithelioma-like carcinoma (LELC) of the lung for circulating EBV DNA. Experimental Design: Prospectively collected serum samples from five female patients with advanced, inoperable LELC of the lung were measured for free circulating EBV DNA using a quantitative PCR technique. EBV-encoded small RNA (EBER)-1 was assayed in serial serum samples of three of the five patients, either from the start or during the initial phase of chemotherapy/radiotherapy until their terminal event or last follow-up. There was only a single-point sample for analysis in the fourth and fifth patients. Six other patients with LELC of the lung were also retrospectively identified, and their sera were tested for EBER-1 at either the first visit plus the last follow-up visit (n = 2), the first visit only (n = 2), or the last follow-up visit only (n = 2). Results: Prospectively collected serum samples from five patients and retrospectively collected serum samples from two patients who had clinical disease at initial serum measurement showed detectable levels of EBER-1. Retrospectively collected serum samples from four patients with no clinical disease had negative sera. There is consistent correlation between the clinical response to treatment and subsequent clinical course of LELC and serum EBER-1 levels in the three prospective patients with longitudinal serum monitoring. Conclusions: This study shows for the first time that free EBV DNA can be detected in the serum of patients with LELC of the lung and further suggests the feasibility of its use for monitoring response to therapy in advanced cases.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/2/755

Purpose: Staging of the clinically N0 neck in patients with head and neck squamous cell carcinoma (HNSCC) using ultrasound-guided, fine needle aspiration cytology (USgFNAC) has a false-negative rate of [~]20% that might be caused by inaccurate cytology. Molecular analysis of
Aspirate residues might reduce the false-negative rate, and we therefore set up a quantitative reverse transcription-PCR (Q-RT-PCR) assay based on TaqMan technology using the squamous cell-specific antigen E48 (Ly-6D) as molecular marker. Experimental design: The detection limit of the assay was determined in reconstruction experiments. The sensitivity of the assay was tested on cytological tumor-positive aspirate residues and the specificity on lymph node aspirate residues of noncancer controls. Subsequently, 235 lymph node aspirate residues of 64 HNSCC patients staged with USgFNAC were examined for the presence of E48 mRNA. E48 Q-RT-PCR results of the aspirated lymph nodes were compared with cytology and clinical outcome. Results: The detection limit of E48 Q-RT-PCR was a single tumor cell in a background of 106 peripheral blood mononuclear cells. From the 41 aspirates that were not evaluable at cytology, 24 (59%) could be diagnosed with E48 Q-RT-PCR. In the 191 aspirates that were tumor negative or not evaluable at cytology, 8 samples from 6 patients were E48 positive. These results were confirmed by histology or clinical outcome in 3 of 6 patients. E48 Q-RT-PCR showed an increase in sensitivity from 56 to 67% and an increase in frequency of reached diagnosis from 97 to 100% compared with cytology. The specificity decreased from 100 to 92%. Conclusions: Real-time E48 Q-RT-PCR is an accurate technique for squamous cell detection in lymph node aspirates of HNSCC patients. The assay shows an increase in sensitivity and frequency of reached diagnosis compared with cytology. The test could be implemented routinely in USgFNAC to diagnose cases for which cytological examination is not conclusive.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/15/4983

Purpose: To test ribozymes targeting mouse telomerase RNA (mTER) for suppression of the progression of B16-F10 murine melanoma metastases in vivo. Experimental Design: Hammerhead ribozymes were designed to target mTER. The ribozyme sequences were cloned into a plasmid expression vector containing EBV genomic elements that substantially prolong expression of genes delivered in vivo. The activity of various antitelomerase ribozymes or control constructs was examined after i.v. injection of cationic liposome:DNA complexes containing control or ribozyme constructs. Expression of ribozymes and mTER at various time points were evaluated by quantitative real-time PCR. Telomerase activity was examined using the telomeric repeat amplification protocol. Results: Systemic administration of cationic liposome:DNA complexes containing a plasmid-expressed ribozyme specifically targeting a cleavage site at mTER nucleotide 180 significantly reduced the metastatic progression of B16-F10 murine melanoma. The antitumor activity of the anti-TER 180 ribozyme in mice was abolished by a single inactivating base mutation in the ribozyme catalytic core. The EBV-based expression plasmid produced sustained levels of ribozyme expression for the full duration of the antitumor studies. In addition to antitumor activity, cationic liposome:DNA complex-based ribozyme treatment also produced reductions in both TER levels and telomerase enzymatic activity in tumor-bearing mice. Conclusions: Systemic, plasmid-based ribozymes specifically targeting TER can reduce both telomerase activity and metastatic progression in tumor-bearing hosts. The work reported here demonstrates the potential utility of plasmid-based anti-TER ribozymes in the therapy of melanoma metastasis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/16/6012

Purpose: The purpose of this study was to analyze whether gene expression levels of folate
enzymes in adjacent mucosa were associated with outcome of colorectal cancer patients. Experimental Design: Real-time PCR was used to quantify expression levels of folate-associated genes including the reduced folate carrier (RFC-1), folylpolyglutamate synthase (FPGS), (gamma)-glutamyl hydrolase (GGH), and thymidylate synthase (TS) in tumor tissue and adjacent mucosa of patients with primary colorectal cancer (n = 102). Furthermore, reduced folates in the tissues were measured with a binding-assay method. Results: Mean gene expression levels of RFC-1, FPGS, GGH, and TS were significantly higher in tumor biopsies compared with mucosa. Univariate and multivariate analyses showed that the FPGS gene expression level in mucosa, but not in tumor, was a prognostic parameter independent of the clinicopathological factors with regard to survival. Patients with high FPGS levels (>0.92) in mucosa also showed significantly higher total folate concentrations (P = 0.03) and gene expression levels of RFC-1 (P < 0.01), GGH (P < 0.01), and TS (P = 0.04) compared with patients with low FPGS levels. The total reduced folate concentration correlated with the gene expression levels of RFC-1 and FPGS but not with TS or GGH. Conclusion: Our results suggest that normal-appearing colonic mucosa adjacent to primary colon cancer can show altered gene expression levels of FPGS that may have bearing on the development of aggressive metastatic behavior of the tumor and on tumor-specific survival.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/11/3468

Purpose: Mutations in the ras genes often occur during tumorigenesis. In malignant melanoma, the most common ras alterations are N-ras codon 61 mutations. This study was aimed to measure the frequency of such mutations in a large series of paired primary and metastatic melanomas to determine their role in melanoma initiation and progression. Experimental Design: Seventy-four primary melanomas and 88 metastases originating from 54 of the primary tumors were screened for N-ras codon 61 mutations using single-strand conformation polymorphism and nucleotide sequence analyses. Results: Twenty-one of the 74 primary tumors (28%) had activating N-ras codon 61 mutations. From 20 of the mutated primary tumors, a total of 34 metastases were analyzed, and all but one showed the same mutation as the primary tumor from which they originated. The remaining 53 primary tumors and corresponding metastases (n = 54) were wild-type for N-ras codon 61. Analysis of the different growth phases of the mutated primary tumors showed that the mutations were already present in the radial growth phase. Mutations were also detected in tumor-associated nevi. N-ras codon 61 mutations were associated with a higher Clark level of invasion (P = 0.012) and a lower age at diagnosis (P = 0.042) but did not affect survival (P = 0.671). Conclusions: This study shows that N-ras codon 61 mutations occur early in primary melanomas rather than in the metastatic stage and that once the mutations have occurred, they persist throughout tumor progression. This suggests that activated N-ras may be an attractive target for therapy in the subset of melanoma patients carrying such mutations.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/12/4475

Purpose: Estrogen exposure has been linked to a risk for the development of testicular germ cell cancers. The effects of estrogen are now known to be mediated by estrogen receptor (ER)- {alpha} and -{beta} receptor subtypes, but only ER-{beta} has been found in human normal testis. The goal of the present study was to compare the localization and expression levels of these ER
subtypes in testicular germ cell cancers (seminomas and nonseminomatous germ cell tumors) with normal testis. For completeness, expression of androgen and progesterone receptors was also investigated. Experimental Design: Immunohistochemistry was used to localize the expression of steroid receptors in 39 archival testicular germ cell cancers and 5 morphologically normal testes. Expression of the steroid receptors at the transcript level was semiquantified by reverse transcription-PCR in 5 paired fresh-frozen specimens of normal and neoplastic testes. Results: ER-(alpha) was not expressed in the human normal testis. It was also absent in all of the testicular germ cell cancers studied. In contrast, ER-(beta) was strongly expressed in various germ cells of the normal testis. However, its expression was markedly diminished in seminomas, embryonal cell carcinomas, and in mixed germ cell tumors, at both transcriptional and translational levels. In contrast, ER-(beta) remained highly expressed in endodermal sinus tumors and teratomas. Progesterone receptor, an estrogen-regulated gene, was localized to spermatagonia of the normal testis, but its expression dramatically reduced in seminomas. With the exception of spermatagonia, androgen receptor was found in all of the germ cells of the normal testis, but, aside from trace staining in 3 of 5 endodermal sinus tumor cells, it was not detected immunohistochemically in any other germ cell cancer. Conclusions: We confirm expression of ER-(beta), but not ER-(alpha), in normal testicular cells, suggesting that only the former ER subtype mediates the action of estrogen in the human male gonad. Our results provide the first evidence that only ER-(beta) is expressed in testicular germ cell tumors. Its expression is down-regulated in seminomas and embryonal cell carcinomas but remains high in endodermal sinus tumors and in teratomas. The observed differences in ER-(beta) expression levels among different testicular germ cell tumors may reflect divergent pathways of differentiation/dedifferentiation of these neoplasms from a common precursor. Collectively, these findings provide a possible mechanistic link between estrogen exposure and testicular cancer risk.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/22/7511

Purpose: A clinical role for nonquantitative reverse transcription-PCR (RT-PCR) using prostate-specific antigen in blood samples from patients with prostate cancer remains undefined. Assay variation and detection of prostate-specific antigen mRNA illegitimate transcription may explain inconsistent results between studies. Defining levels of prostate-specific antigen mRNA expression in blood samples from healthy volunteers and patients with prostate cancer would allow cutoffs to be established to distinguish the two groups. Experimental Design: Quantitative real-time RT-PCR for prostate-specific antigen mRNA was established and levels of prostate-specific antigen mRNA measured in bloods samples from healthy volunteers (n = 21) and patients with localized (n = 27) and metastatic (n = 40) prostate cancer. Results: Levels of prostate-specific antigen mRNA were significantly higher in blood samples from patients with metastatic prostate cancer than in blood samples from patients with localized prostate cancer (P < 0.001) or in blood samples from healthy volunteers (P < 0.01); levels between patients with localized prostate cancer and healthy volunteers were no different. Assay sensitivity to detect patients with metastatic prostate cancer was 68% with specificity of 95%. In patients with newly diagnosed metastatic prostate cancer, monitoring response to hormonal therapy was possible with this assay. No correlation between levels of prostate-specific antigen mRNA and serum prostate-specific antigen protein levels was found, suggesting that prostate-specific antigen mRNA and serum prostate-specific antigen protein levels reflect different features of prostate cancer, i.e., circulating tumor cells and total tumor bulk, respectively. Conclusions: Quantitative RT-PCR discriminates patients with metastatic prostate cancer from healthy volunteers and patients with localized prostate cancer but cannot discriminate patients with localized prostate cancer from healthy volunteers. A role for quantitative RT-PCR has been identified in the
assessment and monitoring of patients with metastatic prostate cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/7/2273

Purpose: Growth factor receptor expression and activation, particularly for epidermal growth factor (EGF) and hepatocyte growth factor (HGF), in many endocrine and nonendocrine tumors is important in determining tumor recurrence, growth, and aggressiveness. Whether this is true of neuroendocrine tumors such as gastrinomas is unclear. Experimental Design: To address this question, we analyzed the extent of EGFR and HGFR expression in gastrinomas from 38 patients with Zollinger-Ellison syndrome and correlated it with clinical and tumor characteristics. EGFR (n = 38) and HGFR (n = 22) mRNA levels were determined by competitive PCR, and immunohistochemistry was performed on a subset. Results: In each of the gastrinomas studied, detectable levels of EGFR and HGFR mRNA were present. Low levels of EGFR protein expression were detected in 40% of gastrinomas and HGFR protein expression in 90%. EGFR mRNA expression varied by 1050-fold and HGFR by 375-fold. Eighteen percent of gastrinomas overexpressed EGFR mRNA and 14% overexpressed HGFR mRNA, compared with normal pancreas. Maximal EGFR and HGFR mRNA levels were 4- and 1.2-fold increased and correlated with the presence of liver metastases (P = 0.034) and decreased long-term curability (P = 0.027) but not tumor location, size, or tumor functional characteristics. Conclusions: These above results indicate that EGFR and HGFR mRNA are universally expressed in gastrinomas. Furthermore, each is overexpressed in a minority (15-20%) of the gastrinomas, and the overexpression correlates with aggressive growth and lower curability.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/12/4150

Purpose: Thrombospondin 1 (THBS 1) is a matricellular protein capable of modulating angiogenesis. However, the actual role of THBS 1 in angiogenesis and tumor progression remains controversial. Hepatocellular carcinoma (HCC) is a hypervascular tumor characterized by neovascularization. The significance of THBS 1 in HCC remains unknown. In this study, the significance of THBS 1 in HCC was evaluated by correlating its expression with clinicopathological data. The possible role of THBS 1 in the angiogenesis of HCC was also studied by correlating its expression with vascular endothelial growth factor (VEGF) expression. Experimental Design: Sixty HCC patients were recruited in this study. THBS 1 and VEGF protein expression in tumorous livers were localized by immunohistochemical staining and quantified by ELISA. THBS 1 mRNA was quantified by quantitative reverse transcription-PCR. Results: Immunohistochemical staining of THBS 1 was positive in HCC cells in 51.7% of patients and in stromal cells in 65% of patients. Tumor THBS 1 protein level was significantly correlated with its mRNA expression (P = 0.001) and was significantly correlated with tumor VEGF protein levels (P = 0.001). Its expression was significantly associated with the presence of venous invasion (P = 0.008) and advanced tumor stage (P = 0.049). High THBS 1 expression was also a prognostic marker of poor survival in HCC patients. Conclusions: This study shows that high expression of THBS 1 is associated with tumor invasiveness and progression in HCC. THBS 1 appears to be a proangiogenic factor that stimulates angiogenesis in HCC in view of its positive correlation with VEGF expression.

http://clincancerres.aacrjournals.org/cgi/content/abstract/9/7/2567

Purpose: Claudin proteins represent a large family of integral membrane proteins crucial for tight junction (TJ) formation and function. Claudins have been shown to be up-regulated in various cancers and have been suggested as possible biomarkers and targets for cancer therapy. Because claudin-3 and claudin-4 have been proposed to be expressed in epithelial ovarian cancer, we have performed a detailed analysis of CLDN3 and CLDN4 expression in a panel of ovarian tumors of various subtypes and cell lines. We also investigated whether high expression of claudin-3 and claudin-4 was associated with TJ function in ovarian cancer cells. Experimental Design: RNA was obtained from a panel of 39 microdissected epithelial ovarian tumors of various histological subtypes for real-time reverse transcription-PCR analysis. In addition, a total of 70 cases of ovarian carcinomas, ovarian cysts, and normal ovarian epithelium from a tissue array were analyzed by immunohistochemistry. Finally, a panel of cell lines was used for Western analysis of claudin expression and TJ permeability studies. Results: Although expressed at low levels in some normal human tissues, including the ovary, CLDN3 and CLDN4 are highly up-regulated in epithelial ovarian cancers of all subtypes. Immunohistochemical analyses using our ovarian tissue array confirmed the high level of expression of claudin-3 and claudin-4 in the majority of ovarian carcinomas, including many tumors exhibiting cytoplasmic staining. Ovarian cystadenoma did not frequently overexpress these proteins, suggesting that the expression of these proteins is associated with malignancy. In ovarian cancer cell lines, claudin-3 and claudin-4 expression was not associated with functional TJs as measured by transepithelial electrical resistance. Conclusions: These results show that CLDN3 and CLDN4 are frequently up-regulated in ovarian cancer and may represent novel markers for this disease. Overexpression of these genes in ovarian cancer also suggests interesting scenarios for the involvement of TJ in tumorigenesis. A better knowledge of the mechanisms underlying ovarian tumorigenesis will likely result in the development of novel approaches for the diagnosis and therapy of this deadly disease.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/14/5299

Purpose: The purpose of the present work was to investigate the relationship between mRNA expression of ERCC1 and XPB, two key genes in the nucleotide excision repair pathway, and clinical resistance of platinum-chemotherapy in histological subtypes of epithelial ovarian cancer. Experimental Design: mRNA levels of ERCC1 and XPB in epithelial ovarian cancer specimens from 126 different individuals were assessed using reverse transcription-PCR and followed by Southern hybridization methodology. Data were analyzed by linear regression analyses and by exhaustive regression analyses. Results: Five different histological types of tumors were examined; serous (n = 76), mucinous (n = 11), clear cell (n = 9), poorly differentiated (n = 9), and endometroid (n = 21). Numerical values for mRNA expression levels were based on internal controls for a stable comparative cell line and for {beta}-actin. Median values for ERCC1 and XPB mRNAs within clear cell tumors were, on average, >2-fold higher than the other histological tumor types. Linear regression analyses suggest a continuum of nucleotide excision repair gene expression among these cell types, and exhaustive regression analyses demonstrate that the higher mRNA levels seen in clear cell tumors are highly statistically significant. Conclusions: We conclude that mRNA levels of ERCC1 and XPB tend to be higher in clear cell tumors as opposed
to other types of epithelial ovarian cancer. This is consistent with the long-standing observation that clear cell tumors are more likely to show de novo drug resistance against DNA damaging agents in the clinic.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/22/7592

Purpose: Methylenetetrahydrofolate reductase (MTHFR) directs intracellular folate toward homocysteine metabolism and away from nucleotide synthesis. Two common MTHFR polymorphisms, C677T and A1298C, are associated with reduced enzyme activity. We evaluated the association of these polymorphisms with risk of relapse and bcr-abl mRNA transcript detection among 336 Caucasian patients who underwent allogeneic hematopoietic cell transplantation for chronic myelogenous leukemia. Experimental Design: Data on the transplant course and folate-related exposures were abstracted from medical records. MTHFR C677T and A1298C genotypes were determined using polymerase chain reaction/restriction fragment length polymorphism and TaqMan assays. Qualitative bcr-abl mRNA testing was conducted using a two-step reverse transcription-polymerase chain reaction assay. Cox regression analysis was used to assess the association between MTHFR genotypes and time to relapse and bcr-abl mRNA detection. Results: A statistically significant decreased risk of relapse was observed in patients with the variant A1298C genotype [1298AC, hazard ratio (HR) = 0.48 and 95% confidence interval (CI) = 0.26-0.88; 1298CC, HR = 0.28 and 95% CI = 0.09-0.84; P-trend < 0.01]. For the joint C677T/A1298C genotype, variant genotypes were associated with a decreased risk of relapse when compared with the wild-type 677CC/1298AA genotype. This risk was lowest for the 677CC/1298CC genotype (HR, 0.23; 95% CI, 0.08-0.72). MTHFR genotypes were not associated with bcr-abl transcript detection. Conclusions: These findings suggest that individuals with the 677CC/1298AA genotype are at higher risk of relapse after hematopoietic cell transplantation and that the balance of intracellular folate metabolites available for nucleotide synthesis (regulated by the relative activity of the MTHFR enzyme) may affect the progression from bcr-abl positivity to clinical relapse.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/10/3886S

Purpose: Two bispecific diabodies (BS1.5 and BS1.5H) and two bispecific trivalent proteins (BS6 and BS8) were produced and tested as potential agents for pretargeted delivery of radiolabeled bivalent haptons to tumors expressing carcinoembryonic antigen. Experimental Design: Each of the four proteins was expressed in Escherichia coli and purified from the soluble fraction. BS1.5 and BS1.5H (a humanized version of BS1.5) were evaluated in the GW-39 human colonic tumor-nude mouse model using a di-HSG-1,4,7,10-tetra-azacyclododecane-N,N',N'' N'''-tetraacetic acid peptide (IMP-241) radiolabeled with 111In. The biodistribution and T/NT ratios were compared with those of hMN-14 x m679 (Fab' x Fab') prepared chemically. Results: In animals, both BS1.5 and BS1.5H cleared more rapidly than hMN-14 x m679 and showed tumor to nontumor ratios far superior to those of hMN-14 x m679. For example, with BS1.5 injected 8 h before 111In-IMP-241, the tumor uptake of 111In was 10.3{+/-} 2.7 and 6.3{+/-} 2.2% ID/g at 3 and 24 h, respectively, with the tumor to blood ratios being 167{+/-} 35 at 3 h and 631{+/-} 231 at 24 h. In comparison, the tumor to blood ratios of 111In observed for hMN-14 x m679 given 24 h earlier were 8{+/-} 2 at 3 h and 16{+/-} 3 at 24 h. Conclusions: These results indicate that BS1.5 and BS1.5H are
promising candidates for use in a variety of pretargeting applications, including tumor therapy with radionuclides and drugs. BS6 and BS8 may be even more attractive because of their potential to achieve higher levels of tumor uptake because of divalent carcinoembryonic antigen binding.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/5/1588

Purpose: The purpose of this study was to better define the role of osteopontin (OPN) and osteonectin (also known as secreted protein acidic and rich in cysteine (SPARC)) in lung tumorigenesis by comparing the expressions of these genes in lung tumor tissue and matched normal tissue and by determining the prognostic significance of the gene expressions.

Experimental Design: Quantitative real-time reverse transcription-PCR was used to analyze OPN and SPARC mRNA expression in normal lung tissue and matching tumor samples from 82 patients with non-small cell lung cancer. Gene expression data for each patient were matched to survival data. Results: The overall median mRNA expression level of OPN was about 20-fold higher in tumor tissues than in matching normal lung tissues (P < 0.001), whereas SPARC gene expression was not significantly different in both tissue types. Forty of 82 patients had high intratumoral OPN expression, and 15 of 82 patients had high SPARC expression. High OPN expression in the tumor tissue was associated with inferior survival (P = 0.014), whereas high SPARC expression showed a trend toward longer survival (P = 0.095). The impact of high OPN and low SPARC expression on patient survival was additive (P = 0.001). Conclusions: The large increase in OPN expression in tumors compared with normal tissue and its association with survival suggest a role for OPN in lung tumorigenesis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/8/2675

Purpose: Double-strand break repair via homologous recombination is essential in maintaining genetic integrity. RAD51 and XRCC3 are involved in the repair of DNA by this pathway, and polymorphisms have been identified in both the RAD51 (RAD51-G135C) and XRCC3 (XRCC3-Thr241Met) genes. The object of this study was to examine whether these polymorphisms may modulate susceptibility to the development of acute myeloid leukemia (AML), a disease that is characterized by genetic instability.

Experimental Design: We studied the distribution of polymorphisms in RAD51 and XRCC3 in 216 cases of de novo AML, 51 cases of therapy-related AML (t-AML), and 186 control subjects using PCR followed by restriction enzyme digestion. The polymorphic deletion of the detoxification gene glutathione S-transferase M1 (GSTM1) was also examined by PCR. Results: The risk of the development of AML was found to be significantly increased when both variant RAD51-135C and XRCC3-241Met alleles are present (odds ratio (OR), 3.77; 95% confidence interval (CI), 1.39-10.24), whereas the risk of t-AML development is even higher (OR, 8.11; 95% CI, 2.22-29.68), presumably because of the large genotoxic insult these patients receive after their exposure to radiotherapy or chemotherapy. If we further divide the AML group into patients in which the burden of DNA damage is increased, because of the deletion of the GSTM1 gene, the risk of development of AML is further increased (OR, 15.26; 95% CI, 1.83-127.27). Conclusions: These results strongly suggest that DNA double-strand breaks and their repair are important in the pathogenesis of both de novo and t-AML.

http://clincancerres.aacrjournals.org/cgi/content/abstract/9/4/1387

Purpose: Families with hereditary nonpolyposis colorectal cancer (HNPCC) have an increased lifetime risk of endometrial (40%) and ovarian (10%) carcinomas. Endometrial and ovarian carcinomas from members of these families frequently display a mutator phenotype as manifest by high levels of microsatellite instability (MSI-H). Microsatellite instability (MSI) occurs in 17-32% of sporadic endometrial carcinomas and 3-17% of sporadic ovarian carcinomas. We hypothesized that there might be a higher rate of MSI in tumors from women with synchronous primary carcinomas of the ovary and endometrium. Experimental Design: We identified 52 cases of synchronous tumors of the ovary and endometrium from the databases of four gynecological oncology units. Archival material and clinical data were available on 45 of these patients. We examined DNA extracted from ovarian and endometrial tumor tissue for MSI using DNA extracted from normal tissue of that patient as a germ-line DNA control. MSI was assessed using a panel of five standard microsatellite markers: D2S123, D5S346, D17S250, BAT25, and BAT26. MSI-H was defined by more than two markers being positive. Low-level MSI (MSI-L) was defined as one or two markers positive and microsatellite stable (MSS) was defined as no markers positive.

Results: The 45 patients had a median age at diagnosis of 53 years. Of a total of 134 samples analyzed, only three samples (3.3%) were MSI-H. No patient had high levels of MSI in both ovarian and endometrial tumors. One ovarian carcinoma had five of five markers positive with the corresponding endometrial carcinoma being MSI-L. Two endometrial carcinomas were MSI-H, and the corresponding ovarian carcinomas were MSI-L and MSS, respectively. Seven ovarian tumors and seven endometrial tumors were MSI-L. The majority of patients had early-stage ovarian carcinoma [International Federation of Gynecology and Obstetrics (FIGO) stage I, 44.4%; stage II, 26.7%; and stage III, 26.6%]. Eighty-two % of the endometrial primaries were FIGO stage I. Progression-free survival was significantly better for patients with synchronous primaries than those presenting with ovarian carcinoma alone [adjusted hazards ratio, 1.94; P = 0.023; 95% confidence interval, 1.096-3.44]. Conclusion: Synchronous primary carcinomas of the ovary and endometrium are unlikely to be part of the HNPCC syndrome unless the family history is in keeping with the modified Amsterdam criteria.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/2/450

Purpose: Gastrointestinal neuroendocrine carcinoma (NEC) is extremely aggressive, but its pathophysiologic features remain poorly understood. There have been no biologically specific markers for this disease. In this study, distinctive up-regulation of human achaete-scute homologue 1 (hASH1) in gastrointestinal NECs was clarified. Experimental Design: Expression of hASH1 in NECs (n=10), carcinoid tumors (n = 10), other tumors (10 adenocarcinomas, 2 squamous cell carcinomas and 1 malignant lymphoma), and the corresponding normal mucosa were investigated by in situ hybridization, reverse transcription-PCR (RT-PCR), real-time RT-PCR, and immunohistochemistry. Results: By in situ hybridization, mild to intense signals of hASH1 mRNA were detected in 9 of 10 NECs, but not in other tumors or normal mucosa, except for focally weak signals in one carcinoid tumor. RT-PCR showed strong expression of hASH1 in a small cell NEC, followed by a moderately differentiated NEC, and a carcinoid tumor, whereas it is undetectable in adenocarcinomas or normal mucosa. By real-time RT-PCR, the amounts of hASH1 mRNA in a small cell NEC were 16,600 times higher than those in adenocarcinomas and 110 times higher than those in a carcinoid tumor. Immunohistochemically, mammalian
homologue of hASH1 was positive in 7 of 10 NECs but was negative in the other tumors. Pan-endocrine markers chromogranin A and synaptophysin were positive in almost all carcinoid tumors, in 4 and 7 of the 10 NECs, respectively. Conclusions: These findings revealed that hASH1 is distinctly up-regulated in gastrointestinal NECs. hASH1 may be used as a more sensitive and specific marker than conventional pan-endocrine markers for clinical diagnosis of gastrointestinal NECs.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/5/1821

Purpose: We aimed to assess the role of CEBP(alpha) mutations in the progression of myelodysplastic syndrome (MDS) to acute myelogenous leukemia (AML) and their cooperating mutations. Experimental Design: Mutational analysis of CEBP(alpha) with direct sequencing for each PCR product was done on matched bone marrow samples obtained from 50 adult patients with MDS at diagnosis and at AML transformation. Cloning analysis was used to determine the allelic distribution. Results: CEBP(alpha) mutations were identified in four patients at diagnosis of MDS, including one with refractory anemia with excess blasts and three with chronic myelomonocytic leukemia. At AML transformation, three patients retained the identical mutant clones as their initial diagnosis, three acquired the mutations, and one lost CEBP(alpha) mutation when she gained FLT3/ITD mutation. Together, seven patients had CEBP(alpha) mutations throughout the disease course; four patients had NH2-terminal mutations resulting in a frameshift and truncation of the protein, three of them had two different mutations either on the same alleles or on different alleles, two had missense mutations, and one had a deletion in the basic region leucine zipper domain. Except for one with coexistence of N-ras mutation, no sample harbored cooperating mutations with FLT3 or N-ras genes. CEBP(alpha) mutations had no influence on the time to AML progression or overall survival. Conclusions: Our results show that CEBP(alpha) mutations play a role in a subset of patients with MDS, especially in chronic myelomonocytic leukemia. The mutation status was heterogeneous, exhibiting identical clone, clonal change, or clonal evolution during the progression to AML.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/6/2156

Purpose: Gene promoter region hypermethylation is a significant event in primary breast cancer. However, its impact on tumor progression and potential predictive implications remain relatively unknown. Experimental Design: We conducted hypermethylation profiling of 151 primary breast tumors with association to known prognostic factors in breast cancer using methylation-specific PCR for six known tumor suppressor and related genes: RASSF1A, APC, TWIST, CDH1, GSTP1, and RAR-(beta)2. Furthermore, correlation with sentinel lymph node (SLN) tumor status was assessed as it represents the earliest stage of metastasis that is readily detected. Hypermethylation for any one gene was identified in 147 (97%) of 151 primary breast tumors. The most frequently hypermethylated gene was RASSF1A (81%). Results: Hypermethylation of the CDH1 was significantly associated with primary breast tumors demonstrating lymphovascular invasion (P = 0.008), infiltrating ductal histology (P = 0.03), and negative for the estrogen receptor (P = 0.005), whereas RASSF1A and RAR-(beta)2 gene hypermethylation were significantly more common in estrogen receptor-positive (P < 0.001) and human epidermal growth factor receptor 2-positive (P < 0.001) tumors, respectively. In multivariate analysis, hypermethylation of GSTP1
and/or RAR-\(\text{beta}\)2 was significantly associated with patients having macroscopic SLN metastasis compared with those with microscopic or no sentinel node metastasis (odds ratio, 4.59; 95% confidence interval, 2.02-10.4; \(P < 0.001\)). In paired SLN metastasis, CDH1 was the most frequently methylated gene (90%) and provides evidence in patients corroborating its role in the clinical development of metastasis. Conclusion: Hypermethylation profiling of primary breast tumors is significantly associated with known pathologic prognostic factors and may have additional clinical and pathologic utility for assessing patient prognosis and predicting early regional metastasis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/10/3831S

Purpose: We had previously reported that certain of our murine (m) antihuman light chain monoclonal antibodies (mAbs) recognized an epitope common to AL and other types of amyloid fibrils. On the basis of this evidence, one such antibody, 11-1F4, was administered to mice bearing AL amyloidomas induced by s.c. injection of human AL extracts. The mAb bound to the amyloid and initiated an Fc-mediated cellular inflammatory response that led to rapid reduction in the tumor masses. To develop this reagent for clinical use, the 11-1F4 mAb was chimerized and its activity compared with that of the unmodified antibody. Experimental Design: The chimeric (c) 11-1F4 mAb was produced in CHOdhfr-stable mammalian cell lines that had been transfected with a supervector DNA encoding the mouse 11-1F4 heavy and light chain variable regions (VH, VL) and human heavy and light chain constant regions (CH, CL). The antibody products were analyzed for their fibril binding activity and ability to effect amyloidolysis in two in vivo experimental models. Results: The capability of the c11-1F4 mAb to interact with amyloid was demonstrated in vitro. Administration of this reagent into mice bearing human AL tumors or those with systemic AA deposits resulted in marked reduction in amyloid burden with no evidence of toxicity in the animals. Conclusions: These results have led to the decision to produce GMP-grade c11-1F4 for a Phase I/II clinical trial in patients with primary (AL) amyloidosis where the effectiveness of the reagent could be determined. The use of amyloid-reactive antibodies would represent a novel approach in the treatment of individuals with this invariably fatal disorder.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/9/2902

Purpose: Overexpression of cyclin D1 mRNA and protein as a result of the chromosomal translocation t(11;14)(q13;q32) is a highly specific molecular marker of mantle cell lymphoma, but cyclin D1 dysregulation can also be found in other B-cell neoplasias. The aim of the study was to develop a precise and reliable tool for quantitation of cyclin D1 mRNA suitable for archival clinical specimens. Experimental Design: A real-time reverse transcription-PCR (RT-PCR) assay was used to quantitate cyclin D1 mRNA copy numbers. Using 2000 microdissected cells as template, 104 formalin-fixed, paraffin-embedded lymph node, spleen, and decalcified bone marrow biopsies from a panel of 95 cases of B-cell non-Hodgkin's lymphomas (B-NHLs) were analyzed. In addition, cyclin D1 protein expression was assessed by immunohistochemistry. Results: Strong cyclin D1 mRNA overexpression was detected in mantle cell lymphomas (23 of 23), hairy cell leukemias (5 of 19), and multiple myelomas (7 of 23) with particularly high levels in 2 of the latter cases. Intermediate transcript levels were found in 5 of 23 multiple myelomas and 7 of 19 hairy cell leukemias. B-cell chronic lymphocytic leukemias (10 of 10), follicular lymphomas (9 of 9),
mucosa-associated lymphoid tissue lymphomas (5 of 5) and reactive lymphoid tissues with the exception of normal spleen had no or very low cyclin D1 expression. In comparison with real-time RT-PCR, immunohistochemistry showed a lower level of sensitivity, more variability, and did not allow accurate quantitation. Conclusions: Real-time RT-PCR for cyclin D1 mRNA is an excellent tool for the differential diagnosis of B-NHLs and, in combination with microdissection, a powerful approach for retrospective trials using archival clinical specimens as tissue source. Furthermore, real-time RT-PCR may help to identify subgroups of B-NHLs according to cyclin D1 mRNA copy numbers and to investigate the possible influence of different chromosomal breakpoints on cyclin D1 expression.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/10/3438

Purpose: The cyclin-dependent kinase inhibitor p21 acts as a main executor of p53-induced growth arrest. Recently, a second transcript, p21B, was found to code for a protein expressing proapoptotic activity. We investigated p21 and p21B for mutations and epigenetic silencing in locally advanced breast cancers treated with doxorubicin or 5-fluorouracil/mitomycin and correlated our findings with treatment response and TP53 status. Experimental Design: We used reverse transcription-PCR to analyze p21/p21B mutation status in 73 breast cancer samples. The p21 promoter region was sequenced and analyzed for hypermethylation by methylation-specific PCR. In addition, a selection of patients were analyzed for mutations in the p21B promoter. Results: The p21 gene was neither mutated nor silenced by promoter hypermethylation in any of the tumors examined. One patient harbored a novel p21 splice variant in addition to the wild-type transcript. We observed two base substitutions in the p21 transcript, C93A and G251A, each affecting six patients (8.2%). The G251A variant had not been reported previously. In 12 patients (16.4%), we observed a novel base substitution, T35C, in p21B. All three base substitutions were observed in lymphocyte DNA and therefore considered polymorphisms. The polymorphisms did not correlate with p21 staining index, treatment response to doxorubicin or 5-fluorouracil/mitomycin, or TP53 status. Conclusions: Our findings do not suggest that genetic or epigenetic disturbances in p21 or p21B cause resistance to doxorubicin or mitomycin/5-fluorouracil in breast cancer. Future studies should assess potential associations between these novel polymorphisms and breast cancer risk.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/4/1579

Purpose: Measles virus (MV) causes the regression of human lymphoma xenografts. The purpose of this study was to determine if canine lymphoid cells could be infected in vitro with MV or canine distemper virus (CDV, the canine Morbillivirus equivalent of MV) and determine if in vitro viral infection leads to apoptotic cell death. Experimental Design: Reverse transcriptase-PCR was used to examine the expression of both signal lymphocyte activation molecule (CD150) and membrane cofactor molecule (CD46) mRNA. An attenuated CDV expressing enhanced green fluorescent protein was used to infect canine cells in vitro. Both flow cytometry and reverse transcriptase-PCR was used to document CDV infection. Cell death was examined using a propidium iodide staining assay and Annexin V binding. Results: Canine lymphoid cell lines and neoplastic B and T lymphocytes collected from dogs with spontaneous lymphoma expressed the Morbillivirus receptor CD150 mRNA. In contrast, only neoplastic lymphocytes expressed
detectable levels of CD46 mRNA. Although MV did not infect canine cells, CDV efficiently infected between 40% and 70% of all three canine lymphoid lines tested. More importantly, CDV infected 50% to 90% of neoplastic lymphocytes isolated from dogs with both B and T cell lymphoma. Apoptosis of CDV-infected cell lines was documented. Conclusions: Attenuated CDV may be a useful treatment for canine lymphoma. As such, dogs with lymphoma may represent a biologically relevant large animal model to investigate the feasibility, safety, and efficacy of Morbillivirus therapy in a clinical setting with findings that may have direct applicability in the treatment of human non-Hodgkin’s lymphoma.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/4/1480

Purpose: Both c-MET and vascular endothelial growth factor (VEGF)-C expression are important factors in primary carcinoma progression. We hypothesized that overexpression of c-MET and/or VEGF-C mRNA in primary colorectal cancer (CRC) can predict tumor invasion and regional metastasis. Experimental Design: The level of c-MET and VEGF-C mRNA expression was assessed using a quantitative RT-RealTime PCR assay on early stage primary CRC tumors (n = 36). Results: The c-MET mRNA copy number ranged from 1.18 x 102 to 1.11 x 106 copies (median 5.17 x 104) per 250 ng of RNA from CRC specimens. c-MET mRNA copies in CRC specimens was significantly higher than that from normal colon mucosal epithelium (P = 0.0001). c-MET mRNA copies significantly correlated with the depth of invasion: T1 versus T2, P = 0.007; T1 versus T3/T4, P = 0.0001; T1 versus T2 versus T3/T4, P = 0.0005; and T1/T2 versus T3/T4, P = 0.011. c-MET copy number in primary CRC of N1/N2 staged patients was significantly higher than N0 cases (P < 0.03). Expression levels of c-MET mRNA were verified with immunohistochemistry analysis of c-MET protein expression in CRC specimens and normal mucosal epithelium. The VEGF-C mRNA copies of primary CRC assessed ranged from 0 to 1.65 x 105 copies (median 580). Although VEGF-C mRNA copies in CRC primary tumors were significantly higher than normal colon mucosal epithelium (P = 0.0008), it did not correlate with any major clinicopathological parameters of CRC. Conclusions: This study indicates c-MET mRNA overexpression in primary CRC may be an important prognostic marker for early stage invasion and regional disease metastasis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/7/2351

Purpose: The chemokine CC-ligand 21/secondary lymphoid tissue chemokine (CCL21/SLC) regulates the homing of naive T cells and dendritic cells that express CC-chemokine receptor 7 (CCR7) from distant sites to lymphoid tissue such as lymph nodes. We hypothesized that CCL21/SLC regulates the migration of CCR7-bearing melanoma cells from a primary lesion to regional tumor-draining lymph nodes. Experimental Design: Quantitative real-time reverse transcriptase-PCR (qRT) assay and immunohistochemistry (IHC) were used to assess the level of CCR7 expression in melanoma cell lines and in primary and metastatic melanoma tumors. Cell migration assay using melanoma cell lines was performed under the induction of CCL21/SLC. The CCL21/SLC expression level in tumor-draining sentinel lymph nodes (SLNs) was assessed by both qRT assay and IHC. Results: Melanoma cell lines and tumors demonstrated heterogeneous expression of CCR7 mRNA by qRT assay. There was strong functional correlation between CCR7 mRNA expression and cell migration induced by CCL21/SLC. IHC evidence of CCR7 expression in primary melanomas significantly (P = 0.02) correlated with
Breslow thickness. Assessment of SLN from 55 melanoma patients by qRT assay demonstrated that CCL21/SLC mRNA expression level was significantly (P = 0.008) higher in pathologically melanoma-negative SLNs than in melanoma-positive SLNs. Conclusions: This report demonstrates a potential mechanism for recruitment and homing of CCR7(+) metastatic melanoma cells to tumor-draining lymph nodes, which express CCL21/SLC. The study also suggests that lymph nodes bearing metastasis may suppress CCL21/SLC production.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/3/938

Purpose: The use of tyrosine kinase receptor inhibitors is increasingly becoming a valuable therapeutic alternative in tumors carrying activated tyrosine kinase receptors. In a previous study, we described a coexpression of KIT and stem cell factor (SCF) mRNA in Synovial sarcomas, (SS) and in a limited number of cases, we demonstrated the presence of an activated receptor. Here, in a wider number of cases, we investigated the expression level and phosphorylation status of two structurally related tyrosine kinase receptors, KIT and platelet-derived growth factor receptor (beta) (PDGFR(beta)), at the light of their role as possible targets of tyrosine kinase receptors inhibitor molecules. Experimental Design: Forty-three SS cases were analyzed for KIT and PDGFR(beta) expression/activation by immunoprecipitation/Western blotting experiments. The cognate ligands, SCF and PDGFB, were detected by reverse transcription-PCR. Results: KIT was observed in 48 and 41% (45% total) whereas PDGFR(beta) in 54 and 33% (45% total) of monophasic and biphasic SS cases, respectively. With respect to the fusion transcript type SYTSSX1 and SYTSSX2, KIT was more expressed in SYTSSX1 carrying cases (48 versus 38%), whereas PDGFR(beta) resulted more frequently expressed in SYTSSX2 ones (54 versus 37%). When expressed, the receptors were phosphorylated. Their ligands were detected in all of the activated cases. Conclusions: About 70% of the cases express one of the two activated tyrosine kinase receptors with a mutually exclusive expression trend. Coexpression is not frequent and seems to be restricted to monophasic subtype. These data indicate that a consistent fraction of this tumor type could represent a good candidate for kinase inhibitor molecules effective on KIT and PDGFR(beta) where their activation is sustained by an autocrine loop.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/24/8214

Purpose: The combinations of various chemotherapeutic drugs currently used to treat advanced small cell lung cancer (SCLC) led to similarly poor survival outcomes, which is why new molecular biology approaches are needed to design and select targeted therapies. Experimental Design: Thirteen stage I SCLC surgical specimens were screened for c-Kit gene mutations by sequencing whole cDNA and for KIT receptor expression/activation by immunoprecipitation and Western blotting. Both the paraffin-embedded and frozen materials were analyzed by immunocytochemistry, and the stem cell factor cognate ligand was assessed by retrotranscription PCR. Results: In all cases, we showed the presence of wild-type KIT receptors by analyzing the entire coding sequence, which together with the detection of the cognate ligand stem cell factor, supports the establishment of an autocrine loop. In addition, the KIT receptor was activated/phosphorylated. The immunoprecipitation/Western blotting data fit the observed immunophenotype. Interestingly, comparison of the level of KIT expression was at least 10 times...
higher in the tumoral specimens than the normal reference lungs. Conclusions: The KIT molecular profile derived from the analysis of SCLC surgical specimens shows that wild-type KIT is overexpressed and phosphorylated in the presence of stem cell factor. This finding, which is consistent with pathological KIT activation driven by an autocrine loop, is particularly interesting in the light of the recent development of new tyrosine kinase inhibitory drugs, which are highly effective in blocking wild-type KIT receptors.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/4/1115

Neutrophil elastase (NE) is a powerful serine protease capable of degrading most protein components of the extracellular matrix. We hypothesize that this elastase may play a significant role in lung cancer development and tested our hypothesis in a study of 348 primary lung cancer cases and 299 controls. Analysis of the entire gene using denaturing high performance liquid chromatography identified two novel single nucleotide polymorphisms (SNPs) in the promoter region: -903 T or G (REP-a) and -741 G or A (REP-b). Allele frequencies of these two SNPs were compared between the cases and controls using {chi}2 statistics. The estimated relative risk in association with the TT at REP-a or the GG at REP-b was measured by odds ratio. Individuals with -903TT or -741GG allele had a 2.3 and 1.4 times higher risk of developing lung cancer than those with TG or AA+AG alleles, respectively. The relative risk for the combined effects of both high-risk alleles at REP-a and REP-b, i.e., TT-GG type, was 24.8. Functional association of the two markers with cancer risk was examined by luciferase activity of the promoter containing different SNPs. We demonstrated a 1.9-fold relative luciferase activity in the promoter construct with -903T/-741G (T-G) compared with the -903G/-741A (G-A) in A549 human non-small cell lung cancer cells, providing evidence that the TT-GG type correlates with a high NE level. In conclusion, our findings support an etiological role of NE in lung cancer development.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/7/2362

Purpose: Chromosome 3p is deleted frequently in various types of human cancers, including lung cancer. Recently, the RASSF1A gene was isolated from the 3p21.3 region homozygously deleted in lung and breast cancer cell lines, and it was shown to be inactivated by hypermethylation of the promoter region in lung cancers. In this study, we investigated the pathogenetic and clinicopathological significances of RASSF1A methylation in the development and/or progression of lung adenocarcinoma. Experimental Design: Association of RASSF1A methylation with clinicopathological features, allelic imbalance at 3p21.3, p53 mutations, and K-ras mutations was examined in 110 stage I lung adenocarcinomas. Results: Thirty-five of 110 (32%) tumors showed RASSF1A methylation. RASSF1A methylation was dominantly detected in tumors with vascular invasion (P = 0.0242) or pleural involvement (P = 0.0305), and was observed more frequently in poorly differentiated tumors than in well (P = 0.0005) or moderately (P = 0.0835) differentiated tumors. Furthermore, RASSF1A methylation correlated with adverse survival by univariate analysis (P = 0.0368; log-rank test) as well as multivariate analysis (P = 0.032; risk ratio 2.357; 95% confidence interval, 1.075-5.169). The correlation between RASSF1A methylation and allelic imbalance at 3p21.3 was significant (P = 0.0005), whereas the correlation between RASSF1A methylation and p53 mutation was borderline (P = 0.0842). However, there was no correlation or inverse correlation between RASSF1A methylation and K-ras mutation (P = 0.2193). Conclusions: These results indicated that epigenetic inactivation of RASSF1A plays an important
role in the progression of lung adenocarcinoma, and that RASSF1A hypermethylation appears to be a useful molecular marker for the prognosis of patients with stage I lung adenocarcinoma.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/8/2612

Purpose: Nasopharyngeal carcinoma (NPC) is highly prevalent in southern China and characterized by a strong association with EBV. We aimed to detect EBV DNA and cancer-related gene promoter hypermethylation in nasopharyngeal (NP) brushing samples and provide a novel noninvasive approach for NPC detection. Experimental Design: Twenty-eight NPC cases and 26 noncancerous subjects were prospectively recruited. NP brushing samples were subjected to quantitative real-time PCR analysis of EBV DNA and methylation-specific PCR analysis of the DAP-kinase, RASSF1A, and p16 genes. Results: EBV DNA quantity in NP brushing samples from NPC patients (median, 8.94 copies/actin) was significantly higher than that of controls (median, 0 copies/actin; P < 0.0001). Twenty-seven of 28 NPC patients had detectable EBV DNA in NP brushes, whereas 25 of 26 controls had undetectable or very low levels of EBV DNA. Elevated EBV DNA level in brushing samples as a tumor marker had a sensitivity of 96.4% and a specificity of 96.2% for NPC detection. Moreover, T1 disease had a significantly lower EBV DNA level as compared with locally more advanced disease (P = 0.037). In brushing samples of NPC patients, the frequencies of DAP-kinase, RASSF1A, and p16 promoter hypermethylation were 50.0%, 39.3%, and 46.4%, respectively. Seventy-eight percent of cases showed methylation of at least one gene. No aberrant hypermethylation was detected in control samples. Conclusions: Our study demonstrated the feasibility of detecting multiple molecular tumor markers in NP brushing samples with a high sensitivity and specificity for NPC detection. It offers a powerful yet noninvasive approach for the diagnosis of NPC in high-risk populations.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/9/3579

Purpose: Vitamin D seems to exert a protective effect against common cancers, although this does not correlate with circulating levels of active 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], indicating a more localized activation of vitamin D. The aim of this study was to investigate the significance of this in breast cancer. Experimental Design: Quantitative reverse transcription-PCR analysis of mRNA expression was carried out for the vitamin D-activating enzyme 1{alpha}-hydroxylase, the catabolic enzyme 24-hydroxylase, and the vitamin D receptor in 41 tumors and paired nonneoplastic tissue as well as breast cancer cell lines. Immunohistochemistry was used to assess 1{alpha}-hydroxylase protein expression, and enzyme assays were used to quantify vitamin D metabolism. Results: Expression of mRNA for 1{alpha}-hydroxylase (27-fold; P < 5 x 10-11), vitamin D receptor (7-fold; P < 1.5 x 10-8), and 24-hydroxylase (4-fold; P < 0.02) was higher in breast tumors. 1{alpha}-Hydroxylase enzyme activity was also higher in tumors (44.3 {+/-} 11.4 versus 12.4 {+/-} 4.8 fmol/h/mg protein in nonneoplastic tissue; P < 0.05). However, production of inactive 1,24,25-trihydroxyvitamin D3 was also significantly higher in tumors (84.8 {+/-} 11.7 versus 33.6 {+/-} 8.5 fmol/h/mg protein; P < 0.01). Antisense inhibition of 24-hydroxylase in vitro increased antiproliferative responses to 1,25(OH)2D3. Conclusion: These data indicate that the vitamin D-activating enzyme 1{alpha}-hydroxylase is up-regulated in breast tumors. However, dysregulated expression of 24-hydroxylase seems to abrogate the effects of
local 1,25(OH)2D3 production in tumors by catalyzing catabolism to less active vitamin D metabolites. The enzymes involved in autocrine metabolism of vitamin D in breast tissue may therefore provide important targets for both the prevention and treatment of breast cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/2/433

Thymidylate synthase (TS), the target enzyme of the fluoropyrimidine class of drugs, has a 28-bp repeat polymorphism in the promoter region that has been associated with response of tumors to 5-fluorouracil-based therapy. Patients homozygous for the double repeat (2R/2R) in the TS gene have an overall better outcome from treatment than patients homozygous for the triple repeat (3R/3R). However, due to loss of heterozygosity at the TS locus on chromosome 18 in cancer cells, heterozygous 2R/3R individuals can acquire the 2R/loss or the 3R/loss genotype in their tumors. The purpose of this study was to determine whether the response of colorectal cancer to fluoropyrimidine therapy is associated with the resulting tumor TS genotype when loss of heterozygosity occurs in tumor DNA. A total of 30 colorectal cancer patients treated with the fluoropyrimidine-based combination S-1, all of whom had stage IV disease, were studied. The response rate to S-1 in this group of patients was 13 of 30 (43%). The heterozygous 2R/3R genotype was found in 22 of 30 normal tissues, whereas 10 (45%) of the matched cancer tissues showed only the 2R-sequence band (2R/loss), and 7 cancer tissues (32%) showed only the 3R-sequence band (3R/loss). The response rate of the 2R/loss tumor genotype patients was 80% (8 of 10) compared with 14% (1 of 7) in the 3R/loss genotype group (P = 0.029). Patients with tumor 3R/loss genotypes had significantly lower survival than 2R/loss genotypes. Heterozygous patients with a 2R/loss tumor genotype had the same survival as 2R/2R patients, whereas patients with a 2R/3R tumor genotype had a short survival similar to homozygous 3R/3R genotypes. These results show that: (a) response to 5-fluorouracil-based therapy is determined by tumor genotype; and (b) the 3R repeat is a direct negative determinant of outcome.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/9/3363

Purpose: Cyclooxygenase-2 (COX-2) is generally elevated in tumors compared with normal tissue and apparently has an important role in tumor development. A number of studies have found high expression of COX-2 to be an unfavorable prognostic factor for overall survival in several cancers. However, the influence of COX-2 expression levels on tumor response to chemotherapy has been relatively little studied. The purpose of this study was to ascertain if COX-2 gene expression is associated with tumor response in the clinical treatment of colorectal cancer with the fluoropyrimidine-based therapy S-1. Experimental Design: Patients with advanced (stage IV) colorectal cancer were treated with S-1 twice daily based on the patient's body surface area (BSA; BSA < 1.25 m2, 80 mg/d; 1.25 m2 [&le] BSA < 1.5 m2, 100 mg/d; BSA [&ge] 1.5 m2, 120 mg/d) for 28 days followed by a 2-week period rest. mRNA was isolated from paraffin-embedded pretreatment primary tumor specimens and expression levels of COX-2 relative to {beta}-actin as the internal reference gene were measured using a quantitative reverse transcription-PCR (Taqman) system. Results: The overall response rate in a group of 44 patients treated with S-1 was 40.9%. Sufficient tumor tissue was available from 40 of these patients for COX-2 mRNA quantitation. COX-2 gene expression was significantly lower in the responding
tumors compared with the nonresponders (P = 0.012, Wilcoxon test). Patients with COX-2 values above the cutoff value of 3.28 x 10^{-3} had a significantly shorter survival than those with COX-2 gene expressions below the cutoff value (adjusted P = 0.031). Conclusions: Intratumoral COX-2 gene expression is associated with likelihood of response to chemotherapy with S-1 and is a prognostic factor for survival of patients after the start of S-1 chemotherapy.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/5/1717

Purpose: Biliary tract cancer is an uncommon malignancy with a poor survival rate. We evaluated p16 gene alteration as a prognostic marker for this disease. Experimental Design: We studied p16 gene alterations by sequencing, methylation, and loss of heterozygosity of chromosome 9p in 118 biliary tract carcinomas, including 68 gallbladder cancers, 33 extrahepatic bile duct cancers, and 17 ampullary cancers. Survival was evaluated in 57 patients with gallbladder carcinomas, 27 with bile duct carcinomas, and 16 with ampullary carcinomas with and without somatic p16 alterations detected by two different methods. Results: p16 gene alterations including silent mutations were present in 61.8% gallbladder cancers, 54.5% bile duct cancers, and 70.6% ampullary cancers. p16 gene nonsilent mutations, p16 methylation, and loss of chromosome 9p21-22 that targets p14, p15, and p16 genes were present in 13 of 53 (24.5%), 8 of 54 (14.8%), and 32 of 44 (72.7%) gallbladder tumors; 5 of 25 (20.0%), 5 of 31 (16.1%), and 12 of 21 (57.1%) bile duct tumors; and 3 of 13 (23.1%), 6 of 15 (40.0%), and 8 of 16 (50.0%) ampullary tumors, respectively. The mean survival of patients with gallbladder cancers without p16 alterations was 21.5 {\pm} 14.8 months compared with 12.1 {\pm} 11.4 months for patients with p16 alterations (P = 0.02). Conclusions: Alteration of p16 gene alone or in combination with alterations of other tumor suppressor genes on chromosome 9p is a prognostic indicator in gallbladder carcinoma, with more favorable survival rates associated with carcinomas lacking p16 gene alterations.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/1/61

Knowledge of the molecular mechanisms involved in metastatic spread is needed to facilitate advances in prognostic evaluation for individual patients and in the design of therapeutic interventions to inhibit the process. In an effort to establish a methodological framework for analysis of molecules and mechanisms involved in this complex multistep process, we have developed a well-defined experimental system, in which the role of candidate genes can be screened and tested. By serial dilution cloning of the MDA-MB-435 breast tumor cell line and screening by orthotopic implantation into the mammary fat pad of athymic mice, we have derived a pair of breast tumor cell lines (M-4A4 and NM-2C5) that originate from the same breast tumor but have diagnostically opposite metastatic capabilities. In 74% of inoculated athymic mice, clone M-4A4 metastasized consistently to the lungs, mimicking a major dissemination route of human breast cancer. Conversely, although equally tumorigenic, clone NM-2C5 did not metastasize to any distal site. We have confirmed that the cell lines originate from a single genetic source by spectral karyotyping and evaluated the expression of a number of proteins previously implicated in cellular transformation and metastasis. The ability of M-4A4 to metastasize was not associated with increased angiogenesis, as measured by immunohistochemical microvessel density analysis. However, RNA and protein analyses revealed that two secreted proteins were differentially expressed: osteopontin expression was increased \( \approx \)30-fold in clone M-4A4 and
thrombospondin-1 expression was increased ~15-fold in clone NM-2C5. These cell lines constitute a stable and accessible model for the identification of genes involved in the multistep process of breast tumor metastasis. Manipulation of candidate genes in these cells will permit evaluation of their functional significance in the geometric progression of breast cancer.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/24/8396

Purpose: NY-ESO-1 and LAGE-1 are homologous cancer-testis antigens, which are expressed in many different cancers. It is essential to type tumors accurately to assess patient suitability for clinical trials which target these. This study evaluates typing strategies used to distinguish these two homologous but distinct antigens and to characterize and quantitate expression of each in clinical samples. Experimental Design: We typed 120 malignant melanomas for the expression of NY-ESO-1 and LAGE-1 with immunohistochemistry, reverse transcription-PCR (RT-PCR), and quantitative real-time (qRT-PCR), which was also used to explore the relationship between NY-ESO-1 and LAGE expression. Results: The two monoclonal antibodies ES121 and E978 had very similar immunohistochemistry reactivities. Both were specific for NY-ESO-1 because neither bound to homologous LAGE-1 peptides despite 84% overall amino acid homology. Of 120 melanomas tested by immunohistochemistry, NY-ESO-1 was expressed in >50% of cells in 23 melanomas (19%), between 11 and 50% cells in 15 (12.5%), <11% cells in 16 (13.5%), and negative in 66 (55%). Although specific for both antigens, the PCR methods did not provide this information about microheterogeneity. Polymorphisms in the LAGE-1 gene resulted in false negative LAGE-1 typing by qRT-PCR by inhibiting binding of oligonucleotide primers, thereby showing the exquisite specificity of qRT-PCR as a typing method. Conclusions: For NY-ESO-1 typing, immunohistochemistry compared favorably with the RT-PCR, with the added advantage of being able to characterize heterogeneity of antigen expression. Because neither mAb bound LAGE and because there was no coordinate expression LAGE and NY-ESO-1, separate typing for each is required.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/18/6265

Purpose: The synthetic retinoid fenretinide (4-HPR) exhibits preventive and therapeutic activity against ovarian tumors. An unidentified polar metabolite was previously found in 4-HPR-treated subjects and in A2780 human ovarian carcinoma cells continuously treated with 4-HPR (A2780/HPR). The metabolite and the enzyme involved in its formation in tumor cells are herein identified. Experimental Design: The metabolite was identified by mass spectrometry in A2780/HPR cell extracts and in plasma from 11 women participating in a phase III trial and treated with 200 mg/d 4-HPR for 5 years. The expression of proteins involved in retinoid metabolism and transport, cytochrome P450 26A1 (CYP26A1), cellular retinol-binding protein I (CRBP-I), and cellular retinoic acid-binding protein I and II (CRABP-I, CRABP-II) were evaluated in tumor cells by reverse transcription-PCR and Western blot analyses. Overexpression of CYP26A1 and retinoic acid receptors (RARs) in A2780 cells were obtained by cDNAs transfection. Results: The polar metabolite was 4-oxo-N-(4-hydroxyphenyl)retinamide (4-oxo-4-HPR) i.e., an oxidized form of 4-HPR with modification in position 4 of the cyclohexene ring. 4-oxo-4-HPR plasma levels were slightly lower (0.52 +/- 0.17 (micro)mol/L) than those of the
parent drug (0.84 {+/-} 0.53 {micro}mol/L) and of the already identified metabolite N-(4-methoxyphenyl)retinamide (1.13 {+/-} 0.85 {micro}mol/L). In A2780/HPR cells continuously treated with 4-HPR and producing 4-oxo-4-HPR, CYP26A1 and CRBP-I were markedly up-regulated compared with A2780 untreated cells. In A2780 cells, not producing 4-oxo-4-HPR, overexpression of CYP26A1 protein and metabolism of 4-HPR to 4-oxo-4-HPR were found in A2780 cells transfected with RAR{beta} and to a lesser extent in those transfected with RAR(gamma). Conclusions: A new metabolite of 4-HPR, 4-oxo-4-HPR, present in human plasma and in tumor cells, has been identified. The formation of this biologically active metabolite in tumor cells was due to CYP26A1 induction and was influenced by RAR expression. Moreover evidence was provided that 4-HPR up-modulates the expression of CRBP-I transcript, which is lost during ovarian carcinogenesis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/1/33

Purpose: ONYX-015 is a chimeric, E1B-deleted adenovirus designed to replicate preferentially in p53-deficient tumor cells; however, little is understood about its actual replication potential in human tumors. We hypothesized that replication of a late viral gene, hexon, would demonstrate replication of virus in human tissues. Experimental Design: In the course of a clinical trial, a patient with paired abdominal wall implants from a primary gall bladder carcinoma was injected with ONYX-015, 1 x 1010 viral particles/lesion, followed by sequential excision of the lesions at 37 h and 7 days. Tissue sections were analyzed for evidence of viral replication. Results: In situ Reverse transcription-PCR was used to measure expression of hexon. Strong signals were obtained in gland-forming tumor cells both at 37 h and at 7 days. Signal was predominantly observed in the cytoplasm. The signal was also observed in adjacent normal stromal cells. Analysis of p53 status of the tumor by immunohistochemistry and Affymetrix Genechip demonstrated an inactivating mutation in p53. Routine H&E staining of the tumor sections revealed no evidence of necrosis at 37 h or 7 days after injection of virus. Presence of viral protein at both 37 h and 7 days was confirmed by immunohistochemistry using antibodies directed against hexon, penton, and fiber proteins. Conclusions: Evidence for replication of hexon confirms that ONYX-015 is not only present but capable of replicating in tumor cells up to 1 week after intralesional injection and that replication is not confined to p53-mutated tumor cells.


http://clincancerres.aacrjournals.org/cgi/content/abstract/10/7/2407

Purpose: The purpose of this research was to evaluate the clinical significance of p16INK4A, p14ARF, p53, and proliferating cell nuclear antigen (PCNA) expression in tumor progression of cervical cancer. Design: Seventeen patients (40 samples) with consecutive cervical lesions from normal squamous epithelium, inflammation of the cervix to cervical intraepithelial neoplasm (CIN) and invasive cervical squamous cell cancer (SCC), or from CIN to SCC were collected for this study. Expression of p16INK4A, p14ARF, p53, and PCNA were detected by immunohistochemistry on paraffin-embedded sections. Human papillomavirus DNA was detected simultaneously with PCR and typed according to its DNA sequence. Results: p16INK4A
overexpression was significantly higher in CIN (75%) and in SCC (75%) than in normal or inflammation of the cervix (12.5%; P < 0.01, P < 0.05, respectively). The positive rate of p14ARF expression was higher in SCC (83%) than in normal/inflammation of the cervix (25%; P < 0.01). PCNA expression was negative in normal or inflammation of the cervix, but an increased expression was seen in 63.2% in CIN and 100% in SCC (P < 0.01, P < 0.05). When the time interval for disease progression from initial biopsy to CIN 3 or invasive cancer was compared with states of p16INK4A expression, cases stained positive for p16INK4A progressed within 64.2 months as compared with 122.3 months among those stained negatively (P < 0.01). Cases with increased p14ARF expression also had a short time interval for disease progression of 78.8 months as compared with 108.3 months in cases that were p14ARF negative. Cases with stable or decreased p53 expression had the shortest time interval for progression of 32.3 months in contrast to cases with no p53 expression (113.9 months). However, cases with increasing p53 expression progressed within 60.8 months. Conclusions: Our results suggested that altered states of p16INK4A, p14ARF, p53, and PCNA may be valuable markers to predict the progression of cervical neoplasia.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/8/2586

Purpose: Nasopharyngeal carcinoma (NPC) has been proven as a cancer associated with Epstein-Barr virus (EBV). This study was performed to examine sequence variations of the EBV nuclear antigen 1 gene (EBNA-1) in primary tumor and peripheral-blood cells of NPC patients from Taiwan. Experimental Design: DNA extracted from freshly frozen tumor tissues and corresponding peripheral-blood cells of 13 previously untreated NPC patients were subjected to PCR and direct sequencing using EBNA-1-specific primers. We compared the sequence data and analyzed the clinical outcomes. Results: We obtained a 100% positive-detection rate of EBV DNA in the primary tumors of all patients irrespective of the degree of differentiation. The EBNA-1 gene of all tumor samples was the "V-val" strain, showing the same clustered point mutations. They included 21 nucleotide exchanges, leading to 14 amino-acid mutations and 6 silent exchanges, relative to B95-8 cell line. Two of 13 tumors exhibited an additional point mutation at codon 585. EBV DNA was also detected in peripheral-blood cells of 9 of 13 patients under our experimental conditions. Direct-sequencing data showed match alterations of EBNA-1 gene between the primary tumor and peripheral-blood cells. Tumor relapse was observed in four of nine patients with detectable EBNA-1 DNA in their peripheral-blood cells, whereas none of the four patients without detectable EBNA-1 DNA in their peripheral-blood cells developed tumor relapse. Conclusions: Results of the current study represents the first demonstration of consistent sequence variation of EBNA-1 in primary tumors and peripheral-blood cells. Clinical observations support that the presence of EBV DNA in the peripheral-blood cells may arise from disseminated cancer cells, resulting in a higher relapse rate and poor prognosis.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/2/483

Purpose: To identify alternative splicing of the liver intestine-cadherin (LI-cadherin) gene in hepatocellular carcinoma (HCC) and correlate its aberrant expression with clinical outcomes. Experimental Design: Reverse transcription-PCR (RT-PCR) and quantitative real-time RT-PCR were used to examine alternative mRNA splicing and mRNA level of LI-cadherin in 50 paired
tumor-peritumor tissues of 50 HCC and 8 normal liver specimens. The minigene exon-trapping strategy was employed to investigate the splicing mechanism introduced by nucleotide polymorphisms. Association of LI-cadherin splicing with tumor venous infiltration, first-year tumor recurrence, and overall survival after partial hepatectomy were determined. Results: Alternative mRNA splicing of LI-cadherin was identified in half of the HCC specimens. Sequencing analysis indicated the loss of exon 7 in the spliced LI-cadherin gene. LI-cadherin mRNA was up-regulated from 2.58-fold to 800-fold in over 80% of HCC samples when compared with normal liver by quantitative PCR. Furthermore, nucleotide polymorphisms were identified in putative branch point at IVS6 + 35 (intron 6) as well as in coding sequence 651 (exon 6) in HCC tissues, which may affect alternative mRNA splicing. Clinically, those patients who harbored the alternative splicing of LI-cadherin were strongly associated with shorter overall survival time (P < 0.01) as well as higher incidences of tumor recurrences and venous infiltration (both P < 0.05) after hepatectomy. Conclusions: Over-expression of LI-cadherin was frequently detected in liver cancer patients. Aberrant alternative splicing of LI-cadherin was detected in 50% of HCC specimens and its clinical significance hinted at early tumor recurrence and poor overall survival of HCC patients.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/1/68

Purpose: To screen and validate the global gene expression in papillary thyroid carcinoma (PTC) using cDNA expression arrays and immunohistochemistry on tumor tissue microarrays in an attempt to find genes that may be of importance in the molecular pathogenesis and malignant progression of PTC. Experimental Design: Eighteen PTC tissue specimens were compared with three morphologically normal thyroid specimens by applying Atlas Human Cancer 1.2 Array membranes printed with cDNAs of 1176 human genes involved in cancer. Results for selected genes were confirmed by reverse transcription-PCR. Protein expression of selected genes was further studied using a tissue microarray consisting of 107 PTCs and compared with histologically normal thyroid tissue samples. Results: By cDNA arrays, two genes [c-MET and matrix metalloproteinase (MMP)-11] were expressed only in tumor tissue, where they were present in >50% of cases. Ten genes [macrophage inhibitory cytokine-1, CGD, fibronectin (FN), hypoxia-inducible factor 1, FC-(epsilon)-receptor (gamma)-chain, lactate dehydrogenase A, HLA-DPB1, AH receptor, tissue inhibitor of metalloproteinase (TIMP-1), and glycyl-tRNA-synthetase] were found to be up-regulated >2-fold in 40-100% of cancers, whereas 9 genes (GADD153, polykystic kidney disease-1, CYR61, DPC4, HBA1, gravin, DLG3, protein tyrosine phosphatase {varsigma}, and heterochromatin protein 1 homologue-(alpha)) were down-regulated to <50% of their normal levels in 40-94% of cases. Conventional reverse transcription-PCR gave consistent results with the cDNA array findings for all four genes selected to be studied (c-MET, FN, TIMP-1, and GADD153). Immunohistochemistry for three selected proteins, FN, MMP-11, and TIMP-1, showed positive staining in 81, 87, and 68% of the tumor samples, respectively. Conclusions: Several novel and previously undetected tumor promoting/inhibiting genes may be of importance in the molecular pathogenesis and malignant progression of PTC. Transcription of these genes may result in overexpression of proteins, such as c-MET, MMP-11, TIMP-1, and FN, which may contribute to the pathogenesis of PTC.

Purpose: The purpose of this study was to profile methylation alterations of CpG islands in ovarian tumors and to identify candidate markers for diagnosis and prognosis of the disease.

Experimental Design: A global analysis of DNA methylation using a novel microarray approach called differential methylation hybridization was performed on 19 patients with stage III and IV ovarian carcinomas. Results: Hierarchical clustering identified two groups of patients with distinct methylation profiles. Tumors from group 1 contained high levels of concurrent methylation, whereas group 2 tumors had lower tumor methylation levels. The duration of progression-free survival after chemotherapy was significantly shorter for patients in group 1 compared with group 2 (P < 0.001). Differential methylation in tumors was independently confirmed by methylation-specific PCR. Conclusions: The data suggest that a higher degree of CpG island methylation is associated with early disease recurrence after chemotherapy. The differential methylation hybridization assay also identified a select group of CpG island loci that are potentially useful as epigenetic markers for predicting treatment outcome in ovarian cancer patients.


Purpose: Because inherited BRCA1 or BRCA2 mutations strikingly increase ovarian cancer risk, polymorphisms in these genes could represent low penetrance susceptibility alleles. Previous studies of the BRCA2 N372H polymorphism suggested that HH homozygotes have a modestly increased risk of both breast and ovarian cancer. We have examined whether BRCA2 N372H or common amino acid-changing polymorphisms in BRCA1 predispose to ovarian cancer.

Experimental Design: A population-based, case control study of ovarian cancer was performed in North Carolina. Cases included 312 women with ovarian cancer (76% invasive and 24% borderline) and 401 age- and race-matched controls. Blood DNA from subjects was genotyped for BRCA2 N372H and BRCA1 Q356R and P871L. Results: There was no association between BRCA2 N372H and risk of borderline or invasive epithelial ovarian cancer. The overall odds ratio (OR) for HH homozygotes was 0.8 [95% confidence interval (CI) = 0.4-1.5] and was similar in all subsets, including invasive serous cases. In addition, neither the BRCA1 Q356R (OR = 0.9, 95% CI 0.5-1.4) nor P871L (OR = 0.9, 95% CI 0.6-1.9) polymorphisms were associated with ovarian cancer risk. There was a significant racial difference in allele frequencies of the P871L polymorphism (P = 0.64 in Caucasians, L = 0.76 in African-Americans, P < 0.0001). Conclusions: In this population-based, case control study, common amino acid changing BRCA1 and 2 polymorphisms were not found to affect the risk of developing ovarian cancer.


Purpose: The RNASEL gene has been proposed as a candidate gene for the HPC1 locus through a positional cloning and candidate gene approach. Cosegregation between the truncating mutation E265X and disease in a hereditary prostate cancer (HPC) family and association between prostate cancer risk and the common missense variant R462Q has been reported. To additionally evaluate the possible role of RNASEL in susceptibility to prostate cancer risk, we performed a comprehensive genetic analysis of sequence variants in RNASEL in the Swedish population. Experimental Design: Using 1624 prostate cancer cases and 801 unaffected controls,
the truncating mutation E265X and five common sequence variants, including the two missense mutations R462Q and D541E, were evaluated for association between genotypes/haplotypes and prostate cancer risk. Results: The prevalence of E265X carriers among unaffected controls and prostate cancer patients was almost identical (1.9 and 1.8% in controls and cases, respectively), and evidence for segregation of E265X with disease was not observed within any HPC family. Overall, the analyses of common sequence variants provided limited evidence for association with prostate cancer risk. We found a marginally significant inverse association between the missense mutation D541E and sporadic prostate cancer risk (odds ratio, 0.77; 95% confidence interval, 0.59-1.00) and reduced risk of prostate cancer in carriers of two different haplotypes being completely discordant. Conclusions: Considering the high quality in genotyping and the size of this study, these results provide solid evidence against a major role of RNASEL in prostate cancer etiology in Sweden.


Purpose and Experimental Design: The role of RASSF1A has been elucidated recently in regulating apoptosis and cell cycle progression by inhibiting cyclin D1 accumulation. Aberrant RASSF1A promoter methylation has been found frequently in multiple adult cancer types. Using methylation-specific PCR and reverse transcription-PCR, we investigated epigenetic deregulation of RASSF1A in primary tumors, adjacent nontumor tissues, secondary metastases, peripheral blood cells, and plasma samples from children with 18 different cancer types, in association with their clinicopathologic features. Results: Regardless of the tumor size, ubiquitous RASSF1A promoter methylation was found in 67% (16 of 24) of pediatric tumors, including neuroblastoma, thyroid carcinoma, hepatocellular carcinoma, pancreaticoblastoma, adrenocortical carcinoma, Wilms' tumor, Burkitt's lymphoma, and T-cell lymphoma. A majority (75%) of pediatric cancer patients with tumoral RASSF1A methylation was male. Methylated RASSF1A alleles were also detected in 4 of 13 adjacent nontumor tissues, suggesting that this epigenetic change is potentially an early and critical event in childhood neoplasia. RASSF1A promoter methylation found in 92% (11 of 12) of cell lines largely derived from pediatric cancer patients was significantly associated with transcriptional silencing/repression. After demethylation treatment with 5-aza-2'-deoxycytidine, transcriptional reactivation was shown in KELLY, RD, and Namalwa cell lines as analyzed by reverse transcription-PCR. For the first time, RASSF1A methylation was detected in 54% (7 of 13), 40% (4 of 10), and 9% (1 of 11) of buffy coat samples collected before, during, and after treatment, correspondingly, from pediatric patients with neuroblastoma, thyroid carcinoma, hepatocellular carcinoma, rhabdomyosarcoma, Burkitt's lymphoma, T-cell lymphoma, or acute lymphoblastic leukemia. Concordantly, RASSF1A methylation was found during treatment in plasma of the same patients, suggesting cell death and good response to chemotherapy. Conclusions: RASSF1A methylation in tumor or buffy coat did not correlate strongly with age, tumor size, recurrence/metastasis, or overall survival in this cohort of pediatric cancer patients. Of importance, epigenetic inactivation of RASSF1A may potentially be crucial in pediatric tumor initiation.


Purpose and Experimental Design: Using real-time quantitative methylation-specific PCR (RTQ-
MSP), we quantified methylated p16INK4a sequences and determined the fractional concentrations of circulating tumor DNA in plasma, serum, and peripheral blood cells collected preoperatively, intraoperatively, and postoperatively from 49 patients with hepatocellular carcinoma (HCC). Results: RTQ-MSP was sufficiently sensitive to detect down to 10 genome-equivalents of methylated p16INK4a sequences. Quantitative MSP data were expressed in terms of the methylation index, which was the percentage of bisulfite converted unmethylated and methylated p16INK4a sequences that consisted of methylated p16INK4a sequences. Quantities of methylated p16INK4a sequences were detected in peripheral circulation of 80% (23 of 29) of HCC patients. No significant difference was seen in the detectability and concentrations of methylated p16INK4a sequences (range: 10-4046 genome-equivalents/ml) between preoperative plasma and serum samples from HCC patients. Preoperatively, the p16INK4a methylation indices ranged from 0.2 to 100% and from 0.012 to 0.075% in the patients' plasma and buffy coat samples, respectively. After surgical resection, the median p16INK4a methylation indices in plasma and buffy coat concordantly decreased 12- and 15-fold, respectively. These results demonstrated the clinical usefulness and effectiveness of peripheral blood RTQ-MSP for detecting and monitoring HCC after treatment. Furthermore, none of the intraoperative plasma samples and only two of the intraoperative buffy coat samples were p16INK4a methylation positive. Conclusions: Quantification of epigenetic changes in peripheral blood by RTQ-MSP is useful for the detection and monitoring of HCC.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/3/1319

Purpose: Treatment with IFN-{alpha} therapy has been shown to exhibit antitumor effects on patients with hepatocellular carcinoma (HCC). However, individual responses remained unpredictable because of the frequent presence of intrinsic or acquired IFN-{alpha} resistance. Hence, delineation of molecular targets implicated in the resistant pathway holds value in refining the therapeutic benefits of IFN-{alpha}. Experimental Design: The current study analyzed the effect of IFN-{alpha} in human HCC cells. Three hepatitis C virus (HCV)-related, five hepatitis B virus (HBV)-related and two non-B non-C-related cell lines were subjected to IFN-{alpha} treatment and the cytotoxic effect on cell viability was measured. Further analysis by cDNA microarray and quantitative reverse transcription-PCR were conducted to examine the gene expression changes that mediated the IFN-{alpha} resistance observed. Results: According to the IC50 values determined, HCV-related cell lines indicated distinct resistance (IC50, 389-1468 units/mL) compared with the HBV-related (IC50, 11-77 units/mL) and non-B non-C-related cell lines (IC50, 24-108 units/mL). Unsupervised hierarchical clustering on array data indicated three HCV-related cell lines to cluster independently from the sensitive cell lines, suggesting discrete features in association with IFN-{alpha} tolerance. Moreover, Significance Analysis of Microarrays analysis indicated the differential expression of 149 expressed sequence tags that represented 51 up-regulated and 98 down-regulated genes in the resistant cell lines. Comparing the temporal pattern of gene expression between 6- and 24-hour treatments, candidate genes that were considerably induced with time were further highlighted in the tolerant HCV-related cell lines. These candidates were verified by quantitative reverse transcription-PCR, which confirmed the down-regulation of UBA2, ZNF185, and FOXF1 and up-regulation of UBE4B in the drug-tolerant cells. Conclusions: Our present study showed that the insensitivity to IFN-{alpha} therapy in HCC cells is associated with drug-inducible transcriptional alterations. Furthermore, our investigation highlighted potential candidate genes in conferring an anti-apoptotic effect toward IFN-{alpha} treatment.

Purpose: We sought to determine whether the -6 exon 13 T>C polymorphism in the DNA mismatch repair gene hMSH2 modulates susceptibility to acute myeloid leukemia after therapy and particularly after O6-guanine alkylating chemotherapy. We also determined the extent of microsatellite instability (MSI) in therapy-related acute myeloid leukemia (t-AML) as a marker of dysfunctional DNA mismatch repair. Experimental Design: Using a novel restriction fragment length polymorphism, verified by direct sequencing, we have genotyped 91 t-AML cases, 420 de novo acute myeloid leukemia cases, and 837 controls for the hMSH2 -6 exon 13 polymorphism. MSI was evaluated in presentation bone marrow from 34 cases using the mononucleotide microsatellite markers BAT16, BAT25, and BAT26. Results: Distribution of the hMSH2 -6 exon 13 polymorphism was not significantly different between de novo acute myeloid leukemia cases and controls, with heterozygotes and homozygotes for the variant (C) allele representing 12.2 and 1.6%, respectively, of the control population. However, the variant (C) hMSH2 allele was significantly overrepresented in t-AML cases that had previously been treated with O6-guanine alkylating agents, including cyclophosphamide and procarbazine, compared with controls (odds ratio, 4.02; 95% confidence interval, 1.40-11.37). Thirteen of 34 (38%) t-AML cases were MSI positive, and 2 of these 13 cases were homozygous for the variant (C) allele, a frequency substantially higher than in the control population. Conclusions: Association of the hMSH2 -6 exon 13 variant (C) allele with leukemia after O6-guanine alkylating agents implicates this allele in conferring a nondisabling DNA mismatch repair defect with concomitant moderate alkylation tolerance, which predisposes to the development of t-AML via the induction of DNA mismatch repair-disabling mutations and high-grade MSI. Homozygosity for the hMSH2 variant in 2 of 13 MSI-positive t-AML cases provides some support for this model.

http://clincancerres.aacrjournals.org/cgi/content/abstract/9/8/3012


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/3/1099
Purpose: This study was designed to evaluate molecular markers for the detection of micrometastasis in esophageal adenocarcinoma, define algorithms to distinguish positive from benign lymph nodes and to validate these findings in an independent tissue set and in patients with pN0 esophageal adenocarcinoma. Experimental Design: Potential markers were identified through literature and database searches. All markers were analyzed by quantitative reverse transcription (QRT)-PCR on a limited set of primary tumors and benign lymph nodes. Selected markers were further evaluated on a larger tissue set and classification algorithms were generated for individual markers and combinations. Algorithms were statistically validated internally as well as externally on an independent set of lymph nodes. Selected markers were then used to identify occult disease in lymph nodes from 34 patients with pN0 esophageal adenocarcinoma. Results: Thirty-nine markers were evaluated, six underwent further analysis and five were analyzed in the external validation study. Two markers provided perfect classification in both the screening and validation sets, although parametric bootstrap analysis estimated 2% to 3% optimism in the observed classification accuracy. Several marker combinations also gave perfect classification in the observed data sets, and estimates of optimism were lower, implying more robust classification than with individual markers alone. Five of thirty-four patients with esophageal adenocarcinoma had positive nodes by multimarker QRT-PCR analysis and disease-free survival was significantly worse in these patients (P = 0.0023). Conclusions: We have identified novel QRT-PCR markers for the detection of occult lymph node disease in patients with esophageal adenocarcinoma. The objective nature of QRT-PCR results, and the ability to detect occult metastases, make this an attractive alternative to routine pathology.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/1/167

Purpose: The purpose of this study was to determine the potential of cancer testis (CT) antigens as vaccines for non-Hodgkin's lymphomas (NHLs). Experimental Design: Ninety-three specimens of NHLs were analyzed for their composite expression of eight CT genes (MAGE-3, MAGE-4, CT-7, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-TES-14/SCP-1, and HOM-TES-85). Thirty-nine of these specimens were also analyzed for their NY-ESO-1 expression. Results: Only 1 of 7 cases of chronic lymphocytic leukemia expressed a CT gene (HOM-TES-14/SCP-1), and 10 follicular lymphomas were negative for all of the CT genes tested. In B-cell lymphomas, the most frequent expression of CT genes was observed in diffuse large-cell lymphomas (HOM-TES-14/SCP-1: 7 of 28; SSX-1: 5 of 28; CT-7: 2 of 28; and HOM-MEL-40/SSX-2 and HOM-TES-85: 1 of 28 positive cases). Only 1 of 8 Burkitt's and 1 of 7 lymphoblastic lymphomas expressed a CT gene (CT7 and HOM-TES-14/SCP-1, respectively). A majority (9 of 15) of T- NHLs (9 peripheral T-cell lymphomas, 2 lymphoblastic T-cell lymphomas, and 4 cases of AILD) expressed HOM-TES-14/SCP-1. Conclusions: HOM-TES-14/SCP-1, and to some degree SSX-1 and CT-7 might be candidates for lymphoma vaccine development. However, the identification of additional tumor-specific antigens with a frequent expression in lymphomas is warranted to allow for the development of widely applicable polyvalent lymphoma vaccines.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/5/1764

Purpose and Experimental Design: CDC25 genes are cell cycle-activating phosphatases that
positively regulate the activity of cyclin-dependent kinase. CDC25A and CDC25B, being oncogenes, are overexpressed in a variety of human malignancies. To investigate the potential roles of CDC25s in hepatocellular carcinoma (HCC), expression of CDC25A and CDC25B was examined in human HCC samples. Results: Reverse transcription-PCR showed that overexpression of CDC25A and CDC25B mRNAs was found in 9 of 13 (69%) and 4 of 13 (31%) HCCs, respectively. Immunohistochemistry of 59 HCCs showed marked increase in CDC25A expression, but not CDC25B, in HCC compared with noncancer tissues, and high expression of CDC25A in 33 of 59 (56%) HCCs. Overexpression of CDC25A in HCC was confirmed by Western blot analysis. High expression of CDC25A was associated with dedifferentiated phenotype and portal vein invasion (P = 0.001 and 0.031, respectively), and expression of CDC25A correlated well with proliferating cell nuclear antigen labeling index (P = 0.005). Univariate analysis indicated that high expression of CDC25A and proliferating cell nuclear antigen were both significant predictive factors for shorter disease-free survival (P = 0.004 and 0.039, respectively). Multivariate analysis indicated that CDC25A was an independent prognostic marker for disease-free survival (risk ratio for cancer relapse, 2.98; P = 0.029), even when analyzed with several clinicopathologic factors. On the other hand, expression of CDC25B did not correlate with any clinicopathological features. Conclusion: Our findings suggest that CDC25A, but not CDC25B, could be used as an independent prognostic marker for HCC. Our data would also contribute to forward understanding of tumor biology of HCC that is associated with cell cycle regulation.


http://clincancerres.aacrjournals.org/cgi/content/abstract/11/7/2471

Background: {beta}-catenin, depending on subcellular localization, plays a dual role in carcinogenesis: as a signaling factor (in the nucleus) and as an adhesion molecule (in cell membrane). In this study, we sought to determine the role of {beta}-catenin in head and neck carcinogenesis. Methods: First, we studied the incidence of mutations of {beta}-catenin in a cohort of 60 head and neck squamous cell cancers (HNSCC). We subsequently evaluated the protein expression levels of {beta}-catenin in a cohort of oropharyngeal squamous cell cancer tissue microarray using a novel in situ method of quantitative protein analysis and correlated those with cyclin D1 levels and clinical and pathologic data. Results: The mean follow-up time for survivors was 45 months and for all patients was 35 months. We found no mutations in the cohort of 60 HNSCC. {beta}-catenin displayed primarily membranous expression pattern. Patients with high tumor-node-metastasis stage were more likely to have high expression of {beta}-catenin (P = 0.040). Patients with low {beta}-catenin expression had a local recurrence rate of 79% compared with 29% for patients with high {beta}-catenin tumors (P = 0.0021). Univariate Cox regression revealed a hazard ratio for low {beta}-catenin tumors of 3.6 (P = 0.004). Kaplan-Meier analysis showed that patients with low {beta}-catenin expressing tumors trended toward worse 5-year disease-free survival (P = 0.06). In multivariate analysis, only {beta}-catenin expression status was an independent prognostic factor (P = 0.044) for local recurrence. Tumors with high {beta}-catenin had low cyclin D1 and vice versa (P = 0.007). Conclusions: The absence of activating {beta}-catenin mutations combined with the inverse correlation between {beta}-catenin levels with cyclin D1 levels and outcome suggest that {beta}-catenin mainly functions as an adhesion and not signaling molecule in HNSCC.


http://clincancerres.aacrjournals.org/cgi/content/abstract/8/4/1087
Purpose and Experimental Design: Glutathione S-transferases, enzymes that defend cells against damage mediated by oxidant and electrophilic carcinogens, may be critical determinants of cancer pathogenesis. In this report, we assess the role of epigenetic silencing of the GSTP1 gene, a gene encoding the \( \pi \)-class glutathione S-transferase, in the pathogenesis of hepatitis B virus (HBV)-associated hepatocellular carcinomas (HCC). The cell lines Hep3B, HepG2, and a cohort of 43 HBV-associated HCC tissue specimens and corresponding nontumor tissues were subjected to analysis for GSTP1 epigenetic alteration and expression. GSTP1 "CpG" island DNA hypermethylation in the liver cell lines, and the tissue specimens were determined by methylation-specific PCR and correlated with expression of the gene using reverse-transcription PCR, immunoblotting, and immunohistochemistry. Results: GSTP1 CpG island DNA hypermethylation was detected in 28 of 43 (65.1%) HCC tissues and 4 of 40 (10%) corresponding nontumor tissues. GSTP1 protein was absent in those cases showing hypermethylation of the gene. Similarly, DNA from Hep3B and HepG2 cell lines displayed complete GSTP1 hypermethylation in the CpG island, and they failed to express GSTP1 mRNA and the corresponding protein product. Treatment of the cell lines with the DNA methyltransferase inhibitor 5-aza-deoxycytidine reversed the hypermethylation, and restored GSTP1 mRNA and polypeptide expression. Conclusions: These data indicate that epigenetic silencing of GSTP1 gene expression by CpG island DNA hypermethylation is common in human HBV-associated HCC. In addition, somatic GSTP1 inactivation via CpG island hypermethylation may contribute to the pathogenesis of this malignancy.


http://clincancerres.aacrjournals.org/cgi/content/abstract/9/9/3376

Purpose and Experimental Design: The human Ras association domain family 1A gene (RASSF1A) is a newly isolated tumor suppressor gene. In this study, we analyzed the methylation status of the promoter region of RASSF1A using bisulfite sequencing and PCR-RFLP in four liver cancer cell lines (Hep3B, HepG2, SK-HEP-1, and Huh-7) and a cohort of 43 hepatitis B virus-associated hepatocellular carcinoma (HCC) tissues and their corresponding nontumor tissue specimens. Results: The methylation of the CpG islands in the RASSF1A promoter was not detected in 4 samples of normal liver tissue or 10 samples of peripheral blood mononuclear cells from normal subjects. However, the CpG islands were completely methylated, and transcription of the RASSF1A was silenced in the four cell lines. Treatment with the DNA methylation inhibitor 5-aza-2'-deoxycytidine reactivated the expression of RASSF1A in the Hep3B and HepG2 cells. In 41 of 43 (95%) HCC specimens studied, the promoter region of RASSF1A was intensively methylated at its CpG sites. Although heterogeneous methylation was also detected in 16 of the 23 (70%) corresponding nontumorous tissues analyzed, the level of methylation was significantly lower than in the corresponding tumor tissues. Conclusions: HCC has the highest incidence of promoter methylation of RASSF1A among all malignancies yet reported suggesting that hypermethylation of the CpG island promoter of RASSF1A may play an important pathological role in this tumor.

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http://www.clinchem.org


http://www.clinchem.org/cgi/content/abstract/48/8/1338

Background: PML/RAR{alpha} fusion transcripts provide a readily accessible marker for diagnosis of acute promyelocytic leukemia (APL) and for monitoring response to therapy. Survival rates are improved by therapies guided by such monitoring. We assessed the potential of DzyNA reverse transcription-PCR (RT-PCR) for measurement of PML/RAR{alpha} fusion transcripts. Methods: Parallel single-tube DzyNA RT-PCR protocols were developed to allow real-time fluorescent quantification of PML/RAR{alpha} fusion transcripts and a low abundance control transcript, normal BCR. Calibration curves, generated using cell line RNA, allowed estimation of these transcripts in RNA from patients with APL at various stages of the disease. Results: DzyNA RT-PCR calibration curves were linear for both transcripts over a broad range and demonstrated interassay variations of 12% (mean, 658 ng) and 10% (mean, 263 ng), respectively. The protocols detected low concentrations of transcripts and resolved twofold dilutions. PML/RAR{alpha} mRNA was quantified in 10 patients at diagnosis and in 1 patient over a 7-year period. Monitoring of transcript concentrations effectively reflected the disease course in one patient and demonstrated that an increase in PML/RAR{alpha} transcripts can be detected 4-6 months before hematologic relapse, with no false-positive results. Conclusion: DzyNA RT-PCR has potential for use in clinical practice as a tool for diagnosis of APL and for subsequent monitoring of minimal residual disease and detection of molecular relapse.


http://www.clinchem.org/cgi/content/abstract/50/9/1544

Background: Transthyretin-associated hereditary amyloidosis (ATTR) is an inherited disease in which variants in the primary structure of transthyretin (TTR; prealbumin) lead to the extracellular polymerization of insoluble protein fibrils, causing organ failure and ultimately death when major organs are involved. We have developed an integrated approach to molecular diagnosis with initial analysis of intact plasma TTR by electrospray ionization mass spectrometry (MS) and referral of positive samples for DNA sequence analysis and real-time PCR to confirm the common Gly6Ser polymorphism. Methods: Samples from 6 patients previously diagnosed with ATTR and from 25 controls with (n = 15) or without (n = 10) polyneuropathy were analyzed in a blinded fashion for the presence of variant TTR. TTR protein was extracted with an immunoaffinity resin from 20 {micro}L of archived plasma samples. The purified TTR was reduced with tris(2-carboxyethyl)phosphine and analyzed by MS. The appearance of two peaks (or a single peak shifted in mass indicative of a homozygous variant), including the wild-type mass of 13 761 Da, was indicative of the presence of a variant, and the individual was referred for DNA sequence analysis. Results: MS analysis of intact reduced TTR correctly identified each of six samples known to contain variant TTR. These results were corroborated by subsequent DNA sequence analysis. Additionally, all Gly6Ser polymorphisms were correctly called based on the +30 mass shift and an equal relative abundance of the +30 polymorphism relative to wild-type
TTR. No false-positive results were seen. Conclusions: This referral method eliminates the necessity of sequencing most samples and allows screening for the familial forms of amyloidosis in a broad patient population in a timely fashion. This method correctly identified all previously known variants and also identified a novel variant, Val94Ala.


http://www.clinchem.org/cgi/content/abstract/49/10/1642

Background: fms-related tyrosine kinase 3 (Flt3) is the most commonly mutated gene in human acute myeloid leukemia (AML) and has been implicated in its pathogenesis. Because screening of Flt3 in AML patients by PCR followed by gel electrophoresis is time-consuming and fails to detect some very small internal tandem duplications (ITDs), we developed a method for screening of FLT3 receptor mutations with PCR plus denaturing HPLC (D-HPLC). Methods: Total mRNAs extracted from 34 AML patients were first analyzed for the presence of juxtamembrane length mutations and tyrosine kinase domain point mutations by a conventional method involving PCR amplification, restriction enzyme digestion, and agarose gel electrophoresis (PCR-RED-AGE). Subsequently, the same patient panel was analyzed by D-HPLC, using specifically designed primers and optimized running temperatures for the length and point mutation analysis. Results: Thirty-four patients were analyzed by PCR-RED-AGE; 9 were positive for known Flt3 mutations: 6 of 34 (18%) for ITDs in exon 14 and 3 of 34 (9%) for point mutations in exon 20. The same patient panel was analyzed by D-HPLC, and additional nucleotide changes were discovered; in total, 14 sequence variations were identified: 7 of 34 (21%) for ITDs in exon 14; 2 of 34 (6%) for point mutations in exon 20; 1 of 34 (3%) for a new point mutation in exon 16; and 4 of 34 (12%) for polymorphisms in exons 13 and 14. Direct sequencing analysis identified nucleotide alterations in each of the "D-HPLC positives" but in none of the "D-HPLC negatives", yielding a specificity and sensitivity of 100% for D-HPLC-based screening. Conclusions: This novel D-HPLC-based procedure, which is optimized for identification of new point mutations in the catalytic and regulatory domains of FLT3 receptor, could potentially be useful for studies involving precise genotype determination, which could be critical for selection of innovative AML therapies targeting the FLT3 protein.


http://www.clinchem.org/cgi/content/abstract/49/12/1981

Background: Hereditary hemochromatosis is a recessive disorder characterized by iron accumulation in parenchymal cells, followed by organ damage and failure. The disorder is mainly attributable to the C282Y and H63D mutations in the HFE gene, but additional mutations in the HFE, transferrin receptor 2 (TfR2), and hepcidin genes have been reported. The copresence of mutations in different genes may explain the phenotypic heterogeneity of the disorder and its variable penetrance. Methods: We used denaturing HPLC (DHPLC) for rapid DNA scanning of the HFE (exons 2, 3, and 4), hepcidin, and TfR2 (exons 2, 4 and 6) genes in a cohort of 657 individuals with altered indicators of iron status. Results: DHPLC identification of C282Y and H63D HFE alleles was in perfect agreement with the restriction endonuclease assay. Fourteen DNA samples were heterozygous for the HFE S65C mutation. In addition, we found novel mutations: two in HFE (R66C in exon 2 and R224G in exon 4), one in the hepcidin gene (G71D), and one in TfR2 (V22I), plus several intronic or silent substitutions. Six of the seven individuals with hepcidin or TfR2 coding mutations carried also HFE C282Y or S65C mutations. Conclusion:
DHPLC is an efficient method for mutational screening for the genes involved in hereditary hemochromatosis and for the study of their copresence.


http://www.clinchem.org/cgi/content/abstract/48/12/2147

Background: The genotype of hepatitis C virus (HCV) is a predictor of antiviral therapeutic response. We describe an approach for HCV genotype determination by real-time PCR and melting curve analysis. Methods: After automated nucleic acid extraction, we used reverse transcription-PCR in a block cycler to amplify nucleotides 6-329 of the 5' untranslated region of HCV. The product was further amplified by single-tube real-time seminested PCR in a LightCycler™ instrument (Roche). The final product was analyzed by melting curves with the use of fluorescence resonance energy transfer (FRET) probes. The FRET sensor probe was directed at nucleotides 151-170 of type 1 HCV and was designed to distinguish types 1a/b, 2a/c, 2b, 3a, and 4, with melting temperatures (Tms) predicted to differ by 1 (degrees)C. Genotypes were compared in a blinded fashion with those of the INNO-LiPATM test (Bayer Diagnostics) on 111 serum samples. Results: In preliminary experiments, the Mg2+ concentration was found to be critical in allowing clear separation of melting points, with the best separation at a Mg2+ concentration of 2 mmol/L. The results for 111 samples clustered at expected Tms for genotypes 1a/b (n = 78), 2a/c (n = 2), 2b (n = 11), 3a (n = 14), and 4 (n = 2). Of the 111 samples, results for 110 were concordant with the comparison method at the level of type 1, 2, 3, or 4. Subtyping results were discordant for two samples, both of type 2. For 108 samples concordant with INNO-LiPA at the genotype and subtype levels, the mean Tms were 64.1, 59.5, 54.2, 52.6, and 50.1 (degrees)C for types 1a/b, 2a/c, 4, 2b, and 3a, respectively, with SDs of 0.2, 0.3, 0.3, 0.2, and 0.3 (degrees)C. All 78 samples identified as type 1 were concordant with results of the comparison method. Conclusions: Melting analysis with a single pair of FRET probes can rapidly provide information about HCV genotypes and identifies type 1 samples with high specificity.


http://www.clinchem.org/cgi/content/abstract/50/1/88

Background: The discovery of fetal DNA in maternal plasma has opened up an approach for noninvasive prenatal diagnosis. Despite the rapid expansion in clinical applications, the molecular characteristics of plasma DNA in pregnant women remain unclear. Methods: We investigated the size distribution of plasma DNA in 34 nonpregnant women and 31 pregnant women, using a panel of quantitative PCR assays with different amplicon sizes targeting the leptin gene. We also determined the size distribution of fetal DNA in maternal plasma by targeting the SRY gene. Results: The median percentages of plasma DNA with size >201 bp were 57% and 14% for pregnant and nonpregnant women, respectively (P <0.001, Mann-Whitney test). The median percentages of fetal-derived DNA with sizes >193 bp and >313 bp were 20% and 0%, respectively, in maternal plasma. Conclusion: Plasma DNA molecules are mainly short DNA fragments. The DNA fragments in the plasma of pregnant women are significantly longer than those in the plasma of nonpregnant women, and the maternal-derived DNA molecules are longer than the fetal-derived ones.

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http://www.clinchem.org/cgi/content/abstract/49/5/719

Background: Recent studies have demonstrated the existence of circulating mitochondrial DNA in plasma and serum, but the concentrations and physical characteristics of circulating mitochondrial DNA are unknown. The aim of this study was to develop an assay to quantify mitochondrial DNA in the plasma of healthy individuals. Methods: We adopted a real-time quantitative PCR approach and evaluated the specificity of the assay for detecting mitochondrial DNA with a cell line (rho0) devoid of mitochondria. The concentrations and physical characteristics of circulating mitochondrial DNA were investigated by experiments conducted in three modules. In module 1, we evaluated the concentrations of mitochondrial DNA in plasma aliquots derived from four blood-processing protocols. In module 2, we investigated the existence of both particle-associated and free forms of mitochondrial DNA in plasma by subjecting plasma to filtration and ultracentrifugation. In module 3, we used filters with different pore sizes to investigate the size characteristics of the particle-associated fraction of circulating mitochondrial DNA. Results: The mitochondrial DNA-specific, real-time quantitative PCR had a dynamic range of five orders of magnitude and a sensitivity that enabled detection of one copy of mitochondrial DNA in plasma. In module 1, we found significant differences in the amounts of circulating mitochondrial DNA among plasma aliquots processed by different methods. Data from module 2 revealed that a significant fraction of mitochondrial DNA in plasma was filterable or pelletable by ultracentrifugation. Module 3 demonstrated that filters with different pore sizes removed mitochondrial DNA from plasma to different degrees. Conclusions: Both particle-associated and free mitochondrial DNA are present in plasma, and their respective concentrations are affected by the process used to harvest plasma from whole blood. These results may have implications in the design of future studies on circulating mitochondrial DNA measured in different disease conditions.


http://www.clinchem.org/cgi/content/abstract/50/3/500

Background: Routine tissue processing has generated banks of paraffin-embedded tissue that could be used in retrospective cohort studies to study the molecular changes that occur during cancer development. The purpose of this study was to determine whether a p53 microarray could be used to sequence the p53 gene in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissues. Methods: DNA was extracted from 70 FFPE breast cancer tissue specimens. p53 was sequenced with an oligonucleotide microarray (p53 GeneChip(R); Affymetrix), and the results were compared with the results obtained from direct sequencing. Results: DNA was extracted from 62 of 70 cases. We identified 26 mutations in 24 of the 62 cases by the p53 GeneChip. No polymorphisms were detected, and exon 4 could not be evaluated in 20 cases. There were 43 genetic alterations detected by direct sequencing in 35 of the 62 cases. These
consisted of 26 polymorphisms and 17 mutations in exons or splice sites. Fifteen mutations were identified by both methods. Direct sequencing detected significantly more gene alterations (43 of 54) in DNA extracted from FFPE tissue than the p53 GeneChip (26 of 54; P = 0.018). However, if the changes in exon 4 were eliminated from this comparison, the p53 GeneChip detected 26 of 27 mutations compared with direct sequencing, which identified 16 of 27 mutations. (P = 0.016).

Conclusions: A combination of oligonucleotide microarray and direct sequencing may be necessary to accurately identify p53 gene alterations in FFPE breast cancer. The p53 GeneChip cannot be used to detect exon 4 polymorphisms (codon 72) in FFPE breast cancer tissue.


young males (age range, 19-35 years) received a single bolus of 2 ng/kg endotoxin [lipopolysaccharide (LPS)] intravenously. Plasma IL-6 was measured by enzyme immunoassay at 0, 2, 6, and 24 h after LPS infusion, and the IL-6 promoter genotype was analyzed by a mutagenic separated PCR assay. Results: IL-6 increased 300-fold 2 h after LPS challenge and returned almost to normal within 24 h. Neither basal IL-6 nor the IL-6 response to LPS was significantly affected by the IL-6 promoter genotype. Conclusions: The IL-6 G(-174)C promoter polymorphism does not significantly influence basal concentrations of IL-6 or peak IL-6 in human endotoxemia.


http://www.clinchem.org/cgi/content/abstract/49/5/732

Background: Genetic risk factors associated with venous thrombosis include mutations in the factor V (Leiden), factor II (prothrombin), and methylenetetrahydrofolate reductase (MTHFR) genes. We evaluated a method using electronically addressable microarrays for the detection of mutations in these genes that have been associated with vascular disease. Methods: The NanoChip(R) Molecular Biology Workstation (Nanogen) uses electronic microarrays for mutation detection. Factor V, factor II, and MTHFR genotypes identified in the NanoChip system on 225 samples were compared with genotypes from LightCycler(R) assays (Roche). We determined within- and between-cartridge signal and ratio variation and analyzed the effect of additional mutations at or near the detection area used for the NanoChip assays. Results: Genotypes determined for all three mutations on the NanoChip platform were in complete concordance with LightCycler results. Within-cartridge signal variation as measured by the CV of fluorescence signals was <10% for each allele when present. The within-cartridge CV for heterozygous mutant/wild-type ratios was <8.5%, and between-cartridge CV was <18%. A dilution study showed that results could be obtained in this assay with 6 ng of nucleic acid per PCR, the lowest input tested. The presence of additional sequence variations near the expected mutations can produce equivocal or discrepant results. Conclusions: Mutation detection using the NanoChip Molecular Biology Workstation was accurate and reproducible for the three assays evaluated.


http://www.clinchem.org/cgi/content/abstract/50/2/313

Background: Scanning for mutations in BRCA1 and BRCA2 in a large number of samples is hampered by the large sizes of these genes and the scattering of mutations throughout their coding sequences. Automated capillary electrophoresis has been shown to be a powerful system to detect mutations by either single-strand conformation polymorphism or heteroduplex analysis (HA). Methods: We investigated the adaptation of gel-based HA of BRCA1 and BRCA2 to a fluorescent multicapillary platform to increase the throughput of this technique. We combined multiplex PCR, three different fluorescent labels, and HA in a 16-capillary DNA sequencer and tested 57 DNA sequence variants (11 insertions/deletions and 46 single-nucleotide changes) of BRCA1 and BRCA2. Results: We detected all 57 DNA changes in a blinded assay, and 2 additional single-nucleotide substitutions (1186 A>G of BRCA1 and 3624 A>G of BRCA2), previously unresolved by conformation-sensitive gel electrophoresis. Furthermore, different DNA changes in the same PCR fragment could be distinguished by their peak patterns. Conclusions: Capillary-based HA is a fast, efficient, and sensitive method that considerably reduces the amount of "hands-on" time for each sample. By this approach, the entire coding regions of
BRCA1 and BRCA2 from two breast cancer patients can be scanned in a single run of 90 min.


http://www.clinchem.org/cgi/content/abstract/48/9/1406

Background: Methods for analysis of the single-nucleotide polymorphism (SNP) known as factor V Leiden (FVL) are described. The technique provides rapid, highly accurate detection of the point mutation that encodes for replacement of arginine-506 with glutamine. After formal assay qualification, 758 clinical samples that had previously been analyzed by the InvaderTM Monoplex Assay were tested as research samples in a commercial clinical laboratory. Methods: Primers specific for factor V (FV) were prepared, and PCR was performed. Samples were analyzed using the NanoChip(R) Molecular Biology Workstation with fluorescently labeled reporters for wild-type and SNP sequences. Results: Of the 635 samples classified by the Third WaveTM assay as FV wild type, 10 were identified as heterozygous FVL by the NanoChip technique. Similarly, of the 114 putative heterozygous samples, 4 were wild type, and of the 9 reported homozygous samples, 6 were homozygous, 2 were heterozygous, and 1 was FV wild type by the NanoChip assay. All 17 results that were discordant with the Third Wave analysis were confirmed by DNA sequencing to be correctly classified by the NanoChip technology. The Nanochip system was 100% accurate in characterizing wild-type, heterozygous, and homozygous samples compared with accuracies of 99.2%, 90.2%, and 100% for the comparable Third Wave analysis.

Conclusions: The NanoChip microelectronic chip array technology is an accurate and convenient method for FVL screening of research samples in a clinical laboratory environment.


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Background: {beta}-Thalassemia is one of the most common genetic diseases in humans. We developed an automated electronic microchip for fast and reliable detection of the nine most frequent mutations accounting for >95% of the {beta}-thalassemia alleles in the Mediterranean area. Methods: We developed a microchip-based assay to identify the nine most frequent mutations (cd39C>T, IVS1-110G>A, IVS1-1G>A, IVS1-6T>C, IVS2-745C>G, cd6delA, -87C>G,
IVS2-1G>A, and cd8delAA) by use of the Nanogen Workstation. The biotinylated amplicon was electronically addressed on the chip to selected pads, where it remained embedded through interaction with streptavidin in the permeation layer. The DNA at each test site was then hybridized to a mixture of fluorescently labeled wild-type or mutant probes. Results: Assays conditions were established based on the analysis of 700 DNA samples from compound heterozygotes or homozygotes for the nine mutations. The assays were blindly validated on 250 DNA samples previously genotyped by other methods, with complete concordance of results. Alternative multiplexed formats were explored: the combination of multiplex PCR with multiple addressing and/or hybridization allowed analysis of all nine mutations in the same sample on one test site of the chip. Conclusions: The open flexible platform can be designed by the user according to the local prevalence of mutations in each geographic area and can be rapidly extended to include the remaining mutations causing {beta}-thalassemia in other regions of the world.


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http://www.clinchem.org/cgi/content/abstract/49/3/415

Background: Optical trapping has traditionally been used to visually select and isolate nonadherent cells grown in suspension because cells grown in monolayers will rapidly reattach to surfaces if suspended in solution. We explored methods to slow cell reattachment that are also compatible with high-fidelity PCR. Methods: Using HeLa cells grown on plates and suspended after trypsinization, we measured the efficiency of capture by retention and movement of the cell by the laser. Success for removing a captured cell by pipette was determined by PCR amplification of the 5S rRNA gene. After optimizing PCR amplification of a 2049-bp region of the p53 gene, we determined PCR fidelity by DNA sequencing. Results: Addition of bovine serum albumin to suspended cells slowed reattachment from seconds to minutes and allowed efficient trapping. The success rate of removing a cell from the trap by pipette to a PCR tube was 91.5%. The 5S PCR assay also revealed that DNA and RNA that copurify with polymerases could give false-positive results. Sequence analysis of four clones derived from a single cell showed only three polymerase errors in 7200 bp of sequence read and revealed difficulties in reading the correct number in a run of 16 A:T. Comparison of the HeLa and wild-type human sequences revealed several previously unreported base differences and an (A:T)n length polymorphism in p53 introns. Conclusions: These results represent the first use of optical trapping on adherent cells and demonstrate the high accuracy of DNA sequencing that can be achieved from a single cell.


http://www.clinchem.org/cgi/content/abstract/48/7/989
Background: NO synthesized from L-arginine by the constitutive endothelial NO synthase (eNOS) plays a key role in the atherosclerotic process. We investigated whether common variants in the NOS3 gene (a T786C mutation in the 5’ flanking region and the polymorphism on exon 7 that produced the Glu298Arg polymorphism in the protein) are associated with an increased risk of moderate to severe internal carotid artery (ICA) stenosis. Methods: We studied 88 patients consecutively operated for ICA stenosis and 133 healthy controls. A T786C mutation in the 5’ flanking region and the polymorphism in exon 7 that produces the Glu298Asp polymorphism in the protein were explored by PCR and fluorescent probe analysis. Results: Genotype distribution was significantly different between patients and controls only for T786C, the CC genotype frequency being 26% and 13%, respectively [odds ratio (OR), 2.26; 95% confidence interval (CI), 1.14-4.46; P = 0.018]. Moreover, the CC genotype was significantly more frequent in a subgroup of patients with ulcerative plaques compared with patients with nonulcerative lesions (44% vs 17%; OR, 3.82; 95% CI, 1.79-8.14; P = 0.003). Multiple logistic regression analysis using the most frequent risk factors and the eNOS gene variant showed that the CC genotype is an independent risk factor for ICA stenosis (P = 0.023). Conclusion: C allele homozygosity in position 786 of the eNOS promoter seems to be an independent risk factor for the development of moderate to severe ICA stenosis, especially ulcerative lesions.


Background: Most pathogenic human mitochondrial DNA (mtDNA) mutations are heteroplasmic (i.e., mutant and wild-type mtDNA coexist in the same individual) and are difficult to detect when their concentration is a small proportion of that of wild-type mtDNA molecules. We describe a simple methodology to detect low proportions of the single base pair heteroplasmatic mutation, A3243G, that has been associated with the disease mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) in total DNA extracted from blood. Methods: Three peptide nucleic acids (PNAs) were designed to bind to the wild-type mtDNA in the region of nucleotide position 3243, thus blocking PCR amplification of the wild-type mtDNA while permitting the mutant DNA to become the dominant product and readily discernable. DNA was obtained from both apparently healthy and MELAS individuals. Optimum PCR temperatures were based on the measured ultraviolet thermal stability of the DNA/PNA duplexes. The presence or absence of the mutation was determined by sequencing. Results: In the absence of PNAs, the heteroplasmic mutation was either difficult to detect or undetectable by PCR and sequencing. Only PNA 3 successfully inhibited amplification of the wild-type mtDNA while allowing the mutant mtDNA to amplify. In the presence of PNA 3, we were able to detect the heteroplasmic mutation when its concentration was as low as 0.1% of the concentration of the wild-type sequence. Conclusion: This methodology permits easy detection of low concentrations of the MELAS A3243G mutation in blood by standard PCR and sequencing methods.
Background: Because cyclosporin A (CsA) and glucocorticoids inhibit the production of interleukin-2 (IL-2) and other cytokines, quantitative analysis of cytokine mRNA might constitute a pharmacodynamic measure for immunosuppressive drug effects. We investigated whether immunosuppressive drugs influence cytokine mRNA expression kinetics during T-cell costimulation. Methods: We used a human whole blood assay to determine basal (unstimulated) IL-2, IL-4, and tumor necrosis factor-α (TNF-α) (TNF-α) mRNA concentrations and expression kinetics after anti-CD3/anti-CD28 monoclonal antibody costimulation in kidney transplant recipients undergoing CsA-based immunosuppressive triple therapy and in healthy controls (ex vivo study I). The effect of CsA on IL-2 mRNA expression kinetics was also determined ex vivo in patients undergoing CsA monotherapy (ex vivo study II) and after in vitro addition of CsA. Results: In ex vivo study I, basal TNF-α mRNA but not IL-2 and IL-4 mRNA was decreased in kidney transplant patients. We observed shifts in peak IL-2 and IL-4 (from 8 to 24 h) and TNF-α (from 4 to 8 h of costimulation) mRNA expression in kidney transplant patients after T-cell costimulation. In patients undergoing CsA monotherapy (ex vivo study II), the inhibitory effect of CsA was detectable as an individually delayed increase in IL-2 mRNA during costimulation. In vitro addition of CsA also induced a dose-independent displacement of IL-2 mRNA expression kinetics (i.e., a delay). Conclusions: A delayed increase in cytokine mRNA expression during T-cell costimulation may represent a sensitive effect of immunosuppression. The single analysis of one absolute or peak mRNA value could be misleading. For prospective studies involving measurement of cytokine mRNA, we therefore suggest the parameter "area of cytokine mRNA expression over time", which should include absolute cytokine mRNA values at two different time points of mRNA kinetics.

Background: To optimize immunosuppressive treatment in individual transplant patients, functional measurements of the effects of tacrolimus (FK 506) are of clinical importance. Previous investigations have demonstrated the occurrence of tacrolimus-resistant production of interleukin-2 (IL-2) in vitro, which may explain in part why rejection episodes are still a frequent problem despite attainment of therapeutic blood concentrations and HLA matching. However, an adequate surrogate marker to define the tacrolimus response in individual patients has not been established. Methods: We investigated the immunosuppressive effects of tacrolimus on anti-CD3/anti-CD28 T-cell costimulation in a human whole-blood assay, analyzing T-cell proliferation, activation marker expression (CD25, CD69), IL-2 protein expression, and cytokine mRNA expression in vitro (n = 11 healthy individuals). We also quantified IL-2 mRNA expression in patients undergoing tacrolimus (n = 4) or cyclosporin A (CsA; n = 4) monotherapy before ex vivo living-donor kidney transplantation. Results: T-cell proliferation; CD25, CD69, and IL-2 concentrations; and IL-4 mRNA were significantly decreased in vitro. In contrast, cytokine mRNA profiles revealed variable tacrolimus sensitivity. Whole-blood samples from 3 of 11 healthy individuals demonstrated marked suppression of IL-2 mRNA expression (>50%) when tacrolimus was administered in vitro. When CsA was added to whole-blood cultures, the influence on IL-2 mRNA expression was comparable to that of tacrolimus in 9 of 11 individuals. Two individuals responded conversely, indicating that differences in the in vitro response to tacrolimus and CsA among individuals may be attributable to potential heterogeneity in the involvement of the CD28


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pathway. Kinetic profiles of IL-2 mRNA expression also revealed individually distinct degrees of calcineurin inhibitor sensitivity in patients undergoing tacrolimus or CsA monotherapy before living-donor kidney transplantation. Conclusions: Our results suggest an individual degree of calcineurin inhibitor sensitivity of activated whole-blood lymphocytes based on IL-2 mRNA expression. Our approach is potentially valuable for identifying transplant patients in whom IL-2 mRNA expression is unaffected or even enhanced after initiation of immunosuppressive therapy. Such individuals may be less sensitive to the immunosuppressive agent and therefore at increased risk of transplant rejection. Prospective studies are necessary to determine the correlation of IL-2 mRNA expression with the clinical risk of transplant rejection.


http://www.clinchem.org/cgi/content/abstract/51/1/113

Background: Increased plasma DNA has been found in cancer patients and may have potential as a tumor marker. The objectives of this study were to develop a controlled, quantitative PCR (QPCR) assay to measure plasma DNA and then evaluate plasma DNA concentrations as a tumor marker in patients with thoracic malignancies. Methods: We developed a QPCR assay for DNA, using the human β-actin gene. Plasma samples were analyzed from 58 patients with esophageal cancer (EC; 20 banked samples and 38 prospectively collected samples) and 25 patients with lung cancer (LC; all prospectively collected). Control groups consisting of 51 patients with gastroesophageal reflux disease (GERD; 23 banked samples and 28 prospectively collected) and 11 healthy volunteers were also analyzed. Results: The assay had an experimental variability <4%. In our banked samples, the mean concentration of plasma DNA in EC was 819.0 μg/L (range, 46.2-4738.0 μg/L) vs 432.0 μg/L (6.0-2888.0 μg/L) in GERD (P = 0.02). However, the prospectively collected samples had lower DNA concentrations, and there was no difference between cancer patients and controls. The mean DNA concentration was 10.6 μg/L (range, 7.0-14.0 μg/L) in healthy volunteers and 10.5 μg/L (range, 4.0-23.5 μg/L) in GERD controls vs 13.0 μg/L (range, 4.5-46.5 μg/L) in EC and 14.6 μg/L (range, 3.0-30.0 μg/L) in LC. Conclusions: Our data indicate that plasma DNA concentrations are of limited diagnostic value when samples are prospectively collected and uniformly handled. This is in contrast to previously published results. Qualitative analysis of DNA may be needed if plasma nucleic acids are to be used as a diagnostic tool in cancer screening.


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http://www.clinchem.org/cgi/content/abstract/48/7/983

Background: Cytochrome P450-dependent monoxygenase 2D6 (CYP2D6) activity can be estimated by investigating the metabolism of model drugs or by genotyping the most common CYP2D6 alleles. For Caucasians, the CYP2D6 allele frequencies are well investigated, and single-step assays are available for genotyping, whereas allele analysis in mainland Chinese is limited. Methods: Two tetra-primer assays and one allele-specific amplification assay were developed to easily genotype the CYP2D6 alleles *8, *10, and *14 previously detected in Asians. Applying these assays in combination with established single-tube assays, we analyzed 223 DNA samples from Chinese volunteers for the CYP2D6 alleles *3, *4, *5, *6, *8, *10, and *14 and for duplication of CYP2D6. Results: Six different alleles were detected in mainland Chinese. The most frequent mutant allele was the intermediate metabolizer allele, CYP2D6*10, with a prevalence of 51.3%, followed by the poor metabolizer alleles CYP2D6*5 (7.2%) and a novel variant of CYP2D6*14. This novel *14B allele (2.0%) differs from the *14 allele by the absence of the C188T substitution and by the additional G1749C substitution. Furthermore, six duplication alleles of CYP2D6 were detected, including one duplication of the *10 allele (*10X2). Conclusions: The CYP2D6 allele frequencies in mainland Chinese shows some genetic diversity compared with Chinese from other regions: a novel *14B allele, a slightly higher frequency of the *5 allele, and a slightly lower frequency of the *10 allele than in most other Chinese populations.


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http://www.clinchem.org/cgi/content/abstract/48/6/818

Background: Congenital adrenal hyperplasia (CAH) is a frequent autosomal recessive disease, with a wide range of clinical manifestations, most commonly attributable to mutations in the 21-hydroxylase gene (CYP21). Large gene deletions, large gene conversions, a small 8-basepair deletion, and eight point mutations in CYP21 account for ~95% of all enzyme deficiencies. We developed a new strategy for a rapid CYP21 analysis. Methods: DNA samples from 40 CAH patients previously genotyped by direct DNA sequencing were reanalyzed by allele-specific amplification of the functional CYP21 gene followed by a multiplex minisequencing reaction using 13 primers. In addition, a second PCR that amplified a part of exon 3 was used to demonstrate the presence or absence of at least one functional gene. Results: The assay detected the P453S mutation and nine of the most common mutations (P30L, intron 2 splice, (Delta)8bp, I172N, exon 6 cluster, V281L, F306t, Q318X, and R356W) caused by microconversions from the CYP21P pseudogene. The concordance was 100% for detecting these mutations, including gene deletions and large gene conversions. The 40 patient DNA samples were analyzed in 1.5 working days by one technician (actual hands-on time, 3.5 h). The material cost for analyzing one sample was approximately 10.00 (US $9.00). Conclusions: This novel mutation screening strategy rapidly detects 90-95% of all mutations associated with CAH and appears applicable as a tool for confirmation of increased 17-hydroxyprogesterone found in neonatal CAH screening.

http://www.clinchem.org/cgi/content/abstract/50/3/522

Background: Multiple endocrine neoplasia type 2 (MEN2) is a cancer syndrome with well-characterized causative mutations. Missense mutations in [-115 codons of the RET gene have been linked to disease phenotypes in the vast majority of cases. These missense mutations range from very simple single nucleotide base changes to more numerous changes at a given codon; they therefore are often tested for by more than one DNA-based diagnostic method. We developed and evaluated a Pyrosequencing technology-based approach for MEN2 mutation testing that allows both simple and complex mutations to be analyzed on one platform. Methods: Archived DNA from peripheral blood of patients referred to the Mayo Clinic Molecular Genetics laboratory for MEN2 testing was selected. One to all of codons 609, 611, 618, 620, 630, 634, 768, 804, and 918 were analyzed by Pyrosequencing technology to match the original analysis of each patient. Template PCRs were set up using an automated liquid handler; the subsequent post-PCR preparation step was performed manually, and the sequencing was performed by a PSQ 96 instrument. Samples were tested in batch sizes expected to occur routinely. Results: We analyzed samples from 217 patients who previously tested negative for MEN2 and 230 patients who previously tested positive, for a total of 1449 sequencing reactions. One discrepant result was found (100% concordant for negatives and 99.6% concordant for positives). A total of 37 unique mutations or alterations of unknown significance were analyzed. Conclusion: Pyrosequencing technology offers an accurate, nonisotopic, simple, and rapid method for the analysis of DNA from patients suspected of having MEN2.


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http://www.clinchem.org/cgi/content/abstract/50/6/1002

Background: Analysis of fetal DNA in maternal plasma has recently been introduced as a new method for noninvasive prenatal diagnosis, particularly for the analysis of fetal genetic traits, which are absent from the maternal genome, e.g., RHD or Y-chromosome-specific sequences. To date, the analysis of other fetal genetic traits has been more problematic because of the overwhelming presence of maternal DNA sequences in the circulation. We examined whether different biochemical properties can be discerned between fetal and maternal circulatory DNA. Methods: Plasma DNA was examined by agarose gel electrophoresis. The fractions of fetal and maternal DNA in size-fractionated fragments were assayed by real-time PCR. The determination of paternally and maternally inherited fetal genetic traits was examined by use of highly
polymorphic chromosome-21-specific microsatellite markers. Results: Size fractionation of circulatory DNA indicated that the major portion of cell-free fetal DNA had an approximate molecular size of <0.3 kb, whereas maternally derived sequences were, on average, considerably larger than 1 kb. Analysis of size-fractionated DNA ([<=]0.3 kb) from maternal plasma samples facilitated the ready detection of paternally and maternally inherited microsatellite markers. Conclusions: Circulatory fetal DNA can be enriched by size selection of fragment sizes less than [~]0.3kb. Such selection permits easier analysis of both paternally and maternally inherited DNA polymorphisms.


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http://www.clinchem.org/cgi/content/abstract/48/11/1938

Background: Lactate dehydrogenase (LD), a tetrameric product of the genes LDHA and LDHB, may be increased in sera of cancer patients. A variant isoenzyme with electrophoretic mobility between LD2 and LD3 (LD2ex) has been described in patients, but its molecular nature is largely unknown. Methods: A newly established retinoblastoma cell line, NCC-RbC-51 (R51), showed an isoenzyme pattern with only two bands, LD1 and LD2ex. We investigated the isoenzymes by Northern blot, Western blot, and methylation analysis and PCR. Results: Northern blot analysis revealed that R51 cells expressed no wild-type/somatic LDHA mRNA, but did express a small amount of LDHA-related mRNA with a slightly higher molecular mass. Western blot analysis confirmed the anti-LDHA-reactive protein with a 3-kDa higher molecular mass. Treatment of R51 cells with the demethylating agent 5-aza-2'-deoxycytidine restored the expression of the LD2, -3, -4, and -5 isoenzymes. PCR analysis of sodium bisulfite-treated genomic DNA revealed that the CpG island in the promoter region around exon a of the LDHA gene was completely methylated. Reverse transcription-PCR analysis and direct sequencing revealed that R51 cells expressed a RNA with the sequence of the human homolog of a murine testis-specific variant that has exon 0 as the 5' noncoding sequence. LDHB was expressed normally in R51 cells. Conclusions: The somatic LDHA in R51 cells is transcriptionally silenced by promoter hypermethylation around exon a, leaving only LDHB to be expressed normally and a testis-specific variant transcript of LDHA containing exon 0. LD2ex possibly results from tetramerization of three wild-type LDHB molecules and one variant LDHA product.


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Background: With the invention of the DNA chip, genome-wide analysis is now a reality. Unfortunately, solid-phase detection systems such as the DNA chip suffer from a narrow range in quantification and sensitivity. Today the best methodology for sensitive, wide dynamic range quantification and genotyping of nucleic acids is real-time PCR. However, multiplexed real-time PCR technologies require complicated and costly design and manufacturing of separate detection probes for each new target. Methods: We developed a novel real-time PCR technology that uses universal energy transfer probes constructed from An Expanded Genetic Information System (AEGIS) for both quantification and genotyping analyses. Results: RNA quantification by reverse transcription-PCR was linear over four orders of magnitude for the simultaneous analysis of {beta}-actin messenger RNA and 18S ribosomal RNA. A single trial validation study of 176 previously genotyped clinical specimens was performed by endpoint analysis for factor V Leiden and prothrombin 20210A mutation detection. There was concordance for 173 samples between the genotyping results from Invader(R) tests and the AEGIS universal energy transfer probe system for both factor V Leiden and prothrombin G20210A. Two prothrombin and one factor V sample gave indeterminate results (no calls). Conclusion: The AEGIS universal probe system allows for rapid development of PCR assays for nucleic acid quantification and genotyping.


Background: Despite considerable advances, DNA sequencing has remained somewhat time-consuming and expensive, requiring three separate steps to generate sequencing products from a template: amplification of the target sequence; purification of the amplified product; and a sequencing reaction. Our aim was to develop a method to routinely combine PCR amplification and cycle sequencing into one single reaction, enabling direct sequencing of genomic DNA. Methods: Combined amplification and sequencing reactions were set up with Big DyeTM sequencing reagents (Applied Biosystems) supplemented with variable amounts of forward and reverse primers, deoxynucleotide triphosphates (dNTPs), and input DNA. Reactions were thermal-cycled for 35 or 45 cycles. Products were analyzed by capillary electrophoresis to detect sequencing products. Results: Reactions using two oligonucleotide primers at a ratio of 5:1 (500 nM primer 1 and 100 nM primer 2), 125 {micro}M supplemental dNTPs, and 35-45 thermal cycles optimally supported combined amplification and cycle sequencing reactions. Our results suggest that these reactions are dominated by PCR during early cycles and convert to cycle sequencing in later cycles. We used this technique for a variety of sequencing applications, including the
identification of germline mutations/polymorphisms in the Factor V and BRCA2 genes, 
sequencing of tumor DNA to identify somatic mutations in the DPC4/SMADH4 and FLT3 genes, 
and sequencing of 16S ribosomal DNA for bacterial speciation. Conclusions: PCR amplification 
and cycle sequencing can be combined into a single reaction using the conditions described. This 
technique allows direct sequencing of genomic DNA, decreasing the cost and labor involved in 
gene sequencing.

Nasis, O., S. Thompson, et al. (2004). "Improvement in Sensitivity of Allele-specific PCR Facilitates 

http://www.clinchem.org/cgi/content/abstract/50/4/694

Background: Cell-free fetal DNA circulating in maternal blood has potential as a safer alternative 
to invasive methods of prenatal testing for paternally inherited genetic alterations, such as cystic 
fibrosis (CF) mutations. Methods: We used allele-specific PCR to detect mutated CF D1152H 
DNA in the presence of an excess of the corresponding wild-type sequence. Pfx buffer 
(Invitrogen) containing replication accessory proteins and Taq polymerase with no proofreading 
activity was combined with TaqMaster PCR Enhancer (Eppendorf) to suppress nonspecific 
amplification of the wild-type allele. The procedure was tested on DNA isolated from plasma 
drawn from 11 pregnant women (gestational age, 11-19.2 weeks), with mutation confirmation by 
chorionic villus sampling. Results: The method detected 5 copies of the CF D1152H mutant allele 
in the presence of up to ~100 000 copies of wild-type allele without interference from the wild-
type sequence. The D1152H mutation was correctly identified in one positive sample; the only 
false-positive result was seen in a mishandled sample. Conclusions: This procedure allows for 
reliable detection of the paternally inherited D1152H mutation and has potential application for 
detection of other mutations, which may help reduce the need for invasive testing.

Palmieri, O., S. Toth, et al. (2003). "CARD15 Genotyping in Inflammatory Bowel Disease Patients by 

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50(4): 702-708.

http://www.clinchem.org/cgi/content/abstract/50/4/702

Background: The orthopox viruses that are pathogenic for humans include variola major virus 
(VAR), monkeypox virus (MPV), cowpox virus (CPV), and to a lesser extent, camelpox virus 
(CML) and vaccinia virus (VAC). PCR is a powerful tool to detect and differentiate orthopox 
viruses, and real-time PCR has the further advantages of rapid turnaround time, low risk of 
contamination, capability of strain differentiation, and use of multiplexed probes. Methods: We 
used real-time PCR with fluorescence resonance energy transfer technology to simultaneously 
detect and differentiate VAR, MPV, CPV/VAC, and CML. An internal control generated by cloning 
and mutating the PCR target gene facilitated monitoring of PCR inhibition in each individual test 
reaction. Results: Strain differentiation results showed little interassay variability (CV, 0.4-0.6%), 
and the test was 100-fold more sensitive than virus culture on Vero cells. Low copy numbers of 
DNA could be detected with [IMG]=" BORDER="0">95% probability (235-849 genome copies/mL
of plasma). Conclusions: The real-time PCR assay can detect and differentiate human pathogenic orthopox viruses. The use of an internal control qualifies the assay for high sample throughput, as is likely to be needed in situations of suspected acts of biological terrorism, e.g., use of VAR.


http://www.clinchem.org/cgi/content/abstract/50/1/67

Background: A novel coronavirus (CoV) was recently identified as the agent for severe acute respiratory syndrome (SARS). We compared the abilities of conventional and real-time reverse transcription-PCR (RT-PCR) assays to detect SARS CoV in clinical specimens. Methods: RNA samples isolated from nasopharyngeal aspirate (NPA; n = 170) and stool (n = 44) were reverse-transcribed and tested by our in-house conventional RT-PCR assay. We selected 98 NPA and 37 stool samples collected at different times after the onset of disease and tested them in a real-time quantitative RT-PCR specific for the open reading frame (ORF) 1b region of SARS CoV. Detection rates for the conventional and real-time quantitative RT-PCR assays were compared. To investigate the nature of viral RNA molecules in these clinical samples, we determined copy numbers of ORF 1b and nucleocapsid (N) gene sequences of SARS CoV. Results: The quantitative real-time RT-PCR assay was more sensitive than the conventional RT-PCR assay for detecting SARS CoV in samples collected early in the course of the disease. Real-time assays targeted at the ORF 1b region and the N gene revealed that copy numbers of ORF 1b and N gene sequences in clinical samples were similar. Conclusions: NPA and stool samples can be used for early diagnosis of SARS. The real-time quantitative RT-PCR assay for SARS CoV is potentially useful for early detection of SARS CoV. Our results suggest that genomic RNA is the predominant viral RNA species in clinical samples.


http://www.clinchem.org/cgi/content/abstract/48/1/35

Background: Fetal DNA has been detected in maternal plasma by the use of genetic differences between mother and fetus. We explore the possibility of using epigenetic markers for the specific detection of fetal DNA in maternal plasma. Methods: A differentially methylated region in the human IGF2-H19 locus and a single-nucleotide polymorphism in this region were chosen for the study. The methylation status in this region is maintained in such a way that the paternal allele is methylated and the maternal allele is unmethylated. The single-nucleotide polymorphism was typed by direct sequencing of PCR products. The methylation status of this region was ascertained by bisulfite conversion and methylation-specific PCR. Differentially methylated fetal alleles were detected in maternal plasma by direct sequencing and a primer-extension assay. Results: Women in the second (n = 21; 17-21 weeks) and third (n = 18; 37-42 weeks) trimesters of pregnancy were recruited. Among these 39 volunteers, the 16 who were heterozygous for the single-nucleotide polymorphism were chosen for further analysis. In 11 of these 16 cases, paternally inherited methylated fetal alleles were different from the methylated alleles of the respective mothers. Using direct sequencing, we detected paternally inherited methylated fetal DNA in 6 of 11 (55%) cases. In 8 of the 16 heterozygous cases, the fetuses possessed an unmethylated maternally inherited allele that was different from the unmethylated allele of the mother. Using a primer-extension assay, we detected fetal-derived maternally inherited alleles in maternal plasma of four of eight (50%) cases. Conclusions: These results represent the first use
of fetal epigenetic markers in noninvasive prenatal analysis. These data may also have implications for the investigation of other types of chimerism.


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http://www.clinchem.org/cgi/content/abstract/48/12/2124

Background: Microelectronic DNA chip devices represent an emerging technology for genotyping. We developed methods for detection of single-nucleotide polymorphisms (SNPs) in clinically relevant genes. Methods: Primer pairs, with one containing a 5'-biotin group, were used to PCR-amplify the region encompassing the SNP to be interrogated. After denaturation, the biotinylated strand was electronically targeted to discrete sites on streptavidin-coated gel pads surfaces by use of a Nanogen Molecular Workstation. Allele-specific dye-labeled oligonucleotide reporters were used for detection of wild-type and variant sequences. Methods were developed for SNPs in genes, including factor VII, \(\beta\)-globin, and the RET protooncogene. We genotyped 331 samples for five DNA variations in the factor VII gene, >600 samples from patients with \(\beta\)-thalassemia, and 15 samples for mutations within the RET protooncogene. All samples were previously typed by various methods, including DNA sequence analysis, allele-specific PCR, and/or restriction enzyme digestion of PCR products. Results: Analysis of amplified DNA required 4-6 h. After mismatched DNA was removed, signal-to-noise ratios were >5. More than 940 samples were typed with the microelectronic array platform, and results were totally concordant with results obtained previously by other genotyping methods. Conclusions: The described protocols detect SNPs of clinical interest with results comparable to those of other genotyping methods.


http://www.clinchem.org/cgi/content/abstract/49/9/1458

Background: Inappropriate quality management of reverse transcription-PCR (RT-PCR) assays for the detection of blood-borne prostate cancer (PCa) cells hampers clinical conclusions.
Improvement of the RT-PCR methodology for prostate-specific antigen (PSA) mRNA should focus on an appropriate numeric definition of the performance of the assay and correction for PSA mRNA that is not associated with PCa cells. Methods and Results: Repeated (RT-)PCR tests for PSA mRNA in single blood specimens from PCa patients and PCa-free controls, performed by four international institutions, showed a large percentage (~50%) of divergent test results. The best estimates of the mean, \( \lambda \) (SD), of the expected Poisson frequency distributions of the number of positive tests among five replicate assays of samples from PCa-free individuals were 1.0 (0.2) for 2 x 35 PCR cycles and 0.2 (0.1) for 2 x 25 PCR cycles. Assessment of the numeric value of the mean can be considered as a new indicator of the performance of a RT-PCR assay for PSA mRNA under clinical conditions. Moreover, it determines the required number of positive test repetitions to differentiate between true and false positives for circulating prostate cells. At a predefined diagnostic specificity of >98%, repeated PCRs with \( \lambda \) of either 1.0 or 0.2 require, respectively, more than three or more than one positive tests to support the conclusion that PSA mRNA-containing cells are present. Conclusions: Repeated nested PCR tests for PSA and appropriate handling of the data allow numeric quantification of the performance of the assay and differentiation between analytical false and true positives at a predefined accuracy. This new approach may contribute to introduction of PSA RT-PCR assays in clinical practice.

http://www.clinchem.org/cgi/content/abstract/49/7/1066

Background: Characterization of fusion gene transcripts in leukemia that result from chromosome translocations provides valuable information regarding appropriate treatment and prognosis. However, screening for multiple fusion gene transcripts is difficult with conventional PCR and state-of-the-art real-time PCR and high-density microarrays. Methods: We developed a multiplex reverse transcription-PCR (RT-PCR) assay for screening and quantification of fusion gene transcripts in human leukemia cells. Chimeric primers were used that contained gene-specific and universal sequences. PCR amplification of fusion and control gene transcripts was achieved with use of an excess of universal primers to allow the ratio of abundance of fusion gene to endogenous or exogenous controls to be maintained throughout PCR. Multiplex RT-PCR products analyzed by an ABI 310 Genetic Analyzer were consistent with those of duplex RT-PCR (single analytical sample plus control). In addition, multiplex RT-PCR results were analyzed by an assay using an oligonucleotide microarray that contained probes for the splice-junction sequences of various fusion transcripts. Results: The multiplex RT-PCR assay enabled screening of >10 different fusion gene transcripts in a single reaction. RT-PCR followed by analysis with the ABI Prism 310 Genetic Analyzer consistently detected 1 fusion-transcript-carrying leukemia cell in 100-10 000 cells. The assay covered a 1000-fold range. Preliminary results indicate that multiplex RT-PCR products can also be analyzed by hybridization-based microarray assay. Conclusions: The multiplex RT-PCR analyzed by either ABI Prism 310 Genetic Analyzer or microarray provides a sensitive and specific assay for screening of multiple fusion transcripts in leukemia, with the latter an assay that is adaptable to a high-throughput system for clinical screening.

http://www.clinchem.org/cgi/content/abstract/48/12/2164

Background: Mutations in codons 12, 13, and 61 of the N-ras gene are common alterations in
cutaneous malignant melanoma. We evaluated pyrosequencing, a simple and rapid method used mainly for single-nucleotide polymorphism analysis, as a possible alternative to single-strand conformation polymorphism (SSCP) analysis and sequencing of N-ras. Methods: We evaluated the sensitivity and accuracy of the pyrosequencing method for identification of mutations in N-ras codons 12, 13, and 61. Nucleotide dispensation orders were created to produce distinct pyrogram peak profiles for the most frequent mutations in codon 61 and codons 12 and 13, respectively. Results: The detection limits for the two most common codon 61 mutations found in malignant melanoma, which code for Arg and Lys, were 30% and 15%, respectively. To evaluate the pyrosequencing method on clinical samples, we performed a parallel analysis of 82 melanoma metastases using SSCP analysis and pyrosequencing. All mutations detected by SSCP analysis and confirmed by sequencing were also correctly identified by pyrosequencing. Codon 61 mutations were identified in 26 of the 82 samples (32%), whereas no mutations were found in codons 12 and 13. Four types of codon 61 mutations, Arg (17%), Lys (10%), Leu (4%), and His (1%), were identified. Conclusion: Pyrosequencing is an attractive alternative to SSCP analysis for N-ras mutation detection in malignant melanoma tumor samples because it displays the same sensitivity and accuracy as SSCP analysis and is simple and rapid.


http://www.clinchem.org/cgi/content/abstract/50/8/1315

Background: The aim of the present study was to investigate the interactions between the circulating concentrations of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and the mRNA concentration of its specific nuclear receptor in human leukocytes. Methods: We measured vitamin D receptor (VDR) mRNA extracted from leukocytes by use of TaqMan fluorescence analysis applied to the reverse transcription-PCR (RT-PCR) technique in 16 volunteers before and after calcitriol administration. VDR mRNA was also measured in leukocytes from calcium-stone-formers (37 hypercalciuric and 34 normocalciuric patients). The relationship between VDR mRNA concentrations and genetic VDR polymorphisms was analyzed in these patients. Results: Imprecision (CV) of RT-PCR was 1.3% within assay (n = 10) and 1.7% between assays (n = 4). Oral 1,25(OH)2D3 increased mean (SE) serum 1,25(OH)2D3 1.6 (0.3)-fold and VDR mRNA 1.6 (0.1)-fold 8 h after administration. The maximum VDR mRNA was reached 3.6 (1.3) h after 1,25(OH)2D3 ingestion. No differences in leukocyte VDR mRNA concentrations were found between normocalciuric and hypercalciuric stone-formers in the absence of stimulation. Finally, no association was found between VDR mRNA concentrations and genetic VDR polymorphisms in stone-formers. Conclusions: The TaqMan RT-PCR assay is a rapid and accurate method to measure VDR mRNA, and leukocytes are a useful model to study VDR and 1,25(OH)2D3 interactions. In humans, VDR mRNA is increased by agonist 1,25(OH)2D3, a finding resembling previously reported results obtained in cellular and animal models.


http://www.clinchem.org/cgi/content/abstract/50/8/1336

Background: Mutations in the retina-specific ABC transporter (ABCA4) gene have been associated with several forms of macular degenerations. Because the high complexity of the molecular genotype makes scanning of the ABCA4 gene cumbersome, we describe here the first use of denaturing HPLC (DHPLC) to screen for ABCA4 mutations. Methods: Temperature conditions were designed for all 50 exons based on effective separation of 83 samples carrying
86 sequence variations and 19 mutagenized controls. For validation, samples from 23 previously characterized Stargardt patients were subjected to DHPLC profiling. Subsequently, samples from a cohort of 30 patients affected by various forms of macular degeneration were subjected to DHPLC scanning under the same conditions. Results: DHPLC profiling not only identified all 132 sequence alterations previously detected by double-gradient denaturing gradient gel electrophoresis but also identified 5 sequence alterations that this approach had missed. Moreover, DHPLC scanning of an additional panel of 30 previously untested patients led to the identification of 26 different mutations and 29 polymorphisms, accounting for 203 sequence variations on 29 of the 30 patients screened. In total, the DHPLC approach allowed us to identify 16 mutations that had never been reported before. Conclusions: These results provide strong support for the use of DHPLC for molecular characterization of the ABCA4 gene.


http://www.clinchem.org/cgi/content/abstract/48/9/1551

Background: 2',5'-Oligoadenylate synthetases (2-5AS) are type I interferon (IFN)-induced proteins with antiviral capacity. Three major forms of 2-5AS with distinct enzymatic activities have been described in IFN-treated human cells. We measured distinct forms of 2-5AS mRNA to analyze the relationship with its enzymatic activity and response to IFN therapy in chronic hepatitis C.

Methods: We established a method to quantify p40/p46 and p69/p71 forms of 2-5AS mRNA by use of reverse transcription followed by competitive PCR. The 2-5AS mRNA concentrations were measured in peripheral blood mononuclear cells from 40 patients with chronic hepatitis C and 28 control individuals. Results: Reconstitution experiments and comparison with Northern blot analyses revealed that our method accurately and linearly quantified 2-5AS mRNA. 2-5AS mRNA concentrations and 2-5AS enzymatic activity were correlated (P <0.03). Our data demonstrated a correlation in 2-5AS mRNA between p40/p46 and p69/p71 (P <0.02), indicating a similar regulation of the expression of these genes. Our data also demonstrated that pretreatment concentrations of 2-5AS mRNA correlated with responses to IFN therapy in chronic hepatitis C. Conclusions: Our method for measuring 2-5AS mRNA concentrations could provide an important marker for selecting patients for IFN therapy and may be useful for the development of more effective therapeutic strategies for chronic hepatitis C.


http://www.clinchem.org/cgi/content/abstract/49/5/761

Background: Malignant hyperthermia (MH) is a fatal autosomal dominant pharmacogenetic disorder characterized by skeletal muscle hypertonicity that causes a sudden increase in body temperature after exposure to common anesthetic agents. The disease is genetically heterogeneous, with mutations in the gene encoding the skeletal muscle ryanodine receptor (RYR1) at 19q13.1 accounting for up to 80% of the cases. To date, at least 42 RYR1 mutations have been described that cause MH and/or central core disease. Because the RYR1 gene is huge, containing 106 exons, molecular tests have focused on the regions that are more frequently mutated. Thus the causative defect has been identified in only a fraction of families as linked to chromosome 19q, whereas in others it remains undetected. Methods: We used denaturing HPLC (DHPLC) to analyze the RYR1 gene. We set up conditions to scan the 27 exons to identify both known and unknown mutations in critical regions of the protein. For each
exon, we analyzed members from 52 families with positive in vitro contracture test results, but without preliminary selection by linkage analysis. Results: We identified seven different mutations in 11 MH families. Among them, three were novel MH alleles: Arg44Cys, Arg533Cys, and Val2117Leu. Conclusion: Because of its sensitivity and speed, DHPLC could be the method of choice for the detection of unknown mutations in the RYR1 gene.


http://www.clinchem.org/cgi/content/abstract/49/1/104

Background: Sensitive monitoring of minimal residual disease may improve the treatment of neuroblastoma in children. To detect and monitor neuroblastoma cells in blood and bone marrow, we developed a quantitative method for the analysis of tyrosine hydroxylase mRNA. Methods: We used real-time reverse transcription-PCR. The calibrator was constructed from a segment of tyrosine hydroxylase mRNA that included the target. Blood and bone marrow samples from 24 children with neuroblastoma and 1 child with ganglioneuroma were analyzed. Controls were blood samples from the cords of 40 babies, from 58 children 6 months to 15 years of age, and from 34 healthy adults, as well as from 12 children with other diseases. Results: The detection limit was \(~70\) transcripts/mL. All 144 blood controls were below this limit. At diagnosis, blood tyrosine hydroxylase mRNA was higher in children with widespread disease (stage 4/4S; \(n=6\); range, 203-46 000 transcripts/mL) than in patients with localized disease (stages 1-3; \(n=6\); \([\lt;\leq]83\) transcripts/mL; \(P=0.002\)). Bone marrow from all five children with localized disease had concentrations \(<72\) transcripts/mL, whereas five of six stage 4 patients had increased concentrations (6000-8 000 000 transcripts/mL; \(P<0.05\)). In nine children in whom tyrosine hydroxylase mRNA was measured repeatedly, the results corresponded to the clinical course. Conclusion: Quantitative analysis of tyrosine hydroxylase mRNA in blood and bone marrow is reliable and easy to perform and may be used for upfront staging, prognostic assessment, and treatment monitoring of neuroblastoma.


http://www.clinchem.org


http://www.clinchem.org/cgi/content/abstract/48/10/1668

Background: Enzymes of the cytochrome P450 3A (CYP3A) family are responsible for the metabolism of \(\geq50\)% of currently prescribed drugs. CYP3A5 is expressed in a limited number of individuals. The absence of CYP3A5 expression in \(~70\)% of Caucasians was recently correlated to a genetic polymorphism (CYP3A5*3). Because CYP3A5 may represent up to 50% of total CYP3A protein in individuals polymorphically expressing CYP3A5, it may have a major role in variation of CYP3A-mediated drug metabolism. Using sequencing, have been identified (Hustert et al. Pharmacogenetics 2001;11:773-9; Kuehl et al. Nat Genet 2001;27:383-91) variant alleles *2 through *7 for CYP3A5. Detection of CYP3A5 variant alleles, and knowledge about their allelic
frequency in specific ethnic groups, is important to establish the clinical relevance of screening for these polymorphisms to optimize pharmacotherapy. Methods: In a group of 500 healthy Dutch Caucasian blood donors, we determined the allelic frequency of the CYP3A5*2, *3, *4, *5, *6, and *7 alleles by use of newly developed PCR-restriction fragment length polymorphism assays. Results: The frequency of the defective CYP3A5*3 allele in the Dutch Caucasian population was 91%, followed by the CYP3A5*2 (1%) and CYP3A5*6 (0.1%) alleles. The CYP3A5*4, *5, and *7 alleles were not detected. Conclusions: On the basis of its allelic frequency, screening for the CYP3A5*3 allele in the Caucasian population is extremely relevant. In addition, screening for the CYP3A5*2 allele may be taken into consideration in individuals heterozygous for the CYP3A5*3 allele. The CYP3A5*4, *5, *6, and *7 alleles have low allelic frequencies that do not support initial screening.


http://www.clinchem.org


http://www.clinchem.org/cgi/content/abstract/49/2/209

Background: {beta}-Thalassemia is endemic to many regions in Southeast Asia and India, and <20 {beta}-globin gene mutations account for [&gt;=]90% of {beta}-thalassemia alleles in these places. We describe a multiplex minisequencing assay to detect these common mutations. Methods: Gap-PCR was used to simultaneously amplify the {beta}-globin gene from genomic DNA and to detect the (Delta)619bp deletion mutation. Multiplex minisequencing was then performed on the amplified {beta}-globin fragment to detect an additional 15 common Southeast Asian and Indian {beta}-thalassemia mutations. Site-specific primers of different lengths were subjected to multiple rounds of annealing and single-nucleotide extension in the presence of thermostable DNA polymerase and the four dideoxynucleotides, each labeled with a different fluorophore. Minisequencing products were separated and detected by capillary electrophoresis, followed by automated genotyping. The optimized assay was subjected to a double-blind validation analysis of 89 {beta}-thalassemia and wild-type DNA samples of known genotype. Results: Homozygous wild-type or mutant DNA samples produced electropherograms containing only a single colored peak for each mutation site, whereas samples heterozygous for a specific mutation displayed two different-colored peaks for that mutation site. Samples were automatically genotyped based on color and position of primer peaks in the electropherogram. In the double-blind validation analysis, all 89 DNA samples were genotyped correctly (100% assay specificity). Conclusions: The described semiautomated multiplex minisequencing assay can detect the most common Southeast Asian and Indian {beta}-thalassemia mutations, is amenable to high-throughput scale up, and may bring population-based screening of {beta}-thalassemia in endemic regions a step closer to implementation.


http://www.clinchem.org

http://www.clinchem.org/cgi/content/abstract/48/11/1873

Background: Electronic microarrays comprise independent microelectrode test sites that can be electronically biased positive or negative, or left neutral, to move and concentrate charged molecules such as DNA and RNA to one or more test sites. We developed a protocol for multiplexed gene expression profiling of mRNA targets that uses electronic field-facilitated hybridization on electronic microarrays. Methods: A multiplexed, T7 RNA polymerase-mediated amplification method was used for expression profiling of target mRNAs from total cellular RNA; targets were detected by hybridization to sequence-specific capture oligonucleotides on electronic microarrays. Activation of individual test sites on the electronic microarray was used to target hybridization to designated subsets of sites and allow comparisons of target concentrations in different samples. We used multiplexed amplification and electronic field-facilitated hybridization to analyze expression of a model set of 10 target genes in the U937 cell line during lipopolysaccharide-mediated differentiation. Performance of multiple genetic analyses (single-nucleotide polymorphism detection, gene expression profiling, and splicing isoform detection) on a single electronic microarray was demonstrated using the ApoE and ApoER2 genes as a model system. Results: Targets were detected after a 2-min hybridization reaction. With noncomplementary capture probes, no signal was detectable. Twofold changes in target concentration were detectable throughout the ([~]64-fold) range of concentrations tested. Levels of 10 targets were analyzed side by side across seven time points. By confining electronic activation to subsets of test sites, polymorphism detection, expression profiling, and splicing isoform analysis were performed on a single electronic microarray. Conclusions: Microelectronic array technology provides specific target detection and quantification with advantages over currently available methodologies for targeted gene expression profiling and combinatorial genomics testing.


http://www.clinchem.org


http://www.clinchem.org/cgi/content/abstract/49/8/1297

Background: Measurement of plasma butyrylcholinesterase (BChE) activity and inhibitor-based phenotyping are standard methods for identifying patients who experience post-succinylcholine (SC) apnea attributable to inherited variants of the BChE enzyme. Our aim was to develop PCR-based assays for BCHE mutation detection and implement them for routine diagnostic use at a university teaching hospital. Methods: Between 1999 and 2002, we genotyped 65 patients referred after prolonged post-SC apnea. Five BCHE gene mutations were analyzed. Competitive oligo-priming (COP)-PCR was used for flu-1, flu-2, and K-variant and direct DNA sequencing analysis for dibucaine and sil-1 mutations. Additional DNA sequencing of BCHE coding regions was provided when the five-mutation screen was negative or mutation findings were inconsistent with enzyme activity. Results: Genotyping identified 52 patients with primary
hypocholinesterasemia attributable to BCHE mutations, and in 44 individuals the abnormalities were detected by the five-mutation screen (detection rate, 85%). Additional sequencing studies revealed mutations in eight other patients, including five with novel mutations. The most common genotype abnormality was compound homozygous dibucaine and homozygous K-variant mutations. No simple homozygotes were found. Of the remaining 13 patients, 3 had normal BChE activity and gene, and 10 were diagnosed with hypocholinesterasemia unrelated to BCHE gene abnormalities. Conclusion: A five-mutation screen for investigation of post-SC apnea identified BCHE gene abnormalities for 80% of a referral population. Six new BCHE mutations were identified by sequencing studies of 16 additional patients.

http://www.clinchem.org


http://cdli.asm.org/cgi/content/abstract/11/3/625

Toll-like receptor 2 (TLR2) is critical in the immune response to mycobacteria. Herein, we report that the frequency of a human TLR2 Arg677Trp polymorphism (C2029T nucleotide substitution) in tuberculosis patients in Tunisia is significantly higher than in healthy controls (P < 0.0001). This finding suggests that this polymorphism could be a risk factor for tuberculosis.

http://cdli.asm.org/cgi/content/abstract/9/2/461

Two PCR protocols targeting the 18S rRNA gene of Cryptococcus neoformans were established, compared, and evaluated in murine cryptococcal meningitis. One protocol was designed as a nested PCR to be performed in conventional block thermal cyclers. The other protocol was designed as a quantitative single-round PCR adapted to LightCycler technology. One hundred brain homogenates and dilutions originating from 20 ICR mice treated with different azoles were examined. A fungal burden of 3 x 101 to 2.9 x 104 CFU per mg of brain tissue was determined by quantitative culture. Specific PCR products were amplified by the conventional and the LightCycler methods in 86 and 87 samples, respectively, with products identified by DNA sequencing and real-time fluorescence detection. An analytical sensitivity of 1 CFU of C. neoformans per mg of brain tissue and less than 10 CFU per volume used for extraction was observed for both PCR protocols, while homogenates of 70 organs from mice infected with other fungi were PCR negative. Specificity testing was performed with genomic DNA from 31
hymenomycetous fungal species and from the ustilaginomycetous yeast Malassezia furfur, which are phylogenetically related to C. neoformans. Twenty-four strains, including species of human skin flora like M. furfur and Trichosporon spp., were PCR negative. Amplification was observed with Cryptococcus amylolentus, Filobasidiella depauperata, Cryptococcus laurentii, and five species unrelated to clinical specimens. LightCycler PCR products from F. depauperata and Trichosporon faecale could be clearly discriminated by melting curve analysis. The sensitive and specific nested PCR assay as well as the rapid and quantitative LightCycler PCR assay might be useful for the diagnosis and monitoring of human cryptococcal infections.


http://cdli.asm.org/cgi/content/abstract/11/4/766

A powerful, cost-effective new method for studying single-nucleotide polymorphisms (SNPs) is described. This method is based on the use of hairpin-shaped primers (HP), which give a sensitive and specific PCR amplification of each specific allele, without the use of costly fluorophore-labeled probes and any post-PCR manipulation. The amplification is monitored in real-time using SYBR Green I dye and takes only 2 h to yield results. The HP assay has a simple design and utilizes a conventional real-time PCR apparatus. The -44 C[-&gt;G] transversion in the DEFB1 gene (which encodes human (beta)-defensin 1) has been previously associated with Candida carriage in oral epithelia. In this study, we analyzed the association between early-onset periodontal disease (EOP) and the -44 SNP. We used an HP assay to study the distribution of the -44 SNP in 264 human DNAs obtained from two cohorts of EOP patients and healthy controls from different ethnic backgrounds. The results indicate that the -44 SNP has a similar distribution between EOP and healthy patients, suggesting that it is not associated with the disease.


http://cdli.asm.org/cgi/content/abstract/9/6/1212

We investigated the expression of membrane-bound CD14 (mCD14) on monocytes and soluble CD14 (sCD14) released into the culture supernatants of peripheral blood lymphocytes (PBMC) from human immunodeficiency virus (HIV)-infected individuals. Monocytes from HIV-positive individuals exhibited both enhanced mCD14 expression and sCD14 production in the PBMC culture supernatants compared to the levels of mCD14 and sCD14 in HIV-negative individuals. This enhanced mCD14 expression and sCD14 production in HIV-infected individuals may be due to the effects of cytokines, the bacterial product lipopolysaccharide (LPS), and/or the HIV regulatory antigens Tat and Nef. Interleukin-10 (IL-10), an immunoregulatory cytokine, as well as LPS enhanced mCD14 expression and the release of sCD14 in the culture supernatants. HIV-Nef, unlike Tat, enhanced mCD14 expression on monocytes but did not induce the release of sCD14 into the culture supernatants. Studies conducted to investigate the mechanism underlying HIV-Nef-induced mCD14 expression revealed that HIV-Nef upregulated mCD14 expression via a mechanism that does not involve endogenously produced IL-10. In contrast, LPS upregulated the expression of mCD14 and increased the release of sCD14 via a mechanism that involves, at least in part, endogenously produced IL-10. Furthermore, dexamethasone, an anti-inflammatory and immunosuppressive agent, inhibited HIV-Nef-induced CD14 expression in an IL-10-independent manner. In contrast, dexamethasone inhibited IL-10-dependent LPS-induced CD14 expression by interfering with IL-10-induced signals but not by blocking IL-10 production. These
results suggest that HIV-Nef and IL-10 constitute biologically important modulators of CD14 expression which may influence immunobiological responses to bacterial infections in HIV disease.


http://cdli.asm.org/cgi/content/abstract/11/3/538

Recurrent respiratory papillomatosis (RRP) is a chronic, debilitating disease of the upper airway caused by human papillomavirus type 6 (HPV-6) or HPV-11. We describe responses of peripheral blood mononuclear cells (PBMC) and T cells from RRP patients and controls to the HPV-11 early proteins E6 and E7. PBMC were exposed in vitro to purified E6 or E7 proteins or transduced with fusion proteins containing the first 11 amino acids of the human immunodeficiency virus type 1 protein tat fused to E6 or E7 (tat-E6/tat-E7). TH1-like (interleukin-2 [IL-2], gamma interferon [IFN-[gamma]], IL-12, and IL-18), and TH2-like (IL-4 and IL-10) cytokine mRNAs were identified by reverse transcription-PCR, and IFN-[gamma] and IL-10 cytokine-producing cells were identified by enzyme-linked immunospot assay. These studies show that HPV-11 E6 skewsl IL-10-IFN-[gamma] expression by patients with RRP toward greater expression of IL-10 than of IFN-[gamma]. In addition, there is a general cytokine hyporesponsiveness to E6 that is more prominent for TH1-like cytokine expression by patients with severe disease. Patients showed persistent IL-10 cytokine expression by the nonadherent fraction of PBMC when challenged with E6 and tat-E6, and, in contrast to controls, both T cells and non-T cells from patients expressed IL-10. However, E7/tat-E7 cytokine responses in patients with RRP were similar to those of the controls. In contrast, E6 inhibited IL-2 and IL-18 mRNA expression that would further contribute to a cytokine microenvironment unfavorable to HPV-specific, T-cell responses that should control persistent HPV infection. In summary, E6 is the dominant inducer of cytokine expression in RRP, and it induces a skewed expression of IL-10 compared to the expression of IFN-[gamma].


http://cdli.asm.org/cgi/content/abstract/12/4/477

Rationale: evaluation of the T-cell receptor (TCR) V(beta)-chain repertoire by PCR-based CDR3 length analysis allows fine resolution of the usage of the TCR V(beta) repertoire and is a sensitive tool to monitor changes in the T-cell compartment. A multiplex PCR method employing 24 labeled upstream V{beta} primers instead of the conventionally labeled downstream C{beta} primer is described. Method: RNA was isolated from purified CD4 and CD8 T-cell subsets from umbilical cord blood and clinical samples using TRI reagent followed by reverse transcription using a C{beta} primer and an Omniscript RT kit. The 24 V{beta} primers were multiplexed based on compatibility and product sizes into seven reactions. cDNA was amplified using 24 V{beta} primers (labeled with tetrachloro-6-carboxyfluorescein, 6-carboxyfluorescein, and hexachloro-6-carboxyfluorescein), an unlabeled C{beta} primer, and Taqgold polymerase. The fluorescent PCR products were resolved on an automated DNA sequencer and analyzed using the Genotyper 2.1 software. Results: V{beta} spectratypes of excellent resolution were obtained with RNA amounts of 250 ng using the labeled V{beta} primers. The resolution was superior to that obtained with the labeled C{beta} primer assay. Also the numbers of PCRs were reduced to 7 from the 12 required in the C{beta} labeling method, and the sample processing time was reduced by half. Conclusion: The method described for T-cell receptor V{beta}-chain repertoire analysis eliminates tedious
dilutions and results in superior resolution with small amounts of RNA. The fast throughput makes this method suitable for automation and offers the feasibility to perform TCR V{beta} repertoire analyses in clinical trials.


http://cdli.asm.org/cgi/content/abstract/10/2/195

Deficiencies of the early components of the classical complement pathway impair the actions of innate and humoral immunity and may lead to increased susceptibility to infections. We have studied the genetic basis of total C4B deficiency in a Finnish patient with recurrent meningitis, chronic fistulas and abscesses. The maternal chromosome carried a four-gene deletion including the C4B gene, and a conversion from C4B to C4A gene was found on the paternal chromosome resulting in complete deficiency of C4B. In the converted C4A gene, mutation screening did not reveal any amino acid changes or prominent mutations, yet a large number of nucleotide variations were found. Further, the patient was heterozygous for structural deficiency of mannan binding lectin (MBL) associating with medium levels of serum MBL. Our data provides new information on the genetic instability of the C4 gene region, and on the association of homozygous C4B deficiency and variant MBL genotype with increased susceptibility to recurrent and chronic infections. Importantly, plasma therapy induced a prompt clinical cure with long-term effects.


http://cdli.asm.org/cgi/content/abstract/10/1/53

Perturbations in the T-cell receptor (TCR) V{beta} repertoire were assessed in the CD4 and CD8 T lymphocytes of human immunodeficiency virus (HIV)-infected children who were receiving therapy during the chronic phase of infection by flow cytometry (FC) and PCR analysis. By FC, representation of 21 TCR V{beta} subfamilies was assessed for an increased or decreased percentage in CD4 and CD8 T cells, and by PCR, 22 TCR V{beta} subfamilies of CD4 and CD8 T cells were analyzed by CDR3 spectratyping for perturbations and reduction in the number of peaks, loss of Gaussian distribution, or clonal dominance. The majority of the TCR V{beta} subfamilies were examined by both methods and assessed for deviation from the norm by comparison with cord blood samples. The CD8-T-lymphocyte population exhibited more perturbations than the CD4 subset, and clonal dominance was present exclusively in CD8 T cells. Of the 55 total CD8-TCR V{beta} families classified with clonal dominance by CDR3 spectratyping, only 18 of these exhibited increased expression by FC. Patients with high numbers of CD8-TCR V{beta} families with decreased percentages had reduced percentages of total CD4 T cells. Increases in the number of CD4-TCR V{beta} families with increased percentages showed a positive correlation with skewing. Overall, changes from normal were often discordant between the two methods. This study suggests that the assessment of HIV-induced alterations in TCR V{beta} families at cellular and molecular levels yields different information and that our understanding of the immune response to HIV is still evolving.

We have applied a newly developed real-time reverse transcriptase (RT) PCR (RT-PCR) assay for quantification of substance P (SP) mRNA expression (the SP real-time RT-PCR assay) in human blood monocyte-derived macrophages, peripheral blood lymphocytes, and microglia isolated from fetal brain. The SP real-time RT-PCR assay had a sensitivity of 60 mRNA copies, with a dynamic range of detection between 60 and 600,000 copies of the SP gene transcript per reaction mixture. The coefficient of variation of the threshold cycle number between the SP real-time RT-PCR assays was less than 1.16%. This assay with an SP-specific primer pair efficiently recognizes all four isoforms of preprotachykinin A (the SP precursor) gene transcripts. In order to use this assay to measure the levels of SP mRNA in the human immune cells quantitatively, we designed a specific probe (molecular beacon) derived from exon 3 of the SP gene. We demonstrated that the real-time RT-PCR quantitatively detected SP mRNA in the human immune cells, among which the microglia isolated from fetal brain had the highest levels of SP mRNA. The SP real-time PCR assay yielded reproducible data, as the intra-assay variation was less than 1%. Thus, it is feasible to apply the real-time RT-PCR assay for quantification of SP mRNA levels in human immune cells, as well as in other nonneuronal cells. Since SP is a major modulator of neuroimmunoregulation, this assay has the potential for widespread application for basic and clinical investigations.


http://cdli.asm.org/cgi/content/abstract/10/6/1123

CCR5, a (beta)-chemokine receptor, plays an important role in human immunodeficiency virus (HIV) infection of human immune cells, as it is a primary coreceptor for HIV entry into macrophages. We have applied a newly developed real-time reverse transcriptase PCR (RT-
PCR) assay for the quantification of CCR5 mRNA in human blood immune cells. The CCR5 real-
time RT-PCR assay has a sensitivity of 100 mRNA copies, with a dynamic range of detection
between 102 and 106 copies of the CCR5 mRNA transcripts per reaction. The assay is highly
reproducible, with an intra-assay coefficient of variation of the threshold cycle of less than 2.07%.
When used for quantification of CCR5 mRNA in human monocyte-derived macrophages (MDM)
and peripheral blood lymphocytes (PBL), the assay has precision and reproducibility. MDM
expressed higher levels of CCR5 mRNA than did PBL. Thus, this assay has the potential and a
wide application for the investigation of the role of CCR5 in inflammatory diseases and viral
infections, including HIV disease.


http://cdli.asm.org/cgi/content/abstract/9/6/1270

Rhodococcus equi infects and causes pneumonia in foals between 2 and 4 months of age but
does not induce disease in immunocompetent adults, which are immune and remain clinically
normal upon challenge. Understanding the protective response against R. equi in adult horses is
important in the development of vaccine strategies, since those mechanisms likely reflect the
protective phenotype that an effective vaccine would generate in the foal. Twelve adult horses
were challenged with virulent R. equi and shown to be protected against clinical disease.
Stimulation of cells obtained from bronchoalveolar lavage fluid with either R. equi or the vaccine
candidate protein VapA resulted in significant proliferation and a significant increase in the level
of gamma interferon (IFN-{gamma}) expression by day 7 postchallenge. The levels of interleukin-
4 expression were also increased at day 7 postchallenge; however, this increase was not antigen
specific. Anamnestic increases in the levels of binding to R. equi and VapA of all immunoglobulin
G (IgG) antibody isotypes [IgGa, IgGb, IgG(T)] examined were detected postchallenge. The
levels of R. equi- and VapA-specific IgGa and IgGb antibodies, the IgG isotypes that preferentially
opsonize and fix complement in horses, were dramatically enhanced postchallenge. The antigen-
specific proliferation of bronchoalveolar lavage fluid cells, the levels of IFN-{gamma} expression
by these cells, and the anamnestic increases in the levels of opsonizing IgG isotypes are
consistent with stimulation of a memory response in immune adult horses and represent
 correlates for vaccine development in foals.


http://cdli.asm.org/cgi/content/abstract/11/6/1194

To investigate a putative link between genetically determined variations in Toll-like receptor 2
(TLR2) and the occurrence of severe Staphylococcus aureus infection, the functional Arg753Gln
single-nucleotide polymorphism and the GT repeat microsatellite in the TLR2 gene were
examined in a large case-control study. No associations with disease or mortality attributable to
these features were found.

Nakagawa, M., R. Viscidi, et al. (2002). "Time Course of Humoral and Cell-Mediated Immune Responses
The time course of cell-mediated and humoral immune responses was elucidated in eight women with human papillomavirus type 16 (HPV-16) infection by performing serial HPV-16 E6 and E7 cytotoxic T-lymphocyte (CTL) assays and HPV-16 virus-like particle (VLP) antibody analyses. Four subjects had a single incident of HPV-16 DNA detection, and four subjects had two periods of HPV-16 DNA detection. In two of the women in the latter group, the second episode of HPV-16 detection occurred in the presence of high titers of HPV-16 VLP antibody, bringing into question the protective role of humoral immunity in preventing repeated infection. However, all four subjects rapidly became HPV-16 DNA negative following the second detection of HPV-16 DNA, suggesting the presence of immunological memory. In addition, one subject rapidly became negative for HPV-16 DNA despite having no evidence of CTL or VLP antibody response prior to the second HPV-16 DNA detection, suggesting the presence of immunological responses at an undetectable level. Overall, seven of eight subjects (88%) had detectable HPV-16 E6 and/or E7 CTL responses and seven of eight women (88%) had detectable HPV-16 VLP antibody responses.


There is considerable controversy concerning the evidence for the presence of Chlamydia pneumoniae in the cerebrospinal fluid (CSF) of both multiple sclerosis (MS) patients and patients with other neurological diseases (OND). In order to clarify this issue, the laboratories at Vanderbilt University Medical Center (VUMC) and the University of South Florida (USF) examined the reproducibility of their respective PCR assays for the detection of C. pneumoniae DNA in the CSF of a common group of MS patients and OND controls. The two laboratories used different DNA extraction and PCR techniques in order to determine the prevalence of the C. pneumoniae genome in both monosymptomatic and clinically definite MS patients as well as in OND controls. In clinically definite MS patients, the VUMC and USF detection rates were 72 and 61%, respectively, and in patients with monosymptomatic MS, the VUMC and USF detection rates were 41 and 54%, respectively. The PCR signal was positive for 7% of the OND controls at VUMC and for 16% at USF. These studies confirm our previous reports concerning the high prevalence of C. pneumoniae in the CSF of MS patients. The presence of C. pneumoniae in patients with monosymptomatic MS would also suggest that infection with the organism occurs early in the course of the disease.


The prevalence of infection with hepatitis A virus (HAV), HBV, HCV, HDV, and HEV was evaluated in 249 apparently healthy individuals, including 122 inhabitants in Ulaanbaatar, the capital city of Mongolia, and 127 age- and sex-matched members of nomadic tribes who lived around the capital city. Overall, hepatitis B surface antigen (HBsAg) was detected in 24 subjects (10%), of whom 22 (92%) had detectable HBV DNA. Surprisingly, HDV RNA was detectable in 20 (83%) of the 24 HBsAg-positive subjects. HCV-associated antibodies were detected in 41 (16%)
and HCV RNA was detected in 36 (14%) subjects, none of whom was coinfected with HBV, indicating that HBV/HCV carriers account for one-fourth of this population. Antibodies to HAV and HEV were detected in 249 (100%) and 28 (11%) subjects, respectively. Of 22 HBV DNA-positive subjects, genotype D was detected in 21 subjects and genotype F was detected in 1 subject. All 20 HDV isolates recovered from HDV RNA-positive subjects segregated into genotype I, but these differed by 2.1 to 11.4% from each other in the 522- to 526-nucleotide sequence. Of 36 HCV RNA-positive samples, 35 (97%) were genotype 1b and 1 was genotype 2a. Reflecting an extremely high prevalence of hepatitis virus infections, there were no appreciable differences in the prevalence of hepatitis virus markers between the two studied populations with distinct living place and lifestyle. A nationwide epidemiological survey of hepatitis viruses should be conducted in an effort to prevent de novo infection with hepatitis viruses in Mongolia.

Clinica Chimica Acta (21)


http://www.sciencedirect.com/science/article/B6T57-3X10S21-4/2/dd9c9049f6e29d71eebe349252d3c45

Three different mutations at codons 330 (TTA to ATA), 365 (GGA to AGA) and 515 (CGT to TGT) of human butyrylcholinesterase (hBChE) were identified in a Japanese family. We correlated alterations in in the patient's hBChE activity with possible structural alterations in the three-dimensional structure of hBChE caused by the point mutations. This study was performed using the published computer-generated three-dimensional structure of hBChE based on the structure of acetylcholinesterase. The amino acid substitution at L330I was adjacent to hydrophobic residues that form the channel domain of the active center. This side chain faced the side opposite the active center. The amino acid substitution at G365R was located at the position most remote from the active center, and this substitution site was exposed to the surface of the BChE protein. [alpha]-Helical structure was present to the active center, and the guanidyl residue of native Arg 515 was hydrogen-bonded to the carboxyl group of Asp 395 in the [alpha]-helix. These point mutations may cause steric effects on the present patient's hBChE activity. This is the first report of three-dimensional structural analysis performed on the L330I, G365R, and R515C mutations of hBChE.


http://www.sciencedirect.com/science/article/B6T57-4DRBFHP-1/2/f8cc14cf436bcff4ae93cc0a05731bb2

BackgroundThe renin-angiotensin system (RAS) and endothelial nitric oxide (NO) affect the pathogenesis of atherosclerosis and prognosis of coronary artery disease (CAD). Previous epidemiologic data suggested that genetic factors are more likely to affect young rather than old people. Our objective was to investigate the association between the polymorphisms of eNOS
(Glu298Asp) and the RAS genes and premature CAD in a Turkish population.

Methods
A total of 115 Turkish patients with premature CAD and 83 controls were included in the study. ACE I/D, AT1R A/C, AGT T/M and eNOS Glu298Asp gene polymorphisms were analysed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results
It was found that increased premature CAD risk is associated with higher frequencies of the ACE DD [OR: 2.600 (CI 95% 1.395-4.847, p=0.002)], AGT MM [OR=2.407 (CI 95% 1.267-4.573, p=0.007)] and eNOS 894TT [OR=17.000 (CI 95% 3.952-73.125, p=0.002)].

Conclusions
This study indicates a synergistic contribution of RAS genes (ACE I/D, AGT T/M, AT1R T/C) and eNOS Glu298Asp polymorphisms to the development of the premature CAD.


http://www.sciencedirect.com/science/article/B6T57-44N9J08-B/2/f92a75075ab0b516216644df6271199

Background: The objective of the present study was to elucidate possible relationships between four polymorphisms of the TGF-[beta]1 gene (-800G>A; -509C>T; Leu10Pro; Arg25Pro) and stage, histological activity grade and progression rate of liver fibrosis, classified according to the METAVIR-score. Methods: Three study groups, i.e. 48 patients with hepatic fibrosis (26 with known duration of hepatitis C virus infection), 47 patients with non-fibrotic diseases and 50 healthy blood donors, were analyzed for TGF-[beta]1 polymorphisms using ARMS-PCR and sequence analysis. The concentrations of total TGF-[beta]1 in plasma and of hyaluronan, P-III-NP and activities of transaminases in serum were measured. Results: The presence of proline at codons 10 and/or 25 was associated with a faster progression of fibrosis than other polymorphisms. Patients with the genotype 25ArgPro developed fibrosis significantly faster (0.23 units/year) than those having 25ArgArg (0.08 units/year). Similarly, the fibrosis progression rate of patients with genotypes 10LeuPro and 10ProPro was almost three times as fast as of those having genotype 10LeuLeu. Stage and histological activity grade of fibrosis in 25ArgPro in comparison to 25ArgArg were higher. Also 10LeuPro showed a higher average stage of fibrosis than 10LeuLeu. The TGF-[beta]1 plasma concentrations of patients with hepatic fibrosis were not significantly different between carriers of 25ArgArg and 25ArgPro genotypes. The frequency of the genotype 25ArgPro in liver fibrotic patients was about three times that of the control group whereas the frequency distribution of the genotype 25ArgArg tended to lower frequency in the fibrosis group. TGF-[beta]1-promoter polymorphisms did not show any correlation with stage, grade or progression of liver fibrosis. Conclusion: Our results indicate that the heterozygous ArgPro of codon 25 predicts significantly faster fibrotic progression of chronic hepatitis C than the homozygous 25ArgArg genotype. The homozygous LeuLeu genotype of codon 10 showed a slow progression of fibrosis.


http://www.sciencedirect.com/science/article/B6T57-44FD01N-12/2/ec1fd57252c7c6cdb43aed356fb9ac97

Background: The prothrombin mutation, a G/A transition at position 20210 in the 3' untranslated region of the prothrombin gene, is associated with an increased risk of deep venous thrombosis.
and obstetrical complications. Several methods have been developed to detect the mutation; however, given the increased demand for this test in risk factor assessment, the development of simple and efficient screening methods has become necessary. Methods: We have used a rapid, sensitive, and precise method developed by Abbott Laboratories to detect the prothrombin mutation. The method employs a polymerase chain reaction (PCR) amplification and the Abbot LCx(R) microparticle enzyme immunoassay (MEIA) for detection. This method is able to detect and identify both homozygous and heterozygous genotypes. Results: Two hundred ninety-six patients with a history of deep venous thrombosis, pulmonary embolism, preeclampsia, or cardiovascular disease and 163 control patients were included in this study. The prevalence of the mutation was 5.74% in the high-risk group and 3.06% in the control group. There was complete agreement between the results from the MEIA detection with those obtained using other detection methodologies, namely standard PCR and restriction fragment length polymorphism (RFLP) analysis. Conclusions: The MEIA detection method of the prothrombin mutation represents a simple, fast, and reliable alternative to standard methods of detection and is well suited for use in routine clinical laboratories. The results of our study confirm others' studies showing a greater incidence of G20210A prothrombin gene mutation in patients with an increased risk of venous thrombosis and pulmonary embolism as well as patients with cardiovascular disease and pregnant women with preeclampsia. It reinforces the necessity of including the screening for prothrombin mutation in populations at risk.


http://www.sciencedirect.com/science/article/B6T57-47P8YNR-9X/2/a20fc7c206e283b5e91b489cccf54d6a

The discovery of a point-mutation, adenine-to-guanine, at position 985 in the gene coding for MCAD (G985), gave the basis for an easy and specific polymerase chain reaction test. We tested the specificity of such a PCR based assay and detected correctly G985 and A985 in sequence verified cDNA clones. We showed that the G985 mutation is present in genomic DNA from 48 of 50 patients with confirmed MCAD deficiency, originating from various European countries, Australia and the USA. On the basis of this high frequency of the G985 mutation among patients, we improved and optimized the assay with respect to reliability and convenience for routine diagnostic and screening purposes. As little as 2 [mu]l blood from filter-paper blood-spots (Guthrie spots) is sufficient for the test.


http://www.sciencedirect.com/science/article/B6T57-458NBXN-12/a51435410c281d44073d16ef6b59f37

Background: The purpose of this study was to examine the relationship between the production of secreted fibrinogen and the synthesis of [gamma]-chain mRNA. Methods: We transfected a [gamma]-chain expression vector into Chinese hamster ovary cells already expressing both A[alpha]- and B[beta]-chains of fibrinogen and measured fibrinogen output concentrations by ELISA. We quantified both [gamma]-chain and B[beta]-chain mRNA concentrations using the recently developed TaqMan fluorogenic detection system. Results: The concentration of secreted fibrinogen into the media positively correlated with the amount of fibrinogen contained in the cell lysates. Additionally, quantitative mRNA assays revealed that the fibrinogen concentration in the
cell lysates correlated well with the concentration of [gamma]-chain mRNA (r=0.7077, pr=0.0224, NS). Conclusions: These results demonstrate that the amount of recombinant fibrinogen produced in cells transfected with the [gamma]-chain vector, also expressing normal A[alpha]- and B[beta]-chains, is dependent on the transcription of [gamma]-chain mRNA. Namely, in this recombinant expression system using a two-step transfection procedure, [gamma]-chain synthesis is the rate-limiting factor for fibrinogen production. This quantitative method to measure mRNA may prove very useful for further in vivo analysis of fibrinogen gene transcription.


http://www.sciencedirect.com/science/article/B6T57-45M0SPC-1/2/c70176d28ea026afa216345ed64e1c79

Background: Mutations in the erythroid-specific 5-aminolevulinate-synthase gene (ALAS2) have been identified in many cases of X-linked sideroblastic anemia (XLSA). Methods: A polymerase chain reaction-mediated restriction fragment length polymorphism (RFLP) assay was used. Results: A G527T point mutation was identified. This resulted in a substitution of tyrosine for asparagine at residue 159 (D159Y). This mutation was also identified in the mother of the two probands. Mutations in all three individuals were confirmed by DNA sequencing analysis. Conclusions: We identified a missense mutation in exon 5 of the ALAS2 gene in two brothers of a consanguineous marriage, who were clinically pyridoxine-responsive.


http://www.sciencedirect.com/science/article/B6T57-4CDRKH3-5/2/04bfdf13bf02a3198ef5f14652f5b73b

Background: trans-Resveratrol, or 3,5,4’trihydroxy-trans-stilbene, is a polyphenolic compound that seems to provide a protective effect against several types of cancer, notably breast cancer. Through its phytoestrogenic properties it regulates the expression of hormone-dependent genes, such as the oncosuppressor BRCA1, in breast cells. This gene is involved in the majority of hereditary breast cancer, as well as sporadic cancers. Methods: We used three human breast tumor cell lines (HBL100, MCF7 and MBA-MB-231) and one breast cell line (MCF10a) derived from a fibrocystic disease to study in vitro the effect of resveratrol on the transcription of a group of genes whose proteins interact in different pathways with BRCA1. BRCA1, BRCA2, ER [alpha], ER [beta], p53, p21waf1/cip1, CBP/P300, RAD51, pS2 and Ki67 mRNA were quantified using real-time quantitative RT-PCR with an ABI 7700 apparatus. Results: Resveratrol modulated the expression of these genes in a pattern dependent on the status of [alpha] and [beta] estrogen receptors. These results show that resveratrol regulates gene expression via the estrogen receptor pathway and also an undetermined pathway. Conclusion: Thus, resveratrol seems to have an effect on breast tumor cell lines, on a fibrocystic cell line by affecting several factors regulating the function of BRCA1.

Background: We examined a technique for detecting point mutations of K-ras codon 12 in stool samples using one-step polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) analysis, in order to determine whether it could be used to screen for colorectal cancer. Methods: DNA was extracted from 200-mg stool specimens of 5 healthy controls and 31 colorectal cancer patients. A 107-base-pair fragment of exon 1 of K-ras was amplified by PCR using mismatched primers. PCR products were digested with Bst NI and analyzed by gel electrophoresis followed by silver staining. Specificity of one-step PCR/RFLP was examined by using synthetic oligonucleotides. The detection limit of K-ras codon 12 mutations was determined by using SW480 and HT29 cells. Results: The K-ras gene was successfully amplified from all healthy controls and colorectal cancer patients studied. Mutations of K-ras codon 12 were not detected in any of the healthy controls, but were identified in 13 (41.9%) of the 31 patients with colorectal cancer. Mutations were detectable in all six synthetic mutant DNAs, while none were detected among the wild type. The detection limit of this method was >=0.1%. Conclusions: PCR/RFLP analysis could be used in mass screening for colorectal cancer, because it is highly specific, has a low detection limit, and is simpler than conventional methods for detecting genetic abnormalities.


Background: Butyrylcholinesterase (BCHE) deficiency is characterized by prolonged apnea after the use of certain muscle relaxants with the genetic defect lying in the BCHE gene. Methods: Two Chinese patients with no serum BCHE activity were studied. The BCHE genes were screened for mutations by polymerase chain reaction and direct DNA sequencing. Results: Of the four mutations detected, two novel mutations were identified in the two patients, i.e., F474L, and an insertion of an adenine between nucleotide positions 395 and 396. This information was used to screen the immediate families of the patients for carrier status. Conclusions: We established the molecular basis of butyrylcholinesterase deficiency in two Chinese patients. The developed mutation detection assay provides a reliable method for identifying mutant BCHE carriers.


Background: The tumour necrosis factor-[alpha] (TNF[alpha]) promoter polymorphism (-308 G/A) has been shown to be associated with the susceptibility to and/or the severity of diverse diseases such as infections, autoimmunity, and malignancies. We developed a genotyping technique based on the mutagenically separated polymerase chain reaction (MS-PCR) which may be useful in the clinical risk assessment. Methods: Different length allele-specific primers and an unspecific complementary strand primer were used in a one-tube assay. At least one PCR product was generated in a single reaction obviating the need for an internal control amplification. Introduction of additional base substitutions into the allele-specific primers led to a clear-cut separation between the alleles through the reduction of cross-reactions during amplification. The only post-PCR step required was the separation of allelic PCR products by size upon agarose gel electrophoresis. Results: The allele frequencies in 300 German healthy Caucasians were 0.84 for TNF1 (-308 G) and 0.16 for TNF2 (-308 A) in accordance with published data obtained with the conventional RFLP method. No significant deviation from Hardy-Weinberg equilibrium was observed. The specificity of MS-PCR was confirmed by sequence-based typing. Conclusions: MS-PCR is a rapid, reliable, and cost-effective technique for genotyping of the TNF[alpha] promoter polymorphism (-308 G/A).


http://www.sciencedirect.com/science/article/B6T57-4384MXN-4/2/4dee516690124c757bbc635338dfac5a

Cytochrome P450 (CYP450) mixed-function mono-oxygenases, consisting of more than 30 enzymes, are responsible for the metabolism of a large number of drugs and metabolites. With the rapid advances in the human genome project, the role of genetic polymorphism in drug metabolism may become an important adjunct for rational drug therapy, and for the explanation of drug toxicity and interactions. This preliminary study modified a previously described procedure for genotyping CYP2D6*3 and *4. An additional step included uracil-DNA glycosylase for the prevention of "carry-over" contamination. DNA was extracted from peripheral blood using PureGene DNA Isolation kit. CYP2D6*3 and *4 sequences were amplified by PCR, followed by digestion with restriction endonuclease Msp1 and Mva1, respectively. Resulting fragments were analyzed by electrophoresis and visualized by ethidium bromide staining. Poor metabolizers of *3 mutation showed 168-, 82- and 20-bp bands, while those of *4 showed a single 355-bp band. Using these protocols, 22 individuals were genotyped, showing the following prevalence for *3 and *4: 0 and 3, respectively--comparable to those of the general population. This method provides a reliable means of genotyping CYP2D6*3 and *4.


http://www.sciencedirect.com/science/article/B6T57-4281031-3/2/72fc5876ff5cf30a0da88a87036e69a4

We quantitatively measured the amount of recombinant molecules formed during PCR when the break point cluster region (BCR) cDNA was coamplified with a homologous internal standard using Taq polymerase. The products were analysed under denaturing conditions using capillary electrophoresis followed by detection of the fluorescently labelled products and the recombinant molecules were differentiated by their size. Early termination of chain synthesis and reannealing of incomplete fragments, to each other as well as to BCR and internal standard, is one mechanism for generating recombinants during PCR since prolonging extension time reduced, but did not totally suppress recombinant molecule formation. Template switching by the extending
chain is another mechanism since recombinant molecules could be detected even after only one round of primer extension. The latter mechanism is probably facilitated by increasing number of templates. Thus, the large increase of recombinant molecules formed in plateau phase is mediated by direct amplification of the recombinants and de novo synthesis by template switching. The effect of additives on recombination could be quantitatively measured and both betaine and DMSO were effective in suppressing recombination. Thus, prolonging extension time, reducing the number of amplification cycles and incorporating additives in the PCR reaction, reduced recombinant molecule formation.


http://www.sciencedirect.com/science/article/B6T57-3T14BGT-5/2/bdb666c01cee12dd74183cc01c35228c

The prevalence of a mutation of the codon for tryptophan 64 to arginine (Trp64Arg) in the [beta]3-adrenergic receptor gene was investigated by genotyping 261 Japanese subjects. The allelic frequency of this mutation was 0.18. Subjects with the homozygous W64R mutant alleles had a significantly higher prevalence of fatty liver, BMI, serum [gamma]-glutamyl transpeptidase, and serum leucine amino transpeptidase levels than those without the mutation. Individuals with this mutation also showed a higher fasting blood glucose level than those without this mutation. However, the prevalence of diabetes mellitus was no different between the three groups. These results suggest a potential association of the Trp64Arg mutation with higher morbidity of fatty liver and mild glucose intolerance.


http://www.sciencedirect.com/science/article/B6T57-4CS4PY4-1/2/0413a48fe94cfbcf93569402d21a6a0

Background: Carboxypeptidase U (EC 3.4.17.20, TAFIa) is a new member of the metallocarboxypeptidase family circulating in human plasma as a zymogen. It is activated during coagulation and is considered as an important player in the regulation of fibrinolysis. Methods: Heterologous expression of human plasma procarboxypeptidase U (proCPU, TAFI) was obtained in mammalian cells (C127 and DON) and in insect cells (Sf21 and H5 cells). Conditioned media were purified by cation-exchange chromatography and plasminogen affinity chromatography to yield an essentially pure protein. Results: All systems gave high expression levels (6-20 mg/l). Due to differences in glycosylation of the activation peptide, the recombinant variants of proCPU migrated differently on SDS-PAGE (52-65 kDa). However, after activation, all active recombinant enzymes migrated at 35 kDa, similar to native CPU and no evidence for post-translational modification of the catalytic domains could be detected. For the mammalian cell produced variants, activation was more efficient after desialylation. After activation, CPU showed low solubility (0.2 mg/ml) but was inhibited similarly as native CPU. Conclusions: Mammalian cell systems were the most efficient for the production of human plasma recombinant proCPU. The obtained zymogen differs with respect to the extent and the heterogeneity of glycosylation but, after activation, the experiments did not reveal any alteration between the recombinant and native protein.

http://www.sciencedirect.com/science/article/B6T57-40R5B2S-4/2/9d60d036eb2e054b73e007af6acd2430

We describe an immuno-polymerase chain reaction (immuno-PCR) assay for the detection of human angiotensinogen using identical first and second polyclonal antibodies. The reporter DNA was initially generated by PCR amplification using a biotinylated primer, and was bound with streptavidin to biotinylated second antibody. Human recombinant angiotensinogen sandwiched by antibodies was detected by amplifying the reporter DNA using PCR. To reduce the effect of nonspecific amplification, the optimal concentrations of streptavidin and DNA label were determined to be 0.1 mg/l and 0.5 ng/l, respectively. The detection limit of the immuno-PCR assay was 0.1 ng/l, an approximately 2.5 x 10^5-fold improvement compared with a conventional enzyme-linked immunosorbent assay. These results indicate that a highly sensitive immuno-PCR for human angiotensinogen can be developed even with identical first and second polyclonal antibodies.


http://www.sciencedirect.com/science/article/B6T57-3SBNJTP-2/2/8f800c80a5ca0e59b53ec6992ba53fa9

The aims of this study were two-fold: first, to assess the relative diagnostic performance of non-isotopic in situ hybridization (ISH) and the nested polymerase chain reaction (nested-PCR) applied to Epstein-Barr virus (EBV) detection in a series of 55 unselected nasopharyngeal carcinoma (NPC) cases and, secondly, to correlate these data with histopathological classification. Our study shows that in 76.36% of NPC cases positive nuclear signals were observed using EBV-ISH. Overall, EBV-ISH positivity varied according to histological type, in that undifferentiated carcinomas showed a higher proportion of positive cases than differentiated cell carcinomas, although ISH results do not show significant differences in relation to histological types when employing two different schemes (WHO and Micheau). However, in adequate quality DNA samples (54 NPC cases), EBV-DNA was detected in 100% of cases using a nested-PCR, supporting the previous view that all histological types of NPC are in reality variants of EBV-infected neoplasia. ISH-negative cases probably reflect a lower sensitivity than PCR, particularly when a small number of viral copies are present, as well as a variable technical effectiveness for detected EBV, independent of the NPC histological type.


http://www.sciencedirect.com/science/article/B6T57-45J9464-3/2/26cc768298e9844c9d24c96dd2f1e212

Background: BRCA1 and BRCA2 are breast cancer susceptibility genes. Recent studies suggest that BRCA1 interacts with a great variety of proteins, including BRCA2, cell-cycle regulators, transcriptional activators and repressors. We investigated the expression of both BRCA1 and BRCA2 during the progression of the cell cycle of human tumor cell lines from different origins.
Methods: First, the growth status was characterized by determination of the cell cycle by flow cytometry analysis. At the same time, immunohistochemistry was performed to follow BRCA1 and BRCA2 protein expression and then, quantification of BRCA1 and BRCA2 transcripts was realized using real-time quantitative RT-PCR. Results: We reported in studied tumor cell lines with 60% of confluency by comparison with 100% of confluency, an increase in the BRCA1 and BRCA2 expression at the level of proteins and transcripts. Conclusion: Therefore, the expression of both BRCA1 and BRCA2 genes at the protein and mRNA levels appear to be up-regulated after cell proliferation in human tumor cell lines from different origins.


Telomerase is an enzyme that synthesizes and adds repetitive telomeric sequences of (TTAGGG)n to the ends of chromosomes. Recently, several telomerase-associated genes have been cloned, making it possible to study the expression of these genes. Quantitative comparisons of the expression of these genes and of telomerase activity might help clarify the regulation of telomerase activity. Therefore, we established the validity of a quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay for the human telomerase catalytic subunit (hTERT) mRNA and telomerase associated protein (TEP1) mRNA using the TaqMan(TM) fluorogenic detection system. Using this assay, we quantitated hTERT mRNA and TEP1 mRNA expression in two human pancreatic cancer cell lines, AsPC-1 and PANIC-1. Our results indicated that the levels of hTERT mRNA and TEP1 mRNA expression in AsPC-1 were 1.50 and 2.31 times higher than in PANC-1 cells. This TaqMan(TM) RT-PCR assay appears to be useful in determining the quantities of hTERT and TEP1 mRNAs in clinical specimens. Taken together, our results indicate that it is possible to measure the expression of the major telomerase genes subunits. Furthermore it is possible to apply this technique to determine the amount of other types of mRNA.


[beta]-2 adrenergic receptor (B2AR) agonists are the most widely prescribed rescue agents used in the treatment of asthma. Recent studies have indicated a relationship between a polymorphism at codon 16 of the B2AR gene, and the response to recurrent [beta]-agonist therapy. The B2AR polymorphism of interest involves a single nucleotide change from A to G, resulting in an amino acid change from Arginine (Arg) to Glycine (Gly). Clinical efforts to further investigate this relationship require an accurate, reliable and inexpensive method for detecting the polymorphism. In this study, we report an LCx(R) assay for the detection of a single nucleotide polymorphism at codon 16 of the [beta]-2 adrenergic receptor. This assay is capable of detecting patients harboring any of the three possible genotypes at this locus, namely, homozygous wild type, homozygous variant or heterozygous individuals with a single genomic DNA sample of 25-500 ng. It requires minimum hands-on time with automated detection. The assay would be suitable for use in research labs for screening of a large number of samples. We believe that this type of assay will facilitate research and clinical investigations in elucidating the association of
SNPs with disease states, diagnosis, prognosis and treatment.

Clinical and Diagnostic Virology (6)


http://www.sciencedirect.com/science/article/B6T58-3YK00V6-F/2/117536cd6cf4716a2682a8cec176d6f9

Background: The presumed latency of cytomegalovirus (CMV) in leucocytes and the sensitivity of the polymerase chain reaction (PCR) raise a question of its clinical value.Objectives: To develop and standardize a CMV PCR as a diagnostic tool for CMV infection in solid organ and bone marrow transplant patients by comparing it to a likewise standardized isolation, rapid isolation and to clinical symptoms.Study design: The material comprised 822 EDTA peripheral blood samples from 96 solid organ and 119 bone marrow transplant patients. One sample from each of 21 healthy bone marrow donors and 25 blood donors were used as controls. Two million leucocytes were lysed and one-tenth of a volume was used in a nested PCR employing immediate early gene primers.Results: The limit of detection was [ap] 10 gene copies of a CMV DNA clone and 1 TCID50 of extracted DNA from a cell suspension. The specificity was >=0.99 when tested in CMV seronegative individuals. The positive and negative predictive values were 0.62 and 1.00, respectively. When PCR was compared to virus isolation/rapid culture in individual patients, PCR was positive more frequently in solid organ transplant patients than was CMV isolation/rapid culture, but the difference was not significant in bone marrow transplant patients. In isolation-positive patients, PCR became positive in samples taken 1-2 weeks earlier. In 54 solid organ transplant patients with PCR-positive samples, CMV-associated symptoms were present in 29/31 patients with CMV isolated from blood but in only 5/23 patients without viraemia. In 17 bone marrow transplant patients treated with ganciclovir, PCR became negative during or immediately after treatment in 14/20 (70%) episodes. This was true of 5/12 (42%) solid organ transplant patients.Conclusion: Screening of transplant patients with CMV PCR can be standardized at a clinically relevant level so that antiviral therapy can be instituted early.


http://www.sciencedirect.com/science/article/B6T58-3W3FH73-3/2/c29eefd226a58b0bf9cfa7bc012788fe

Background: Multiplex polymerase chain reaction (PCR) has been established as a general technique for the simultaneous amplification of different target sequences. Uses of multiplex include pathogens identification, linkage analysis and genetic disease diagnosis. The high sensitivity of PCR may produce false-positive results due to contamination with previously amplified material.Objectives: To develop a multiplex PCR technique that can simultaneously detect and discriminate human immunodeficiency virus types 1 and 2 (HIV-1/2) and human T-lymphotropic virus types 1 and 2 (HTLV-I/II) proviral sequences. Such a method should incorporate a system that prevents the occurrence of false-positive results.Study design:
Combinations of four primer pairs, one for each retrovirus, were assayed in order to determine the combination of oligonucleotides as well as the PCR conditions that yield the most specific and sensitive coamplification of proviral sequences. To prevent contamination with DNA from previous PCR amplifications, the uracil N-glycosylase (UNG) system was incorporated into the coamplification format.

Results: A combination of primer pairs from the gag region of HIV-1, env of HIV-2, pol of HTLV-I and tax of HTLV-II yielded specific and sensitive coamplification of proviral sequences. The UNG system was incorporated and shown to be efficient in the degradation of contaminating DNA. In the evaluation of a serologically well established panel of singly and dually infected individuals, the assay detected 20/22 HIV-1, 8/10 HIV-2, 8/8 HTLV-I and 8/8 HTLV-II infections.

Conclusions: A multiplex PCR method for the detection and discrimination of HIV-1/2 and HTLV-I/II has been developed. Under standardized conditions, all four proviral sequences were detected in a specific and sensitive manner. The evaluation of a panel of clinical specimens from infected individuals by one or more retroviruses showed that the technique detected most of the infected individuals. A low viral load may explain cases where multiplex PCR failed to detect target sequences.


http://www.sciencedirect.com/science/article/B6T58-3SRKJF-6/2/7c7ce8be870bf4c55329e2dd5e1e16

Background: The Amplicor(TM) HBV Monitor Test for quantitative determination of serum hepatitis B virus (HBV) DNA has recently been introduced. This assay is based on PCR and a non-radioactive hybridization and detection system on microwell plates. Objective: The performance of the Amplicor(TM) HBV Monitor Test was evaluated in a routine diagnostic laboratory. The Amplicor(TM) HBV Monoitor assay was compared to the Digene Hybrid Capture(TM) System HBV DNA assay for the quantitation of HBV in patient sera. Study design: Sensitivity and reproducibility were determined with 10-fold dilution series of two Eurohep HBV reference plasma specimens. Furthermore, 196 sera from 14 children with chronic HBV infection and interferon therapy were tested with both assays. Results: The detection limit was found to be 103 copies/ml with the Amplicor(TM) PCR assay compared to 106 to 107 copies/ml with the Digene(TM) hybridization assay. Both assays were quasi-linear over the measurable ranges. The new PCR assay proved to be very reliable. With the Amplicor(TM) PCR assay, 26.2% of the HBV DNA-positive clinical samples were found between 103 and 107 copies/ml and all of them tested below the detection limit with the hybridization assay. Conclusion: The Amplicor(TM) HBV Monitor Test shows excellent sensitivity and provides a valuable tool for the detection of HBV DNA in serum. It can be used for recognizing those patients who might benefit from antiviral therapy, for evaluation of the efficacy of anti-HBV therapy, and for validation of blood products.


http://www.sciencedirect.com/science/article/B6T58-3VW7BDK-B/2/02152d1a99b2c226cf65468e69c49dfc

Background: Insulin-dependent diabetes mellitus or type 1 diabetes is a disease with a diverse aetiology. Epidemiological studies examining newly diagnosed, recent onset IDDM patients have suggested a role for viruses in the aetiology of IDDM (Yoon, 1995, Diabetes/Metabolism Reviews 11, 83-107). Important candidates are the enteroviruses, in particular coxsackieviruses B3 and B4. The latter can cause diabetes in animals (Clements et al., 1995, Lancet 346, 221-
Objectives: We have developed a quantitative PCR method for the detection of enterovirus genomes in biological samples. The quantitative PCR will be used to screen for enteroviruses in blood of diabetes patients and their relatives by testing a Blood Diabetes Register.

Study design: A substantial amount of data has been collected on enterovirus induced IDDM, our study is original in so far as it will be: (1) a quantitative study, not only the presence of viral genome sequences in blood will be determined, but also their concentrations (viral load); and (2) a longitudinal study, samples are and will be collected as a function of time. Positive PCR samples will be quantified using the standard addition method.

Results: The test is specific for enteroviruses, since all enteroviruses were detected with equal sensitivity. Viruses belonging to other picornavirus genera scored negative (even up to 3 x 10^6 genome copies). An equal detection limit of 10 genome copies was found for all enteroviruses.

Conclusions: The developed method will permit us to generate quantitative and longitudinal data of enterovirus genomes in blood of diabetes patients and their relatives, which might help in the elucidation of the relationship between enteroviruses and IDDM.


http://www.sciencedirect.com/science/article/B6T58-3W3FH73-5/2/9a052a75d6c7f2609d02d6a0d3e5bbb3

Background: Rapid laboratory methods for the early detection of cytomegalovirus (CMV) are needed for the prevention of CMV disease in transplant recipients. These methods should not only be able to detect the virus but also be highly predictive for CMV disease. Objective: The clinical value of a simple and rapid nested plasma polymerase chain reaction (PCR) was evaluated by comparing the results with CMV pp65 antigen detection in leukocytes (CMV antigenemia assay), virus isolation from leukocytes, CMV IgG and IgM antibody response and clinical data. Study design: A total of 471 EDTA blood samples were collected from 85 kidney transplant patients during a 3-4 month period after transplantation. CMV DNA was amplified directly from 10 [mu]l of plasma while 150 000 separated leukocytes were stained for CMV pp65 antigen by each of two monoclonal antibodies. A total of one million leukocytes were used for virus isolation. The PCR protocol used in the present study involves a simple alkaline lysis technique for isolating DNA directly from plasma which is easy and rapid to perform. Results: Twenty-eight patients developed symptomatic CMV infection while asymptomatic infection occurred in 29 patients. CMV pp65 antigen detection had a 75% sensitivity and a 57% positive predictive value for CMV disease development, compared with 64% and 79% sensitivity and 49% and 46% positive predictive value for CMV DNA and viremia, respectively. The median time until detection of CMV in patients with symptomatic CMV infection was 26 days after transplantation, compared with 49 days in asymptomatic patients by any of the methods used. Early appearance (within 8 weeks) of CMV pp65 antigen and CMV DNA had high predictive values for symptomatic infection; repeated detection of pp65 antigen and CMV DNA were more common in symptomatic patients.

Conclusions: CMV antigenemia assay and plasma PCR can be used for pre-symptomatic diagnosis of CMV infection. Virus isolation and CMV serology in most cases provide a post-symptomatic diagnosis. The best marker for monitoring kidney transplant patients might be the quantitative CMV antigenemia assay.

Background: The detection of proviral DNA by Polymerase Chain Reaction (PCR) is regarded as an important tool in the diagnosis of HIV-1 infection, specially among adults at risk of AIDS and children born to seropositive mothers. However, application of PCR in routine testing is hampered by the need to use radioactive probes. Objectives: In this study, a non-radioactive test based on a microtiter plate (DNA Enzyme ImmunoAssay, DEIA) was used for the detection of proviral sequences of HIV-1 in peripheral blood cells of different patients. The results of the PCR-DEIA assay were compared to those obtained by liquid hybridization (PCR-LH), virus isolation (VI) and Western blot (WB).

Study design: The study population included 92 patients belonging to three different groups: seropositive subjects with a well-defined clinical status and WB profile; adults at risk of infection with negative or indeterminate WB; children born to seropositive mothers with still unestablished HIV-1 infection.

Results: In the seropositive subjects, both PCR-LH and PCR-DEIA confirmed infection and gave the same results as WB. In adults at risk of infection, PCR with both methods anticipated the seroconversion in one patient with indeterminate WB and confirmed the absence of infection among seronegative and other indeterminate patients. In children born to seropositive mothers, both PCR systems as well as VI permitted an early diagnosis of infection, as confirmed by the clinical follow-up.

Conclusion: This study has shown that in subjects at risk of AIDS and in children born to seropositive mothers, the non-isotopic DEIA method presents the same sensitivity and specificity for the detection of HIV-1 infection as the radioactive procedure. The DEIA method appears to be particularly useful for the detection of PCR products in routine diagnostic analyses.

Clinical Biochemistry  (24)


Objective: We used single-strand conformational polymorphism (SSCP). To screen for mutations/polymorphisms in exon 4 of the apolipoprotein C III in 45 patients with hypertriglyceridemia and 46 control individuals, single-strand conformational polymorphism was investigated using restriction endonuclease and amplification refractory mutations systems (ARMS). Results: SSCP identified six patterns corresponding to six genotypes. We confirmed that the different genotypes result form the two polymorphic sites at positions 3175 and 3206 of the apo C III gene. Only three of four possible haplotypes were found in the study population. This resulted in the identification of 6 of the 10 possible genotypes. Conclusions: SSCP is a useful method to screen for both known and unknown mutations/polymorphims and should have increasing applications in clinical laboratories involved with the study of genetic markets of a wide variety of diseases.

Objective: We examined several critical parameters that must be optimized when converting between the ABI Prism 7700 real-time PCR platform and the Cepheid SmartCycler[trademark] II while using the same primer and probe sequences. Design and methods: A lyophilized master mix, MgCl₂ concentration, PCR cycling conditions, and ramp times were evaluated. Results: Optimization of each parameter, including use of the OmniMix[trademark] HS-lyophilized beads, 6 mM MgCl₂ concentration, changes in PCR cycling parameters, and increased ramp time were necessary to convert this real time PCR assay to a new platform. Conclusion: We conclude that careful consideration of several analytical parameters can result in a smooth transition of assays between real time PCR platforms.


Objectives: To assess the impact of the human Fc[gamma]RIIA and Fc[gamma]RIIIB gene polymorphisms on the risk of rheumatic fever (RF). Designs and methods: Fc[gamma]RIIA-R/H-131 and Fc[gamma]RIIIB-NA1/NA2 genotypes were determined using polymerase chain reaction in 66 RF cases and 117 healthy controls in this case control study. Results: Compared with healthy controls, the RR genotype was enriched in the entire group of RF cases (odds ratio [OR] 4.98, 95% confidence interval [95% CI] 1.81-13.70). RF patients were more frequently HR heterozygotes rather than HH homozygotes (OR 3.09 vs. 0.11). The results of this study show that patients who have RF are more likely to have the RR and HR genotypes than control children. These probabilities show that RR is associated with the greatest risk for rheumatic fever and HR is associated with an intermediate risk. For the distribution of Fc[gamma]RIIIB NA2 genotypes, a nonsignificant increase was found in RF patients (39.31% vs. 51.51%; OR 1.64, P = 0.1226). Conclusion: The Fc[gamma]RIIA-R/H-131 polymorphism may be an important marker in determining predisposition to RF.

tissue samples from 115 head and neck cancer patients were assayed to ascertain the relative levels of the growth factors. Results: The CVs of within-run and between-run assays for VEGF, PDGF and bFGF were all less than 3%. The correlation coefficient of the RNA concentrations and Ct values were 0.9987, 0.9977, and 0.9996 respectively for VEGF, PDGF and bFGF. The assay was sensitive to as little as 10-3 ng of RNA. All three growth factors were significantly increased in tumor tissue as compared to normal tissue. VEGF, PDGF and bFGF levels were elevated in 71.3%, 58.2% and 54.0% of cancerous tissue samples, with average levels of over-expression of 35.1, 24.6 and 13. sixfold, respectively. Conclusion: This method provides sensitive, quantitative, high-throughput analysis for direct comparison of gene expression levels between samples, while adjusting for factors that may influence quantity determination. It should be applicable to molecules other than angiogenic growth factors, as well.


http://www.sciencedirect.com/science/article/B6TDD-47P82KP-6/2/dc214d4d2ed2a23a4a5c12493d89231

More than 95% of the patients with chronic myelogenous leukemia (CML) carry translocations between protooncogene abl of chromosome 9 and bcr gene of chromosome 22, resulting in the Philadelphia chromosome (Ph1). After allogeneic bone marrow transplantation (BMT) it is important to detect possible residual malignant cells in CML patients. A new sensitive hybridization method combined with polymerase chain reaction (PCR), based on the detection of the europium (Eu3+) label by time-resolved fluorescence, was applied for the detection of Ph1 chromosome. Total RNA from 106 peripheral blood leukocytes was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction. After cDNA synthesis by reverse transcriptase, the PCR amplification (30 cycles) was carried out. In the detection phase two oligonucleotide probes were used in the hybridization reaction, one biotinylated (bcr gene, exon 2) and one (abl gene) labeled with Eu3+. The hybrids were collected in a streptavidin-coated microtitration well and the bound Eu3+ was measured in a time-resolved fluorometer. To assess the sensitivity of the method, different numbers of CML cell line K562 cells were mixed with 105 apparently normal human leukocytes. Five K562 cells/105 leukocytes could be detected. Six patients with CML confirmed by clinical and cytogenetic criteria were studied. Three of the patients underwent an allogeneic BTM 6-18 months before the investigation and all of them were Ph1-negative. The other three patients who were nontransplanted were positive as expected.


http://www.sciencedirect.com/science/article/B6TDD-4287BN8-6/2/0492c4a24beff7f1362c9030e524c6236

Objectives: The major RNase activity of leukocytes has been attributed to eosinophil-derived neurotoxin EDN. Depletion of eosinophils enables RT-PCR from 105 leukocytes without RNA extraction. In this study we introduced streptavidin-coated PCR tube strips for the selection of eosinophil-free leukocytes for RT-PCR analysis. Design and methods: Polypropylene 0.2 ml PCR tube strips were coated with streptavidin and biotinylated antibodies against cell surface antigens were attached to the tubes. CD7-positive T-lymphocytes, CD19-positive B-lymphocytes and CD16-positive cells (mainly neutrophils and monocytes) were positively selected by incubating of 1-2 x 105 leukocytes in the antibody-coated PCR tubes for 30 min at 23[deg]C. Results: The mean amount of cells bound into a tube was 31,500 (CV25%) T-cells and 8,600 (CV61%) B-cells from 12 blood samples, and 23,600 (CV22%) CD16+ cells from 17 samples. The influence of
selected cell lysate on the RT-PCR analysis of Philadelphia chromosome (bcr/abl translocation) from 100 K562 cells was small: 78% (CV28%) of the leukocyte-free signal was obtained in the presence of CD16+ cells or 89% (CV15%) and 99% (CV11%) and in the presence of T-cells and B-cells, respectively. Conclusions: These results suggest that through the introduction of eosinophil-free cell population into RT-PCR a reproducible method with reasonable leukocyte yield and avoiding RNA extraction was developed.


http://www.sciencedirect.com/science/article/B6TDD-46HG2R3-3/2/d2108cab5786f9f29482c90b6fa4e7c

Objective: To develop tetra-primer PCR assays for detection of the CCR2-V64I, CCR5-A59029G and SDF1-G801A polymorphisms associated with HIV pathogenesis. Design and Methods: For each assay, two primers for the amplification of the gene locus are combined in one tube with two primers for the subsequent allele specific amplification (ASA). In the first set of cycles, pre-amplification of the gene region of interest is ensured by the gene specific primers. In the second set of cycles, lowering the annealing temperature allows ASA on the newly produced template. Results: Analysis of 90 DNA samples resulted in allele frequencies for CCR2-V64I, CCR5-A59029G and SDF1-G801A which are similar to other Caucasian cohorts. Furthermore, re-analysis of sequenced genomic DNA by tetra-primer PCR analysis (7-11 times) always showed identical results. Conclusion: Our set of single-tube assays allows rapid and reproducible genotyping of the CCR2-V64I, CCR5-A59029G and SDF1-G801A polymorphisms. These inexpensive but accurate assays are valuable for screening these polymorphisms in cohorts of HIV-infected patients.


http://www.sciencedirect.com/science/article/B6TDD-3V4C29HG2/2/6004ae66a60c327a2f797f2339a35f21


http://www.sciencedirect.com/science/article/B6TDD-45GJ7R5-6/2/a7092d0c2f05e17a31420f987eb08545

Objectives: Variable number of tandem repeat polymorphisms (VNTR) are frequently analyzed by PCR in genetic, epidemiologic and forensic studies. We wanted to explore the validity of these PCR analyses. Design and Methods: The amplification of the different alleles of the 17- and the 44-bp VNTR of the serotonin transporter gene and the 39-bp VNTR of the glycoprotein Ib[alpha] gene was analyzed. We studied the effects of the parameters magnesium, dimethylsulfoxide, 7-deaza-dGTP, formamide, betaine, PCR temperatures and different types of polymerases. Results: In all three VNTR polymorphisms selective amplification of one of the alleles of heterozygous individuals could be obtained by change of the magnesium concentration. This problem could be minimized by a combination of Taq- and Pwo-polymerases and by use of 7-deaza-dGTP. Conclusion: PCR analysis of all of these VNTRs may give reproducibly wrong results in
truly heterozygous subjects due to selective amplification of only one of the alleles.


http://www.sciencedirect.com/science/article/B6TDD-3X1VV26-9/2/d548eeee19c4e077f09592f21c52448f2


http://www.sciencedirect.com/science/article/B6TDD-453FWWW-C/2/1abe0ce93f7da5479e4f5daace32a3b5

Objective: To develop a real-time PCR technique for detection of the insertion/deletion (I/D) polymorphism of angiotensin-converting enzyme (ACE) gene. Design and methods: Three primers were designed for performing real-time PCR in the presence of SYBR Green I as fluorochrome followed by melting curve analysis. Forty human genomic DNA that have been genotyped by two-rounds of conventional PCR were used for evaluation of this technique. Results: Melting curve analysis indicated the melting peak at 73.9[deg]C and 76.2[deg]C corresponding to the presence of I and D alleles, respectively. Comparable genotyping results were obtained by both conventional and real-time PCR. Besides, the mistyping of ID allele individuals by the first run of conventional PCR were accurately genotyped by single-tube real time PCR. Conclusions: The real-time PCR method presented in this study provides a rapid and sensitive way for genotyping of ACE gene that may be suitable for large-scale clinical and epidemiologic study.


http://www.sciencedirect.com/science/article/B6TDD-41MB0F4-9/2/8189242a09d84ac229e372efb884fee2


http://www.sciencedirect.com/science/article/B6TDD-3XR7YG-3/2/6cb9e17e87fe117573c2304f588e371b

Objectives: Acute intermittent porphyria (AIP) is an autosomal dominant inherited disease caused by a decreased activity of hydroxymethylbilane synthase (HMBS). As far as the gene abnormalities of the HMBS, many different mutations have been reported. In this work, we investigated the presence of mutations in a Japanese family with AIP. Design and Methods: A 44-year-old Japanese male and nine members of his family were investigated. All of them were screened by traditional biochemical markers. Mutational analysis was performed using polymerase chain reaction-single strand conformation polymorphism method followed by DNA sequencing. A reliable restriction enzyme cleavage assay was established for the pedigree analysis. Results: The mutation was a splicing mutation, a C to G transversion at position -3 of the
acceptor site of intron 11 of the HMBS gene, resulting in the exon 12 skipping. The patient is heterozygous for the mutation, and his father appeared to be the source of the mutant allele. This mutation created a new cleavage site of the Nla III restriction enzyme and could be screened by a amplified fragment from genomic DNA with digestion. Using this cleavage assay, an asymptomatic carrier in the family was definitively identified.

Conclusions: This mutation was first found among Japanese AIP patients, but happened to be the same as reported previously from Europe. A similarity of gene abnormality may suggest that those European and Japanese AIP families have a common ancestor. Molecular investigations on the family members should be applied not only for more accurate diagnosis, but also for understanding the molecular genetic heterogeneity underlying this dominantly inherited enzymopathy.


http://www.sciencedirect.com/science/article/B6TDD-3YGV4BT-3/2/7c2fa733ba0cb8072cf99b7f40b41aef

Objectives: In the clinical laboratory, identification of Streptococcus pneumoniae can be confused with other streptococci. Conventional biochemical tests such as optochin sensitivity and bile solubility can give inconsistent results. This report presents a method to distinguish true S. pneumoniae from other upper respiratory tract streptococci when conventional tests fail.

Design and Methods: We used arbitrarily primed polymerase chain reaction with the single primer M13 universal as a method to distinguish S. pneumoniae from other upper respiratory tract streptococci.

Results: The fingerprint pattern of S. pneumoniae was established by amplifying DNA of S. pneumoniae type strains 1-48 and of other common upper respiratory tract streptococci at three different DNA concentrations with the single primer M13 universal. From these type strains, a common arbitrarily primed-polymerase chain reaction pattern was identified characterized by two predominant bands of equal intensity at 800 base pairs and at 1100 base pairs. Fingerprint patterns of viridans streptococci were easily distinguishable from those of S. pneumoniae. Many of the clinical isolates used in this study were equivocal by conventional tests but were distinguishable by their fingerprint patterns.

Conclusions: Our results indicated that the fingerprint pattern of S. pneumoniae is species specific and distinguishes true S. pneumoniae of clinical isolates from other streptococci when conventional biochemical tests are unclear.


http://www.sciencedirect.com/science/article/B6TDD-47P83BX-5/2/77ae74caabf601dce723f36bf0ee386

Familial defective apolipoprotein B-100 (FDB) is a genetic disorder resulting from a mutation in the apolipoprotein B-100 (apo B-100) gene, most frequently at position 3500, in which arginine is substituted for glutamine in the mature protein. This mutation drastically decreases the affinity of the mutant apo B-100 particle for the low-density lipoprotein (LDL) receptor, and hence decreases the clearance of cholesterol from the circulation. Familial hypercholesterolemia (FH), also a disorder of lipid metabolism, results from mutations in the gene for the LDL receptor. Both FDB and heterozygous FH occur at approximately the same frequency (1 in 500) among Caucasians and both produce clinical symptoms and signs that can be indistinguishable. Polymerase chain reaction (PCR) amplification and subsequent restriction analysis have been used to detect the substitution at codon 3500 in the apo B-100 gene using mutagenic PCR primers. At least one proband from 10 unrelated families with a history of hypercholesterolemia
was screened by mutagenic PCR for FDB. Only one of 10 patients demonstrated the mutation for FDB. The mutant apo B-100 allele was shown to segregate with other clinically affected family members. These results demonstrate that molecular analysis is essential to distinguish between FDB and heterozygous FH in hypercholesterolemic families.


promoter region of FR-[alpha] may harbor much more genetic variation than its highly conserved exons, and not just isolated, unique mutations. This could be a new factor contributing to gene-food interaction explaining part of the hyperhomocysteinemia panorama. Extended searches for polymorphisms further upstream in the FR-[alpha] gene are warranted.


http://www.sciencedirect.com/science/article/B6TDD-3VCR30B-6/2/629617a7ccd0a6751fcccc63b3c896e0

Objective: The presence of small numbers of cells of donor origin in the circulation of recipients of organ transplants (microchimerism) may correlate with immunologic tolerance. As part of our ongoing studies on microchimerism, we evaluated the utility of seven PCR-based assays for the detection of the less abundant DNA in paired mixtures (100 ng total DNA). Design and methods: DNA samples were screened to identify pairs informative for one or more PCR assays. DNA mixtures from the informative pairs were then analyzed using at least one assay. The assays were based on the X-Y homologous region; a Y chromosome microsatellite locus; three autosomal microsatellite loci; the D1S80 minisatellite locus; and sequence specific oligonucleotide probe (SSOP) analysis of the HLA DRB1 locus. Results: About 0.1% of male DNA against a background of female DNA was detectable using primers for the X-Y homologous region, but the sensitivity was increased to 0.0001% using nested primers for the Y chromosome microsatellite marker. Analysis of the minor DNA component was difficult with the three autosomal microsatellite assays because of the presence of shadow bands. Similar problems with the D1S80 assay were resolved using more stringent PCR conditions, and the sensitivity was 0.1%. Using the DRB1 locus, we were able to detect 1% DNA in the mixed samples. Conclusions: These studies show that: (a) nested PCR for the Y chromosome is the most sensitive assay for the detection of microchimerism; (b) D1S80 is a useful marker for microchimerism; (c) additional optimization of analytical conditions is required if autosomal microsatellite markers and the SSOP assay are to be used for microchimerism analysis.


http://www.sciencedirect.com/science/article/B6TDD-47RR63T-4/2/94737dd27e74a6b56280f2e0c4907ae0

We have developed a method to genotype variable number of tandem repeats (VNTRs) and insertion/deletion polymorphisms using an integrated microfluidic chip-based system. We used this method to analyze a) a highly polymorphic pentanucleotide repeat (CCTTT)n locus within the 5'-putative promoter region of the human inducible nitric oxide synthase gene (iNOS5) which is associated with diabetic complications and infectious diseases; b) a bi-allelic 27 bp VNTR region within intron 4 of endothelial nitric oxide gene (eNOS27) which is associated with hypertension in type 2 diabetes patients with coronary heart disease and excess risk of advanced diabetic nephropathy in type 1 diabetes patients and c) an insertion/deletion polymorphism within the gene encoding angiotensin-converting enzyme (ACE/ID) which is associated with cardiovascular pathology and nitric oxide activity, and is in strong linkage disequilibrium with functional variants. Following amplifications, samples were mixed with gel-dye and markers and loaded into commercially available microfluidic chips designed for DNA sizing applications. In the study (N = 230), 95 (41%) of the DNA samples were homozygous and 135 (59%) were heterozygous for the iNOS5 repeats. For eNOS27, 173 (75%) of the genotyped DNA samples were homozygous for the larger 4b allele and the remaining 57 samples (25%) were heterozygous (4b/4a). No DNA
samples were homozygous for the shorter 4a allele with four 27 bp repeats. In case of ACE/iD, 47 (20\%) of the DNA samples were homozygous for the insertion, 65 (28\%) were homozygous for the deletion and the remaining 118 (51\%) were heterozygous. The results obtained were verified by analyzing random amplicons using bi-directional sequencing and GeneScan(R) 3.0 analyses with 100\% concordance being observed. Using the microfluidic chip-based method, separation and DNA sizing and genotyping are rapidly accomplished. The DNA fragments are resolved clearly and the system allows quantitation. Finally, the microfluidic chip-based method may be used for both large- and small-scale genotyping studies.


http://www.sciencedirect.com/science/article/B6TDD-3W2T7PV-C/2/e40c4c4e2eff34dd9fb186ac2e6b2d4a

Objective: We used single-strand conformational polymorphism (SSCP) to screen for mutations at nucleotides 833 and 919 of the cystathionine [beta]-synthase (CBS) gene in 13 patients with homocystinuria and 11 of their relatives.

Methods: Exon 8 of genomic DNA was selectively amplified by PCR using primers derived from intronic sequences of the human CBS gene. SSCP analysis was performed on the amplified products. Genotypes identified by SSCP were confirmed by DNA sequencing and an allele-specific PCR method.

Results: SSCP identified 5 patterns corresponding to five genotypes. We confirmed that the different genotypes result from mutations at nucleotides 833 and 919 of the CBS gene, and that these 2 mutations account for approximately 50\% of affected alleles in homocystinuria patients.

Conclusion: Our recent elucidation of intron-exon borders and intronic sequences of the CBS gene has made possible the use of SSCP to screen for known/unknown mutations in the CBS gene. Because T833C and G919A represent the two most common mutations and both are located within exon 8 of the CBS gene, SSCP of exon 8 allows screening of the heterozygous carrier state of these mutations in a large population, to determine the importance of heterozygosity of CBS mutations as the cause of mild hyperhomocyst(e)inemia associated with premature vascular diseases.


http://www.sciencedirect.com/science/article/B6TDD-41MB0F4-4/2/6a78544008a5d1adff1ed38fc03841e9

Objective: We developed a quantitative reverse-transcription polymerase chain reaction (RT-PCR) to determine CK20 expression in colorectal tumor and hematopoietic tissue.

Design and Methods: Our method incorporates a calibrated PCR with an internal competitor and an external standard.

Results: The RT-PCR assay is sensitive detecting 10 target molecules of CK20 in solution with one round of 38 amplification cycles. Genomic DNA contamination was eliminated by Dnase I digestion of total RNA. The inclusion of a calibrator in the quantitative RT-PCR analysis allowed for a high throughput of unknown samples within the same assay improving comparative analysis between the samples tested. Analysis of peripheral blood and bone marrow from 20 healthy volunteers revealed a low level of CK20 expression in all samples.

Conclusion: To study the clinical significance of CK20 expression as a marker of systemic metastatic disease it is essential to measure CK20 mRNA levels in hematopoietic tissue with sensitive quantitative RT-PCR. A sensitive and reproducible method, which is easily performed, is described.

http://www.sciencedirect.com/science/article/B6TDD-41MB0F4-D/2/8d5543b1fede6e32119b6d625cc0cc68


http://www.sciencedirect.com/science/article/B6TDD-4BDC5C4-1/2/e37f8194e288112501ae90b093e7e63

Objectives: (1) To compare two stool antigen EIAs (HpSA, FemtoLab) and PCR of ureaseA and cagA in feces, with 13C-urea breath test (UBT). (2) To ascertain whether a simplified UBT (breath collection TIME = 10 min) is as reliable as the standard assay (30 min). Design and Methods: Helicobacter pylori status was recorded in Group 1 (n = 187) by UBT, H. pylori stool antigen, ureA and cagA PCR in feces. UBT with 10, 20 and 30 min sampling was performed in Group 2 patients (n = 283). Results: The sensitivity and specificity of HpSA, FemtoLab, and ureA were 67% and 99%, 90% and 96%, 35% and 98%, respectively. cagA results were positive in 16/48 H. pylori-positive, and in 5/100 H. pylori-negative patients. The results of UBT with a 10- and 30-min sampling strictly overlapped. Conclusion: UBT with 10 min breath collection and FemtoLab stool antigen assay are the most reliable non-invasive tests to diagnose H. pylori infection.

Clinical Immunology  (2)


http://www.sciencedirect.com/science/article/B6WCJ-4FH0D8G-1/2/abcc89c737c6c969e09e6994d5dba34f

After the provision of highly active antiretroviral therapy (HAART), the level of circulating CD4+ T cells increases in many adults infected with the human immunodeficiency virus, type 1 (HIV). To study factors involved in immune reconstitution, we have measured thymic abundance by CT scans, circulating naive-phenotype CD4+ T cells by flow cytometry, and T cell receptor (TCR) rearrangement excision circles (TRECs) by quantitative PCR in 40 virologically suppressed, HIV-infected adults and 33 age-matched, HIV-uninfected controls. In HIV-uninfected subjects, naive T cell numbers, thymic abundance, and the frequency of circulating naive CD4+ T cells bearing TRECs decreased with age, as expected. When corrected for this relationship with age, naive T cell numbers correlated significantly with naive T cell TREC frequencies. Virologically suppressed HIV-infected subjects had higher TREC frequencies, and subjects over the age of 39 were more likely to have abundant thymus compared to age-matched, HIV-uninfected adults. Nevertheless, all HIV-infected subjects had reduced absolute numbers of naive T cells, irrespective of thymic size, age, or TREC frequencies. These data illustrate the complex relationship between these measures of thymic size and function and underscore the need to develop more definitive
measures of thymic function in the future.


http://www.sciencedirect.com/science/article/B6WCJ-4CWRKX2-1Z/2/ce0f0981aafcf1d0c7b962da3bb60c4

T-cells are causally involved in the pathogenesis of inflammatory bowel disease (IBD). The tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) regulates T-cell proliferation and survival. We show in this report that IDO mRNA is markedly induced in lesional colonic biopsies of IBD patients. IDO is primarily expressed in CD123+ mononuclear cells infiltrating the submucosal areas of the inflamed lesions. In Crohn's disease (CD), IDO is also strongly expressed in peril follicular regions of lymphoid follicles. Upregulation of IDO is of functional significance, as we detected an increase of kynurenine and of the kynurenine/tryptophan ratio in supernatants from colonic explant cultures (CECs) of CD patients. Immunohistochemistry of colonic biopsies taken from CD patients prior and after treatment with the TNF-blocking antibody Infliximab revealed reduced IDO expression in patients with good clinical response to Infliximab. In summary, high local expression of IDO may represent an anti-inflammatory mechanism tempting to counterbalance the tissue-damaging effects of activated T-cells infiltrating the colonic mucosa in IBD.

Clinical Neurology and Neurosurgery (1)


http://www.sciencedirect.com/science/article/B6T5F-3RSP3FH-2Z/2/1fe4109af4ea69742beaed9b0600dc80

Hyperekplexia (MIM: 149400), or startle disease, is an autosomal dominant neurological disorder characterized by an extreme generalized stiffness immediately after birth, normalizing during the first years of life. Other features of this disorder are excessive startle reactions to unexpected, particularly auditory, stimuli together with a short period of generalized stiffness during which voluntary movements are impossible. Linkage analysis mapped a gene for this disorder to chromosome 5833-q35 Subsequently, mutations in the GLRA1 gene encoding the [alpha]1-subunit of the glycine receptor proved to be causally related to the disease. In the present study, mutation analysis of all exon and flanking intron sequences of this gene was performed in sporadic patients and their parents. Moreover, a branch of the original Dutch hyperekplexia family with a very severely affected individual was screened for an additional mutation in the GLRA1 gene. Except for two polymorphisms, of which one results in an amino acid change, no potentially disease causing mutations were found in the [alpha]1-subunit of the glycine receptor. Together with haplotype analysis these results exclude a recessive inheritance or new mutation etiology in these hyperekplexia-like syndromes and emphasize that hyperekplexia-like syndromes can be caused by other genetic factors. The involvement of other genes encoding subunits of the functional glycine receptor complex has not been excluded.
Clinical Nutrition  (1)


http://www.sciencedirect.com/science/article/B6WCM-48M7V31-5/2/ec6220d5448bf0424de753db663340fb

Objective: The aim of our study is to determine the effect of a 30-day-period caloric restriction (CR) upon the immune response of rats and the influence of glutamine upon mononuclear cells proliferation and cytokine production.

Methods: Male albino Wistar rats were submitted to CR receiving an amount of food equivalent to 50% of the mean amount consumed by the control animals. We measured the incorporation of [2-14C]-thymidine by lymphocytes obtained from the spleen and mesenteric lymph nodes, plasma glucose and glutamine concentration, as well as cytokine production by cultivated cells, in the presence of glutamine.

Results: Rats submitted to CR presented reduced body weight (49%) and decreased splenic leukocyte number. CR led to a reduction in the proliferative response of lymphocyte. Spleenocytes from CR animals produced less [gamma]-interferon and interleukins 1, 4 and 10 in 48 h culture than did those from control rats. The same pattern is observed in cells obtained from the mesenteric lymph nodes. The addition of glutamine 2 mM to the culture medium restored spleen and mesenteric lymph node cells' proliferative response and the production of interleukin 2 by cells obtained from the spleen and from the mesenteric lymph nodes.

Conclusions: The present data reinforce that undernutrition decreases in vitro immune cell function and indicates that, in such circumstances, glutamine supplementation could reverse some of the changes observed in the functionality of cultured immune cells. The presence of the amino acid at physiological concentration, however, reinforces the diversion of the immune response towards a Th1-like response.

Clinical Pharmacology & Therapeutics  (14)


http://www.sciencedirect.com/science/article/B6WCN-4F53HJD-9/2/74f757101b8f996450e056bf8eb806c5

Background and objective: Surfactants used in pharmaceutical formulations can modulate drug absorption by multiple mechanisms including inhibition of intestinal P-glycoprotein (P-gp). Our objective was to analyze the effect of 2 surfactants with different affinity for P-gp in vitro on the intestinal absorption and bioavailability of the P-gp substrate talinolol in humans.

Methods: In vitro, the influence of surfactants on talinolol permeability was studied in Caco-2 cells. In vivo, an open-label 3-way crossover study with 9 healthy male volunteers was performed. Subjects were intubated with a 1-lumen nasogastrointestinal tube. The study solution, containing either talinolol (50 mg), talinolol and D-[alpha]-tocopheryl polyethylene glycol 1000 succinate (TPGS) (0.04%),

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or talinolol and Poloxamer 188 (0.8%), was administered through the tube. Results: TPGS, but not Poloxamer 188, inhibited the P-gp-mediated talinolol transport in Caco-2 cells. In healthy volunteers TPGS increased the area under the plasma concentration-time curve with extrapolation to infinity (AUC0-[infinity]) of talinolol by 39% (90% confidence interval, 1.10-1.75) and the maximum plasma concentration (Cmax) by 100% (90% confidence interval, 1.39-2.88). Poloxamer 188 did not significantly alter the AUC0-[infinity] or Cmax of talinolol. Conclusions: This in vivo intraduodenal perfusion study showed that low concentrations of TPGS, close to the concentrations that showed P-gp inhibition in vitro, significantly increased the bioavailability of talinolol. The study design excluded modulation of solubility by TPGS and unspecific surfactant-related effects. The latter was supported by the absence of modulation of the talinolol pharmacokinetics by Poloxamer 188, which does not modulate P-gp. Therefore we consider intestinal P-gp inhibition by TPGS as the major underlying mechanism for the increase in talinolol bioavailability.


http://www.sciencedirect.com/science/article/B6WCN-45SR82H-15/2/ee76cuda07b3fee22c8c27d13993ea71b

Background: P-glycoprotein, the gene product of MDR1, confers multidrug resistance against antineoplastic agents but also plays an important role in the bioavailability of common drugs in medical treatment. Various polymorphisms in the MDR1 gene were recently identified. A silent mutation in exon 26 (C3435T) was correlated with intestinal P-glycoprotein expression and oral bioavailability of digoxin. Objective: We wanted to establish easy-to-use and cost-effective genotyping assays for the major known MDR1 single nucleotide polymorphisms and study the allelic frequency distribution of the single nucleotide polymorphisms in a large sample of volunteers. Methods: In this study, the distribution of the major MDR1 alleles was determined in 461 white volunteers with the use of polymerase chain reaction and restriction fragment length polymorphism. Results: Five amino acid exchanges were found with allelic frequencies of 11.2% for Asn21Asp and 5.5% for Ser400Asn. Strikingly, in exon 21 three variants were discovered at the same locus: 2677G (56.4%), 2677T (41.6%), and 2677A (1.9%), coding for 893Ala, Ser, or Thr. A novel missense Gln1107Pro mutation was found in two cases (0.2%). The highest frequencies were observed for intronic and silent polymorphisms; C3435T occurred in 53.9% of the subjects heterozygously, and 28.6% of individuals were homozygous carriers of 3435T/T with functionally restrained P-glycoprotein. Conclusion: This study provides the first analysis of MDR1 variant genotype distribution in a large sample of white subjects. It gives a basis for large-scale clinical investigations on the functional role of MDR1 allelic variants for bioavailability of a substantial number of drugs. (Clin Pharmacol Ther 2001;69:169-74.)


http://www.sciencedirect.com/science/article/B6WCN-4F02D2W-B/2/bdd068908ec347f5d374abd63c24c27

Purpose: Irinotecan, a drug widely used in the treatment of advanced colorectal cancers, is a prodrug requiring activation to 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterase 2 (hCE2). The existence of functional polymorphisms in the gene encoding this enzyme could explain the individual variability in drug efficacy and toxicity. We have explored this possibility in
looking for single nucleotide polymorphisms and their functional consequence. Methods In a series of 115 human deoxyribonucleic acid samples, we have explored the 12 exons of the hCE2 gene, the intron-exon junctions, and the 5'- and 3'-untranslated regions, by denaturing HPLC and sequencing of polymerase chain reaction products. The functionality of the variations identified was studied in 60 human liver samples by measuring hCE2 gene expression by real-time reverse transcriptase-polymerase chain reaction of messenger ribonucleic acid extracts and carboxylesterase activity by use of irinotecan as a substrate. Results We have identified a total of 11 single nucleotide polymorphisms, none of them able to alter the amino acid sequence of the protein. They are distributed in 10 distinct genotypes in addition to the wild type. The most frequent variation (localized in IVS10) has an allele frequency of 0.17 and has been identified at the homozygous state in 1 sample. hCE2 gene expression and carboxylesterase activity in the variants identified were not significantly different from those measured in wild-type samples. Conclusion The hCE2 gene presents several polymorphisms, none of which seems to be involved in significant variations in protein activity and, therefore, in irinotecan activation.


Objective: To investigate the CYP2C19 polymorphism in Tanzanians because this enzyme shows large interindividual differences in activity and metabolizes several drugs of importance in Africa, especially the antimalarial agent chloroguanide (INN, proguanil). Methods: Two hundred fifty-one Tanzanian healthy volunteers were phenotyped with respect to CYP2C19 with use of a single oral dose of mephenytoin (n = 106), a single oral dose of omeprazole (n = 207), or both. Thirty-two were phenotyped with both probe drugs. The urinary 0- to 8-hour S/R-mephenytoin ratio and the plasma omeprazole metabolic ratio (MR) (omeprazole/hydroxyomeprazole) 3 hours after drug intake were determined. The genotype was determined by analysis for CYP2C19*1 (wt), CYP2C19*2 (m1), and CYP2C19*3 (m2). Ten subjects with high omeprazole MR were screened for new mutations in the CYP2C19 gene by searching for single-strand conformation polymorphisms (SSCP). Results: Eight subjects were classified as mephenytoin poor metabolizers (7.5%). Only 5 of these were homozygous for mutated alleles. The S/R ratio was skewed to the right (lower CYP2C19 activity) compared with other ethnic groups studied previously. No new mutations were found with polymerase chain reaction (PCR)-SSCP. We found 30 volunteers (14.5%) with an MR > 7, which is the antimode found previously in white subjects and Asian subjects. Of the 251 volunteers genotyped, 3.2% were homozygous for mutated alleles and 66.1% were homozygous for the wild-type allele. The allele frequencies of CYP2C19*1, *2, and *3 were 81.5%, 17.9%, and 0.6%, respectively. The correlation between the S/R-mephenytoin ratio and the omeprazole MR was significant (Spearman r = 0.59; P Conclusion: Tanzanians have a decreased capacity to metabolize both omeprazole and mephenytoin when their genotype is compared with metabolic capacity and genotype in other previously studied populations. We identified a low frequency of the Asian allele (CYP2C19*3). Although we did not find any new mutations, our results may be consistent with the presence of yet-unidentified mutations of CYP2C19 that causes decreased CYP2C19 activity in the Tanzanian population.

Ieiri, I., T. Kubota, et al. (1996). "Pharmacokinetics of omeprazole (a substrate of CYP2C19) and comparison with two mutant alleles, CYP2C19m1 in exon 5 and CYP2C19m2 in exon 4, in Japanese subjects." Clinical Pharmacology & Therapeutics 59(6): 647.
The pharmacokinetic profile of omeprazole was examined in 27 healthy Japanese volunteers, and the results were analyzed in relation to genotype for the two mutations, CYP2C19m1 in exon 5 and CYP2C19m2 in exon 4, associated with the poor metabolizer phenotype. Of the 27 individuals analyzed, 10 were homozygous for the wild-type (wt) allele in both exon 5 and exon 4 (wt/wt; 37.0%, pattern G1), five were heterozygous for the CYP2C19m1 (wt/m1; 18.5%, G2), five were heterozygous for the CYP2C19m2 (wt/m2; 18.5%, G3), two were heterozygous for the two defects (m1/m2; 7.4%, G4), and five were homozygous for the CYP2C19m1 (m1/m1; 18.5%, G5). The allele frequencies of the m1 and m2 mutation were 0.31 and 0.13, respectively. A correlation between the rate of metabolism of omeprazole and genotype was observed. The mean clearance values of omeprazole in patterns G1, G2, G3, G4, and G5 were 1369.0, 332.7, 359.0, 70.8, and 89.5 ml/hr/kg, respectively. The relative area under the serum concentration-time curve (AUC) ratio of omeprazole to 5-hydroxyomeprazole in patterns G1, G2, G3, G4, and G5 was 1:2.8:3.4:16:17.2. A similar relation was observed in the omeprazole/5-hydroxyomeprazole serum concentration ratio, determined 3 hours after drug intake (1:3:4:18.8:20.3). There were significant (p < 0.05 to 0.01) differences in the disposition kinetics of omeprazole between the subjects with patterns G1, G2, and G3 and the subjects with patterns G4 and G5. The results indicate that the 5-hydroxylation pathway of omeprazole is clearly impaired in subjects with m1/m2 and m1/m1.


http://www.sciencedirect.com/science/article/B6WCN-45SR862-38/2/12eb0943c758280e3cb606718251a7da

MDR1 (P-glycoprotein) is an important factor in the disposition of many drugs, and the involved processes often exhibit considerable interindividual variability that may be genetically determined. Single-strand conformational polymorphism analysis and direct sequencing of exonic MDR1 deoxyribonucleic acid from 37 healthy European American and 23 healthy African American subjects identified 10 single nucleotide polymorphisms (SNPs), including 6 nonsynonymous variants, occurring in various allelic combinations. Population frequencies of the 15 identified alleles varied according to racial background. Two synonymous SNPs (C1236T in exon 12 and C3435T in exon 26) and a nonsynonymous SNP (G2677T, Ala893Ser) in exon 21 were found to be linked (MDR1*2) and occurred in 62% of European Americans and 13% of African Americans. In vitro expression of MDR1 encoding Ala893 (MDR1*1) or a site-directed Ser893 mutation (MDR1*2) indicated enhanced efflux of digoxin by cells expressing the MDR1-Ser893 variant. In vivo functional relevance of this SNP was assessed with the known P-glycoprotein drug substrate fexofenadine as a probe of the transporter's activity. In humans, MDR1*1 and MDR1*2 variants were associated with differences in fexofenadine levels, consistent with the in vitro data, with the area under the plasma level-time curve being almost 40% greater in the *1/*1 genotype compared with the *2/*2 and the *1/*2 heterozygotes having an intermediate value, suggesting enhanced in vivo P-glycoprotein activity among subjects with the MDR1*2 allele. Thus allelic variation in MDR1 is more common than previously recognized and involves multiple SNPs whose allelic frequencies vary between populations, and some of these SNPs are associated with altered P-glycoprotein function. (Clin Pharmacol Ther 2001;70:189-99.)


http://www.sciencedirect.com/science/article/B6WCN-4FBYNB9-C/2/ecb5ae21d76a5fead169831142fa5999
Background: Ritonavir is a potent inhibitor of cytochrome P4503A4 that strongly increases saquinavir bioavailability. In this study we assessed the safety and antiretroviral efficacy of the combination of these two compounds in patients pretreated and receiving continued treatment with zidovudine and lamivudine who were protease inhibitor naive and who had a CD4 cell counts below 200/mm3. Methods: In this 48-week pilot study, all patients received 600 mg ritonavir and 400 mg saquinavir twice daily. Administration of zidovudine and lamivudine was continued without a change in previous doses. Viral load, CD4 cell count, and the emergence of resistance to the two protease inhibitors were evaluated repeatedly up to week 48. Results: Sixteen patients were included in the study. Previous nucleoside analog treatment duration was 48 +/- 22 months (mean +/- SD). Two patients quit taking both protease inhibitors within 2 weeks. The ritonavir dose had to be reduced in 10 other patients because of side effects. Between inclusion and week 48, plasma viremia varied from 4.87 +/- 0.43 to 3.00 +/- 1.29 log10 copies/mL and CD4 cell counts ranged from 98 +/- 61 to 250 +/- 139/mm3. Ten patients (63%) had viral loads below 200 copies/mL and 7 (44%) had viral loads below 50 copies/mL. A single key mutation that conferred ritonavir resistance 184V and V82A/V developed in two patients. A mutation at codon 54 developed in another patient. These mutations were associated with repeated cessations of antiretroviral treatment. No lipodystrophy was observed. Conclusion: Ritonavir and saquinavir in combination are quite well tolerated and induce a high and sustained antiretroviral efficacy. A four-drug combination that includes these two protease inhibitors should be considered as a first line of treatment in patients with low CD4 cell counts.


http://www.sciencedirect.com/science/article/B6WCN-4FCJHTR-N/2/712acd37afa5912a686336e4f5fdace

The frequency of various genotypes of arylamine N-acetyltransferase (NAT2) was investigated in 248 Polish unrelated children. Allele-specific polymerase chain reaction (PCR) was applied for mutation at 341 nucleotide (nt) of NAT2 coding sequence and PCR/restriction fragment length polymorphism for the other mutations. Genotypes coded for slow acetylation in 62.9% (56.6% to 68.9%). The frequency of specific NAT2 alleles was *4 (wild-type), 22.0%; *5A (341C, 481T), 5.2%; *5B (341C, 481T, 803G), 33.1%; *5C (341C, 803G), 6.0%; *6A (282T, 590A), 30.0%; *7B (282T, 857A), 3.4%; and *12A (803G), 0.2%. No mutations were found at 191, 434, and 845 nt. By a molecular-genetic procedure, genotypes *4/*6A were confirmed not to mask *6B/*13 (590A/282T). *6B and *13 were absent in a composite sample representative of 826 alleles (95% confidence limits, 0% to 0.45%). Five cases of genotype-phenotype discrepancy were sequenced and their mutation allocation confirmed; 21 further genotypes were confirmed by sequencing. This first evaluation of NAT2 genes among a Slavic population should provide a basis for clinical and epidemiologic investigations of NAT2 in the Polish population.


http://www.sciencedirect.com/science/article/B6WCN-4FCJHTR-D3/2/fa0bfb000936a627dcd7cf9263cdd583

Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of thiopurine drugs. TPMT activity is regulated by a common genetic polymorphism that is associated with large individual variations in thiopurine toxicity and efficacy. We previously cloned the functional gene for human TPMT and reported a common variant allele for low enzyme activity, TPMT*3A, that contains
point mutations at cDNA nucleotides 460 and 719. In the present study, we set out to determine the number, types, and frequencies of TPMT variant alleles associated with low enzyme activity in clinical laboratory samples in the United States and to compare those results with data obtained from two different ethnic groups. We identified a total of six different variant alleles for low TPMT activity in the 283 clinical laboratory samples studied. The most common variant was *3A; the second most frequent variant allele, *3C, contained only the nucleotide 719 polymorphism; and four other variant alleles were detected. TPMT*3A also appeared to be the most common variant allele in a Norwegian white population sample, but it was not found in a population sample of Korean children. However, *3C was present in samples from the Korean children, as was a novel allele, *6. Characterization of variant alleles for low TPMT enzyme activity will help make it possible to assess the potential clinical utility of deoxyribonucleic acid-based diagnostic tests for determining TPMT genotype.


http://www.sciencedirect.com/science/article/B6WCN-4CNTHGM-H/2/4cef8a27f46ae515ec92f0487733a606

Aim: The flavin-containing monoxygenase 3 (FMO3) has been shown to be genetically polymorphic. In vitro, the enzyme contributes to the \( N \)-oxidation of clozapine, caffeine, and several other drugs. We therefore wanted to analyze population frequencies and allelic linkage of FMO3 mutations and their functional effect on the metabolism of clozapine and caffeine.

Methods: This study included 204 patients treated with clozapine for schizophrenia and 192 healthy volunteers receiving a 100 mg oral test dose of caffeine. FMO3 polymorphisms M66I, P153L, E158K, V257M, E305X, E308G, and R492W were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. Ratios of serum clozapine \( N \)-oxide over clozapine and of urine theobromine versus paraxanthine were used as in vivo indicators of FMO3 activity. Results: From the known FMO3 amino acid variants, only K158 (frequency 0.426), G308 (0.225), and M257 (0.069) were found; mutations I66, L153, X305, and W492 were not found in the 396 subjects. Linkage analysis revealed seven different alleles; the most frequent of these was the wild-type E158-V257-E308 (0.534), followed by K158-V257-G308 (0.199) and K158-V257-E308 (0.192). Subjects with these frequent variants of FMO3, however, did not differ in clozapine \( N \)-oxidation or caffeine oxidation compared with the wild-type.

Conclusion: There are several genetic polymorphisms for the FMO3 enzyme. The effects on the metabolism of caffeine or clozapine could not be shown, indicating that the mutations have only minor functional effects or that substrate affinity is too low to be clinically relevant. (Clin Pharmacol Ther 1999;66:431-8.)


http://www.sciencedirect.com/science/article/B6WCN-4FCJHTR-F8/2/49766d3ecaaddab7a2b545d654851852

Objective: To determine the possible impact of CYP2D6 polymorphism on the pharmacokinetics and pharmacodynamics of selegiline. Methods: Five poor metabolizers and 8 extensive metabolizers of debrisoquin (INN, debrisoquine) were given 10 mg selegiline hydrochloride. The concentrations of selegiline and its main metabolites in serum were determined for 4 days. The pharmacodynamics were quantitated by measuring platelet monoamine oxidase type B activity for 3 weeks. In addition, the effect of selegiline and its main metabolites on the CYP2D6-
catalyzed dextromethorphan O-demethylase activity and the effect of quinidine on the metabolism of selegiline were studied in human liver microsomes. Results: Peak serum concentrations of selegiline were reached rapidly and ranged from 1 to 32 nmol/L. The metabolite concentrations were considerably higher and remained so for a longer period. There were no significant differences in the pharmacokinetic parameters of selegiline, desmethylselegiline, and l-amphetamine between poor metabolizers and extensive metabolizers. However, the area under the serum concentration-time curve (AUC) values of l-methamphetamine were, on average, 46% higher (P = .01) in poor metabolizers than in extensive metabolizers. No significant correlations were found between debrisoquin metabolic ratio and AUC values of selegiline or its metabolites, except for l-methamphetamine (rs = 0.90; P l-methamphetamine toward dextromethorphan O-demethylase was very low (50% inhibitory concentration values from 160 to 580 [μmol/L]. Quinidine (l-methamphetamine from selegiline. Conclusions: CYP2D6 is not important in the primary elimination of selegiline, and the biological effect of selegiline seems to be similar in poor metabolizers and extensive metabolizers of debrisoquin. The inhibitory effect of selegiline and its main metabolites on CYP2D6 activity seems to be negligible.


http://www.sciencedirect.com/science/article/B6WCN-45SR862-36/2/2882c8c6f9a8cbde6f8ea28bee4cbb68

Objective: Genetic polymorphisms were identified in the 5'-flanking region of the human CYP2C9 gene, and their effects on the phenotype were evaluated on the basis of the luciferase reporter gene assay and the in vivo pharmacokinetics of phenytoin. Methods: Genetic polymorphisms were screened by polymerase chain reaction-single-strand conformational polymorphism analysis, following sequencing with DNA samples obtained from 50 healthy volunteers and 133 adult epileptic patients. HepG2 hepatoma cells were cotransfected with various sequence patterns of 5'-flanking region-luciferase reporter gene constructs. Pharmacokinetic parameters of phenytoin in relation to the corresponding sequence patterns were estimated by the Bayesian method, and the results were compared with in vitro activities. Results: Genetic analysis revealed the existence of 7 single nucleotide polymorphisms (SNPs). Allele frequencies of T>C transition at position -1912 (T-1912C), C-1886G, C-1566T, G-1538A, C-1189T, G-982A, and A-162G were 0.019, 0.019, 0.077, 0.019, 0.579, 0.019, and 0.003, respectively. Some mutations occurred simultaneously, and a total of 6 sequence patterns (patterns 1-6) were observed. The luciferase reporter gene assay indicated that the presence of mutation(s) resulted in a reduction in luciferase activity of 41.4% (pattern 2) to 86.8% (pattern 5) compared with the activity of the wild-type construct. The calculated intrinsic clearance of phenytoin was also lower (up to a 40% reduction for pattern 2) when a mutation(s) was present. Conclusion: In addition to the two major mutations in the coding region (CYP2C9*2 and CYP2C9*3), mutations in the 5'-flanking region of the human CYP2C9 gene appear to contribute to the large interindividual variability in drug metabolism activity. (Clin Pharmacol Ther 2001;70:175-82.)


http://www.sciencedirect.com/science/article/B6WCN-4CRXGHG-8/2/af1acce29116a250b18ee88bdc9e6631

Objective: The adenosine triphosphate-binding cassette transporter ABCG2 (breast cancer resistance protein [BCRP]) functions as an efflux transporter for many drugs, including the
topoisomerase I inhibitor diflomotecan, and is expressed at high levels in the intestine and liver. We performed an exploratory analysis to evaluate the effects of the natural allelic variant ABCG2 421C>A on the pharmacokinetics of diflomotecan. Methods The drug was administered to 22 adult white patients with cancer as a 20-minute infusion (dose, 0.10-0.27 mg), followed 2 weeks later by an oral solution (dose, 0.10-0.35 mg). Results The ABCG2 421C>A genotype significantly affected the pharmacokinetics of diflomotecan; in 5 patients heterozygous for this allele, plasma levels after intravenous drug administration were 299% (P =.015) of those in 15 patients with wild-type alleles, at mean values of 138 ng. h/mL. mg-1 (95% confidence interval, 11.3-264 ng. h/mL. mg-1) versus 46.1 ng. h/mL. mg-1 (95% confidence interval, 25.6-66.7 ng. h/mL. mg-1), respectively. Diflomotecan levels were not significantly influenced by 11 known variants in the ABCB1, ABCC2, cytochrome P450 (CYP) 3A4, and CYP3A5 genes. Conclusion These findings provide the first evidence linking variant ABCG2 alleles to altered drug exposure and suggest that interindividual variability in substrate drug effects might be influenced, in part, by ABCG2 genotype.


http://www.sciencedirect.com/science/article/B6WCN-49YDGK4-1P/2/419431acd232077be688af53b6bcde42

Objective: To evaluate the relationship between the metabolic ratio (MR) of metoprolol, CYP2D6*10B genotype, and the disposition of paroxetine in Korean subjects. Methods: A single 40-mg dose of paroxetine was administered orally to one poor metabolizer and 15 healthy subjects recruited from 223 Korean extensive metabolizers whose phenotypes were predetermined by use of the metoprolol MR. Genotypes were determined by allele-specific polymerase chain reaction and the GeneChip microarray technique. Pharmacokinetic parameters were estimated from plasma concentrations of paroxetine for more than 240 hours after the oral dose. Results: The oral clearance and area under the plasma concentration versus time curve (AUC) of paroxetine were best described by a nonlinear relationship with metoprolol MR at correlation coefficients of 0.82 and 0.91, respectively (P <.05). Nine extensive metabolizer who were either homozygous or heterozygous for CYP2D6*10B had significantly lower oral clearance values of paroxetine than six extensive metabolizers with CYP2D6*1/*1. The AUC of paroxetine in subjects who were homozygous for CYP2D6*10B (666.4 +/- 169.4 ng/mL. h) was significantly greater than that of subjects who were homozygous for the wild type (194.5 +/- 55.9 ng/mL. h). Unexpectedly, the average AUC of subjects who were heterozygous for CYP2D6*10B was greater with wide variation (789.8 +/- 816.9 ng/mL. h) than that of subjects who were homozygous CYP2D6*10B/*10B mainly because of two atypical subjects whose metoprolol MR was not associated with the CYP2D6*10B genotype and who showed greater AUC and lower oral clearance than subjects with homozygous CYP2D6*10B. Conclusions: The CYP2D6 activity measured by metoprolol MR was a strong predictor of paroxetine disposition in Korean extensive metabolizers. In general, the extensive metabolizers with the CYP2D6*10B allele seemed to have higher plasma concentrations of paroxetine than extensive metabolizers with the wild-type CYP2D6 genotype. However, quantitative prediction of paroxetine disposition from the CYP2D6*10B genotype alone was not perfect because several Korean extensive metabolizers had metoprolol MRs that were not associated with the genotype. (Clin Pharmacol Ther 2000;67:567-76.)

http://www.sciencedirect.com/science/article/B6VNH-42D2CR1-8/2/00ac8deda34cc6939ca02776d319241d

Natriuretic peptide receptors in the central vasculature of the toad, Bufo marinus, were characterized using autoradiographical, molecular, and physiological techniques. Specific 125I-rat ANP binding sites were present in the carotid and pulmonary arteries, the lateral aorta, the pre- and post-cava, and the jugular vein, and generally occurred in each layer of the blood vessel. The 125I-rat ANP binding was partially displaced by the specific natriuretic peptide receptor C ligand, C-ANF, which indicates the presence of two types of natriuretic peptide receptors in the blood vessels. This was confirmed by a RT-PCR study, which demonstrated that guanylyl cyclase receptor (NPR-GC) and NPR-C mRNAs are expressed in arteries and veins. An in vitro guanylyl cyclase assay showed that frog ANP stimulated the production of cGMP in arterial membrane fractions. Physiological recordings from isolated segments of the carotid and pulmonary arteries and the lateral aorta, which had been pre-constricted with arginine vasotocin, showed that rat ANP, frog ANP and porcine CNP relaxed the vascular smooth muscle with relatively similar potency. Together, the data show that the central vasculature contains two types of natriuretic peptide receptors (NPR-C and NPR-GC) and that the vasculature is a target for ANP and CNP.
available sequences, show that the bovichtids are paraphyletic. Pseudaphritis is the sister group of all the non-bovichtid notothenioids. The same results are found from two independent genetic markers, the nuclear 28S rDNA and the 12S and 16S mitochondrial rDNA. This reliably refutes a previous hypothesis that placed Pseudaphritis as the sister group of all the remaining notothenioids (including Cottoperca and Bovichtus). Bootstrap analyses show that the Notothenioidei are monophyletic (although members of the suborder Trachinoidei have not been surveyed). Subsequent data from hemoglobin composition confirm the present relationships. After discussions between members of the European Science Foundation (ESF) network during its last two meetings, we point out here some fundamental aspects of comparative biology to improve understanding between the physiologist community and phylogeneticists. The most important points are differences in how the concept of homology is used and differences in the consideration of adaptation. When adaptation is evoked or questioned, endless speculations and untestable scenarios are often developed. We strongly advocate the use of phylogenetic trees for testing hypotheses of adaptation (through multiple character mapping). Such a "research program" in comparative biology has the power to improve knowledge because it can potentially lead to new experiments for testing adaptive hypotheses.

**Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology** (18)


http://www.sciencedirect.com/science/article/B6T2R-43CCB4Y-8/2/f4b467e5f59b93266223439c30d00580

Transferrin was isolated from plasma of the ascidian Halocynthia roretzi by ion-exchange chromatography. The molecular weight of the plasma transferrin was determined to be 52K by SDS-polyacrylamide gel electrophoresis and gel filtration. Ascidian plasma transferrin was found to bind one mole of iron ion per mole of protein. The reductive S-pyridylethylated transferrin was subjected to Edman degradation analysis for determination of the N-terminal amino acid sequence, and it was also subjected to proteolytic fragmentation to yield peptide fragments, whose amino acid sequences were determined by Edman degradation analysis. Using the above amino acid sequences, a cDNA clone (1880 base pairs) encoding a protein of 372 amino acids containing a signal peptide of 21 amino acids was isolated from an H. roretzi hepatopancreas cDNA library. The reduced amino acid sequence contains the same sequences of the peptide fragments. A comparison of the amino acid sequence of ascidian transferrin with those of other members of the transferrin family revealed that the ascidian transferrin is composed of only the N-terminal lobe of two-lobed vertebrate transferrins. Thus, a one-lobed transferrin is present in the ascidian H. roretzi.


http://www.sciencedirect.com/science/article/B6T2R-3WK3DWX-V2/e9f40deab41fa0cf1d32538907f4dd39
A cDNA encoding a rainbow trout homologue of mammalian heart fatty acid binding protein (H-FABP) was isolated. The deduced protein sequence is 75% identical to that of rat H-FABP. The structural conservation of H-FABPs and their evolutionary relationship are discussed.


Using degenerative primers, partial cDNAs of a TNF (tumor necrosis factor) receptor and two TNF ligands were obtained by PCR of zebrafish and trout cDNAs, or cDNA libraries. These fragments were then used to screen cDNA libraries of appropriate tissues to obtain clones containing full coding sequences. A zebrafish cDNA was obtained that presumably codes for a 438 amino acid ovarian TNF receptor (OTR) that was identified as a death-domain-containing member of the TNF receptor family. On Northern blots, the OTR cDNA hybridized with a 3.4-kb transcript that is abundant in the zebrafish ovary but lightly detected in all other tissues tested. A zebrafish cDNA presumably coding for a 214 amino acid protein with sequence similarity to mammalian TRAIL (TNF-related apoptosis inducing ligand), was also isolated. In addition, a fragment of the brook trout TRAIL homologue was obtained. Finally, a full-length brook trout cDNA, that presumably codes for a 255 amino acid protein with sequence similarity to mammalian TNF-alpha and lymphotoxin-alpha, was isolated. This study is the first report of a death-domain-containing TNF receptor and the first published report of a TNF ligand in fish.


The full-length growth hormone receptor (GHR) of gilthead sea bream (Sparus aurata) was cloned and sequenced by RT-PCR and rapid amplification of 5'and 3'ends. The open reading frame codes for a mature 609 amino acid protein with a hydrophobic transmembrane region and all the characteristic motifs of GHRs. Sequence analysis revealed a 96 and 76% of amino acid identity with black sea bream (Acanthopagrus schlegeli) and turbot (Scophthalmus maximus) GHRs, respectively, but this amino acid identity decreases up to 52% for goldfish (Carassius auratus) GHR. By means of real-time PCR assays, concurrent changes in the hepatic expression of GHRs and insulin-like growth factor-I (IGF-I) was evidenced. Moreover, their regulation occurred in conjunction with the summer spurt of growth rates and circulating levels of GH and IGF-I. Search of alternative splicing was carried out exhaustively for gilthead sea bream GHR, but Northern blot and 3' RACE failed to demonstrate the occurrence of short alternative messengers. Besides, RT-PCR screening did not reveal deletions or insertions that could lead to alternative reading frames. In agreement with this, cross-linking assays only evidenced two protein bands that match well with the size of glycosylated and non-glycosylated forms of the full-length GHR. If so, it appears that alternative splicing at the 3'end does not occur in gilthead sea bream, although different messengers for truncated or longer GHR variants already exist in turbot and black sea bream, respectively. The physiological relevance of this finding remains unclear, but perhaps it points out large inter-species differences in the heterogeneity of the GHR population.

http://www.sciencedirect.com/science/article/B6T2R-49F9WVK-2/2/69a2bd20bd9edf34e1ebdee08166d01a

Gene transcripts and enzyme activities were quantified for a selection of functionally important aminopeptidases at 2-day intervals throughout the first 72 days of development in the Pacific oyster Crassostrea gigas. Leucine aminopeptidase (LAP) and cathepsin B (CathB) gene transcripts were quantified using fluorogenic ('real time') PCR. LAP and CathB gene transcripts were detected at all time points. The proportion of CathB transcripts remained essentially constant and low throughout development (CtCtCt~23). CathB and cathepsin D (CathD) enzyme activities were measured biochemically. Whilst CathD activity peaked at day 19, LAP and CathB activities both peaked at day 24. The closely coupled increase in transcript and enzyme activity for LAP indicates regulation at the transcriptional level. Alternatively, the peak in enzyme activity for CathB without enhanced transcriptional activity suggests post-transcriptional regulation. Similar mechanisms of regulation for LAP and CathB have been observed in both plants and mammals, indicating widespread conservation.


http://www.sciencedirect.com/science/article/B6T2R-43CCCV5-1N/2/e54d30dade81f9454ccf3c89fc94a1e1

Retinol-binding protein (RBP) is the specific carrier of retinol in vertebrates and forms a 1:1 complex with transthyretin (TTR). A cDNA encoding serum RBP was cloned from liver and 7-day larvae of the marine fish Sparus aurata. The mature protein is 176 amino acids long and shows sequence identity of 77-78%, 56%, 63% and 62% with rainbow trout, Xenopus, chicken and human RBP, respectively. Northern blot analysis of hepatic RBP revealed two transcripts: a major one of approximately 1.4-1.5 kb and a minor of approximately 0.7 kb. Distribution of RBP mRNA in various tissues was studied by RT-PCR and showed high expression in liver and skin, and low expression in brain, kidney and gill filament (20-35% of the level in liver). RBP expression in intestine, pyloric caeca, muscle and pituitary was estimated to be approximately 7-14% of the level in liver. The ontogeny of RBP expression in S. aurata was examined in unfertilized eggs, embryos and larvae by using RT-PCR followed by hybridization with a specific probe. RBP transcript was found in all larval stages studied. Very low levels of RBP mRNA were detected in unfertilized eggs and in embryos 8 h after fertilization with a gradual increase at 12 h and 15-16 h post-fertilization. A single injection of estradiol-17[beta] to S. aurata immature, bisexual fish or to adult males reduced steady-state levels of hepatic RBP by 37 and 25%, respectively. The same treatment induced vitellogenin expression. The present data suggest that in fish, liver is the main site of RBP synthesis, but that RBP may have an important function in fish skin. RBP is expressed early in embryonic development and in fish its expression can be down regulated by estrogen.

The full-length cDNA for the cod (Gadus morhua) StAR was cloned by RT-PCR and library screening using ovarian RNA. From the library screening, two size classes of cDNA were obtained; a 1577 bp cDNA (cStAR1) and a 2851 bp cDNA (cStAR2). The cStAR1 cDNA presumably encodes a protein of 286 amino acids. The cStAR2 cDNA was composed of 6 separated sequences that contained all of the coding regions of cStAR1 when added together, but also contained 5 noncoding regions not observed in cStAR1. Polymerase chain reactions of cod genomic DNA produced products slightly larger than cStAR2. The sequence of these products were the same as cStAR2 but revealed one additional noncoding region (intron). Thus, the fish StAR gene contains the same number of exons (7) and introns (6) as observed in mammals, but is approximately half the size of the mammalian gene. Using Northern analysis and RT-PCR, cStAR1 expression was observed only in testes, ovaries and head kidneys. Polymerase chain reaction products were also observed using cDNA from steroidogenic tissues and primers designed to regions specific for cStAR2, indicating that cStAR2 is expressed in tissues and may account for the presence of larger transcripts observed on Northern blots.
The apparent high degree of homology of a blood protein with a unique dual binding affinity for two distinct hormones, thyroxin (T4) and vitamin D, isolated from a turtle, Trachemys scripta (Family Emydidae) and mammalian vitamin D binding protein (DBP) prompted further interspecific comparison to better understand the structure of functional binding sites. Using polymerase-chain reaction (PCR) with primers derived from the putative nucleotide sequences encoding peptides from the degradation of the T. scripta protein, we cloned the cDNA. The mature turtle protein contains 466 amino acids, about eight residues more than in mammalian DBP. The nucleotide sequence of the coding region showed 63% nucleotide and 73% amino acid homology (53% identity) to mammalian DBP (human, rat, mouse, and rabbit). However, there was no significant homology to mammalian T4-binding globulin (TBG) or transthyretin (TTR). Comparisons with mammals help define further the requirements for the vitamin D and actin binding sites. Northern blots of RNA isolated from turtle tissue probed with the 5' portion of cDNA established expression of the transcript in liver, kidney, and brain (in order of abundance), in contrast to mammal sequences in which expression of DBP is largely confined to the liver.


A -galactose binding lectin (SLL-2) was isolated from Sinularia lochmodes, an octocoral, by a combination of affinity chromatography on acid-treated agarose and FPLC on Superdex 200. SLL-2 agglutinated rabbit and horse erythrocytes while SLL-1, a minor component, reacted only with rabbit erythrocytes. SLL-2 is a glycoprotein with a molecular mass of 122 kDa and is composed of eight identical subunits (15 kDa). The sequence of the amino terminal region of SLL-2 did not show any apparent homology to the sequences of other animal and plant lectins. -Galactose, N-acetyl--galactosamine, lactose, and melibiose were moderate inhibitors to the agglutination of rabbit erythrocytes. In contrast, horse erythrocytes were much more susceptible to agglutination by SLL-2, which was inhibited by sugars and glycoproteins such as -galactose, N-acetyl--galactosamine, lactose, melibiose, and porcine stomach mucin. SLL-2 showed considerable tolerance to heating and kept its activity after heating at 80[deg]C for 60 min. In immuno-histochemical studies using an anti-SLL-2 antiserum and protein A gold conjugate, SLL-2 was found to be present in high amounts in the nematocysts. SLL-2 was also detected on the surface of symbiotic dinoflagellate, Symbiodinium sp. cells irrespective whether they were surrounded with or without host cells. These observations suggest the presence of lectin-mediated interaction between symbiotic dinoflagellates and S. lochmodes.


The success of rainbow trout as an aquaculture species is dependent on the ability to produce fish with large amounts of high-quality lean muscle. It is therefore important to understand not only the best conditions under which to raise the fish but also the molecular control of muscle
growth. Vertebrate muscle growth is initiated by the specification of myogenic precursor cells into myoblasts. The myoblasts proliferate and fuse to form multinucleated myotubes, which mature into myofibers. A family of basic helix-loop-helix (bHLH) transcription factors, the Myogenic Regulatory Factors (MRFs), controls these events. In trout, two MRF-encoding genes, TMyoD (of which there are two) and Tmyogenin, have been identified. However, the primary MRF-encoding Myf5 is not yet sequenced. Here, using degenerate PCR and 5' and 3' RACE, the cDNA sequence of trout Myf5 (TMyf5) is identified. Translation of the cDNA reveals that TMyf5 is a bHLH protein with homology to Myf5 and MRFs in other organisms. It is expressed mainly in red and white muscle, suggesting that it shares functional homology to Myf5 in other species. The molecular control of muscle growth has been well-characterized in mammals, but there are differences in the growth of fish muscle, highlighting the need for characterization of MRFs in fish species, particularly those in which understanding muscle growth will have a positive impact on the economic potential of the species.


channel were detected but not until 1-2 days after the feeding event. It is concluded that the transient increase in Na, K-ATPase activity is not attributable to increases in the abundance of [alpha]- and [beta]-subunit mRNAs but must be associated with some, as yet unknown, post-transcriptional activation mechanism.


http://www.sciencedirect.com/science/article/B6T2R-40X8D55-W/2/e48e74efee413fe726ed820a895a84b

The fish otolith is a hard tissue consisting of calcium carbonate and organic matrices. The matrix proteins play important roles in otolith formation, but little is known about the nature of these proteins. In this study, matrix proteins were extracted from the otoliths of rainbow trout, Oncorhynchus mykiss, and chum salmon, Oncorhynchus keta. EDTA-soluble matrix proteins were separated by SDS-PAGE, revealing two major components in the otoliths of both species with apparent molecular masses of 55 and 43 kDa. N-terminal and some internal amino acid sequences of the 55-kDa otolith matrix protein were determined. A cDNA fragment encoding this protein of O. mykiss was amplified by reverse transcription PCR using two degenerate primers designed from the amino acid sequences. A cDNA encoding this protein was obtained by screening a saccular cDNA library using the amplified cDNA fragment as a probe. Nucleotide sequence analysis revealed that the cDNA clone has a sequence of 2.5 kb and the open reading frame encoding 344 amino acid residues. Northern blot analysis showed that mRNA of this protein is expressed specifically in the saccus, and consistently during the day.


http://www.sciencedirect.com/science/article/B6T2R-3VBB5X0-W/2/80f949892e87f7788956e1f831490183

The molecular weight of the liver-type subunit (L) of bovine ferritin is much larger than that of the heart-type subunit (H) as determined by SDS-PAGE (L, 20.5 kDa; H, 18.4 kDa). The migration of these two subunits on SDS-PAGE gels, relative to each other, is opposite to that reported for ferritin L and H subunits in other mammalian species (L, 19 kDa; H, 21 kDa). To determine the cause of this anomaly, full-length cDNA clones of the bovine L and H chains were isolated from a bovine spleen [lambda] gt11 cDNA library and sequenced. The amino acid sequences of the L and H chains of bovine ferritin, deduced from their cDNA sequences, contained open reading frames coding for 174 and 180 amino acid residues with calculated molecular weights of 19,856 and 20,920 Da, respectively. The deduced amino acid sequence of the L chain shows 96%, 84%, 87%, 83% and 83% homology with the amino acid sequences of horse, human, rabbit, rat and mouse L chains, respectively. The H chain displays a higher homology with the human, rat and mouse H chains (91%, 92% and 93%, respectively). In addition, the bovine L chain did not contain the extra octapeptide present in rodent L chains, and bovine L and H chains did not react with concanavalin A. The bovine L and H chains expressed using a baculovirus expression system showed almost the same mobilities as those of bovine spleen ferritin, respectively, by SDS-PAGE. These results suggest that the much slower mobility of the bovine L chain compared with other mammalian L chains on SDS-PAGE cannot be attributed to insertion(s) of amino acid(s) or peptide(s) into the L chain, to the deletion(s) of them or to the addition of carbohydrate chain(s) but may result from significant differences in the binding affinity of SDS for
bovine ferritin L chains.


http://www.sciencedirect.com/science/article/B6T2R-4B945XP-5/2/4f62be2bb1b204ec6710890f3553012c

Purified cathepsin L from carp, Cyprinus carpio, consists of a 28 kDa single-chain form that is different from the 24 and 5 kDa mammalian two-chain form. We cloned cathepsin L from carp hepatopancreas. The sequence consisted of a 1490 bp cDNA and a 1014 bp open reading frame, encoding a deduced protein of 337 amino acids that is likely processed to an active enzyme (single-chain form) with 222 amino acids. Its similarity to other types of vertebrate cathepsin L is less than 69%. Mammalian cathepsin L is further processed to a two-chain form, but possibly this is not the case with carp cathepsin L: the P1 site where cleavage occurred in the two-chain form of mammalian cathepsin L contains a serine, while carp cathepsin L processes a valine. Therefore, carp cathepsin L may have a different mechanism of action from mammalian cathepsin L.


http://www.sciencedirect.com/science/article/B6T2R-3SG3M4Y-C/2/2b8887aac95a7610c5421163d6d81d21

A bombyxin gene encoding precursor molecule for bombyxin-IV, one of the insulin-related neurosecretory peptide of the silkmoth Bombyx mori, has been cloned and characterized. The nucleotide sequence of this gene and its deduced amino acid sequence deviate moderately from those characterized previously for the family A, B, C and D bombyxin genes. The gene encoding the bombyxin-IV precursor was therefore defined into a novel family E and designated as gene E1. The bombyxin E1 transcript in Bombyx brain was shown to locate in four pairs of medial neurosecretory cells, which also produce other bombyxin family mRNAs, and the amount of the E1 transcript did not change markedly during the fifth larval instar. Genomic Southern hybridization indicated that the Bombyx haploid genome contained a single copy of the bombyxin family E gene.


http://www.sciencedirect.com/science/article/B6T2R-43C5N6W-7/2/119b6f4d4206747868e776d22def314a

This study examined connexin (Cx) gene activity in relation to oocyte maturation in the red seabream (Pagrus major) ovary. Mixed primers for the polymerase chain reaction (PCR) were designed based on the high sequence homology of selected regions of known Cx genes. PCR-
amplified cDNA fragments generated by 3' and 5' rapid amplification of cDNA ends (RACE) were combined to generate full-length cDNA sequences. The 1212-bp cDNA has an open reading frame encoding 282 amino acids, with a molecular mass of 32.3 kDa (red seabream Cx32.3). Hydropathy plots of red seabream Cx32.3 show the four typical major hydrophobic and four major hydrophilic regions of Cx proteins. Typical Cx consensus sequences are observed in the first and second extracellular loops. The ovarian follicles of matured female seabream were incubated in the presence of 17[alpha],20[beta]-dihydroxy-4-pregnen-3-one (DHP, 10 ng/ml), gonadotropin (GtH)-I (300 ng/ml) and GtH-II (300 ng/ml). Northern blot analysis of poly(A)+ RNA extracted from the ovarian follicles were hybridized with red seabream Cx32.3 and [beta]-actin probes. The transcription level of PmCx32.3 in the presence of DHP, PmGtH-I and PmGtH-II was significantly higher than in the control.

Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology (1)


http://www.sciencedirect.com/science/article/B6T2S-41TNDY4-M/2/de2b1b2c98a1101b2ea4d72f106948ae

Several studies have shown that the heart of species from each vertebrate class contains natriuretic peptide binding sites which suggests that ANP released from the heart may act in a paracrine/autocrine fashion. The present study used a set of techniques to study cell surface receptors in order to investigate the presence and nature of NPRs in the heart of the cane toad, Bufo marinus. Autoradiographical studies of both atria and ventricle showed no variation between total and non-specific binding, indicating a lack of NP binding sites in these tissues. This was confirmed with in vitro binding studies in which increasing concentrations of ANP did not compete for any specific binding. Increasing concentrations of ANP did not increase cGMP generation and physiological experiments showed that both ANP and CNP had no effect on the force or rate of contraction of a sino-atrial preparation. Molecular expression studies, however, showed that mRNA for NPRs was expressed in the heart, in spite of the lack of evidence for NPR on the cell surface. Overall, this study showed that no functional NPRs are present in the heart, and provided evidence that the heart is not a target organ for NP action in B. marinus.

Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology (5)


http://www.sciencedirect.com/science/article/B6W89-4DF44HD-
Herein we report Xiphophorus DNA polymerase [beta] (XiphPol[beta]) mRNA and protein expression levels in brain, liver, gill, and testes tissues from Xiphophorus maculatus, Xiphophorus helleri, and Xiphophorus couchianus parental line fish and two different tumor-bearing Xiphophorus interspecies hybrids. Polymerase [beta] protein levels in the Xiphophorus tissues were measured by Western blot, and mRNA was measured with a quantitative real time RT-PCR method which employed cRNA construction to produce accurate calibration curves. We found significant differences in both mRNA and protein levels between the tumor-bearing hybrid animals and the three parental species. However, there were no significant differences in either mRNA levels or protein expression observed between the parental species. Thus, interspecies hybridization results in dysregulation of Pol[beta] expression and this may manifest a modulation in DNA repair capability and susceptibility to latent tumorigenesis.


http://www.sciencedirect.com/science/article/B6W89-4FHJYDV-3/2/81949daa546c8b1c2f675d42f072e88

Xenoestrogens such as 4-nonylphenol (4-NP) have been shown to affect the parr-smolt transformation, but their mechanisms of action are not known. We therefore examined effects of 4-NP and estradiol-17[beta] (E2) on expression of estrogen receptor (ER) [alpha] gene in the liver, gill, pituitary and brain of sockeye salmon to elucidate molecular mechanisms of 4-NP and E2 and developmental differences in response during smolting. Fish were treated twice within a week with 4-NP (15 and 150 mg/kg BW), E2 (2 mg/kg BW) or only vehicle at three stages of smolting, pre-smolting in March, early smolting in April and late smolting in May. The absolute amounts of ER[alpha] mRNA were determined by real-time PCR. The basal amounts of ER[alpha] mRNA peaked in April in the liver, gill and pituitary. In March, E2 extensively increased the amounts in the liver, while 4-NP had no effects at this stage. In contrast, 4-NP (but not E2) decreased liver ER[alpha] mRNA in April. 4-NP also decreased the amount of ER[alpha] mRNA in the gill in April. In the pituitary, 4-NP increased ER[alpha] mRNA in March but decreased it in May. There were no significant effects in the brain. Changes in basal ER[alpha] mRNA observed in this study indicate that estrogen responsiveness of tissues may change during salmon smolting. Furthermore, 4-NP and E2 have different effects on expression of ER[alpha] gene in the liver and gill during smolting, and the response is dependent on smolt stage.


http://www.sciencedirect.com/science/article/B6W89-437B3GV-3/2/0ff593c4ee66675e08951a6a2a49174

Pituitary adenylate cyclase-activating peptide (PACAP), a novel compound with vasoactive intestinal polypeptide-like activity, was recently shown to be localized in the neuronal endings of the human uterus. The purpose of the present study was to assess the functional presence of PACAP mRNA in the decidual endometrium and its relationship to the expression levels of decidual prolactin-related protein (dPRP) and the progesterone receptor mRNAs during
decidualization and pregnancy in Sprague-Dawley rats. PACAP was constitutively and temporally expressed in the decidual endometrium and gravid uterus. The time-dependent correlated expression levels of PACAP, dPRP and the progesterone receptor were induced by the neurogenic reproductive signals, i.e. the vagino-cervical/deciduogenic stimuli of decidualization and by the normal equivalent stimuli of mating/blastoct implantation of gestation. Correlation among the mRNA expression levels of PACAP, dPRP and the progesterone receptor and the coordinated inhibitory actions of the anti-progesterone (RU-486) suggest that there is also correlated time-dependent steroid regulation of the mRNA levels of PACAP, dPRP and the progesterone receptor in the decidual and pregnant uteri. One possible functional meaning for the time-related localization of endometrial/uterine PACAP could be to facilitate endometrial blood flow and increase the availability of metabolic substrates to the developing deciduoma or embryo. The study demonstrates the potential importance of PACAP expression in the regulation of the maternal feto-placental component and suggests a prominent reproductive role for the neuropeptide in mammalian pregnancy.


As the immune system is known to be influenced by the endocrine system, the effects of hypophysectomy on immune functions were examined in the rainbow trout (Oncorhynchus mykiss). Superoxide anion (O2-) production, accompanied by phagocytosis, was significantly decreased in leucocytes isolated from the head kidney 7 days after hypophysectomy. Significant reduction was also observed in plasma immunoglobulin (Ig) M levels, whereas no change was observed in plasma lysozyme activity. The number of Ig-secreting leucocytes in peripheral blood had decreased after hypophysectomy, although total leucocyte number was not affected. The percentage of Ig-producing leucocytes as assessed by flow cytometry using a monoclonal antibody to trout IgM showed significant reduction in the head kidney. However, hypophysectomy did not affect the number of Ig-producing leucocytes in spleen, thymus or peripheral blood. By RT-PCR, expression of two growth hormones (GH I and II) and prolactin (PRL) mRNA was detected in lymphoid tissues, such as head kidney, spleen, thymus and intestine, as well as in leucocytes from blood and head kidney, indicating the local production of these hormones. These results indicate important roles of hypophyseal hormones produced not only in the pituitary, but also in the lymphoid tissues, in the maintenance of the immune functions in trout.


In vivo and in vitro effects of prolactin (PRL) and growth hormone (GH) on plasma levels of lysozyme and ceruloplasmin were examined in the rainbow trout (Oncorhynchus mykiss). Hypophysectomy had no effect on the plasma lysozyme level. Implantation of PRL- or GH-containing cholesterol pellets increased the lysozyme level in a dose-related manner. After hypophysectomy and sham operation, plasma ceruloplasmin was elevated above the level in intact fish, suggesting inflammation caused by the surgery. PRL or GH treatment significantly attenuated the increased level of ceruloplasmin in the operated fish. Expression of lysozyme mRNA was detected in the leucocytes isolated from the peripheral blood by RT-PCR. In vitro
administration of PRL or GH showed no effect on the proliferation of isolated leucocytes or on the total protein content; however, lysozyme activity in the medium increased in a dose-related manner. These results suggest that PRL and GH directly stimulate lysozyme production without affecting the proliferation of leucocytes, and the attenuated ceruloplasmin level increased in response to inflammation.


http://www.sciencedirect.com/science/article/B6T5H-3Y6PFXX-2S/2/e5089bb3dd74d67f490fb1955627b0db

A polymerase chain reaction (PCR) technique was used to assay the presence of the aerolysin gene in a total of 89 Aeromonas hydrophila and A. sobria strains isolated from drinking water, fish and foods. These strains were also characterized for the production of virulence factors such as haemolysin, protease and cytotoxin. The primers used in the PCR targeted a 209-bp fragment of the aer gene coding for the [beta]-haemolysin and detected template DNA only in haemolytic A. hydrophila strains. The cell-free culture supernatants of these aerolysin-positive A. hydrophila strains were also cytotoxic to the HeLa and McCoy cells. The haemolytic A. sobria and non-haemolytic A. hydrophila were consistently negative in the PCR assay. Primer specificity was determined in the PCR by using a control haemolytic Escherichia coli, Streptococcus pyogenes and a restriction endonuclease assay. The PCR clearly identified the aerolysin-producing strains of A. hydrophila and may have application as a rapid species-specific virulence test.


http://www.sciencedirect.com/science/article/B6T5H-469W2K8-4/2/3e0dec198f147d0e00f2156417e3187c


http://www.sciencedirect.com/science/article/B6T5H-45XR89W-1/2/b836789fd99cbcd03f053aba3ffce5b8

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E. coli strains isolated from pigs with postweaning diarrhea or edema disease were tested by phenotypic and genotypic methods for the presence of virulence antigens and genes, respectively. The slide agglutination and ELISA analyses were used for determination of F4, F5, F6, F17, and F41 fimbriae whereas the prevalence of fimbrial fedA and toxin eltI, estI, estII, stx1, stx2 and stx2e genes were recorded by the means of PCR. Only F4 antigen (ac variant) was found in strains of the serogroup O149:K91 isolated from pigs with diarrhea. PCR analyses showed that the fedA gene encoding F18 fimbriae was present in 61.9% of strains isolated from pigs with diarrhea and in 84.2% of strains isolated from pigs with edema disease. The eltI genes encoding heat-labile toxin I (LTI) were present only in 9 out of 21 strains recovered from pigs with diarrhea. Shiga toxin 2 variant (stx2e) genes were found in six isolates from edema disease and also in one strain from diarrhea. The PCR test used in the study was a sensitive and valuable method for determination of virulence factors of E. coli strains.


http://www.sciencedirect.com/science/article/B6T5H-487MTS9-2/2/dfdeccb352d2b2dd63e79c83ff5c24d27

Three goats from a group of five caprine herpesvirus 1 (CpHV.1) seronegative pregnant goats were inoculated intranasally with a virulent BA.1 strain of CpHV.1. Goat n.1 was infected on day 45 of pregnancy, goat n.2 on day 92 and goat n.3 on day 127. Each of the three goats produced a single foetus 10-60 days after infection. Foetus n.1 was never found and so it could not be examined for virological findings. Goat n.2 delivered at term of gestation and CpHV.1 was detected by PCR and isolated from most of the foetal organs. Foetus n.3 was partially autolysed and the virus was only detected by PCR but not isolated from foetal organs. The results confirm the damaging effect of CpHV.1 infection on pregnancy, the difficulty in diagnosing the CpHV.1 induced abortion, and the importance developing appropriate prophylactic programmes.


http://www.sciencedirect.com/science/article/B6T5H-3WK3RT6-1/2/086df03f6f7877d35002768d34d91081


http://www.sciencedirect.com/science/article/B6T5H-3VM10K4-5/2/80a635d5b374e1b8020fd7df3bbbc24d2e

Inhibition of isolation of Listeria monocytogenes by bacteriocin-like substance (BLS)-producing Listeria innocua after enrichment culture was investigated. When 26 L. monocytogenes strains...
were examined in combination with eight L. innocua strains using the spot on lawn method, 52/208 (25.0%) combinations showed the growth inhibition of L. monocytogenes. When two Listeria species were cultured simultaneously in selective enrichment broth, inhibition of isolation of L. monocytogenes was observed in 12/52 of the combinations at 24 h (23.1%), in 24/52 at 48 h (46.2%) and in 30/52 (57.7%) after 7 days of incubation. The randomly amplified polymorphic DNA profiles showed no interstrain similarities between either strains of the BLS-producing L. innocua or the BLS-sensitive L. monocytogenes strains. Therefore inhibition by BLS-producing L. innocua of isolation of L. monocytogenes after enrichment culture is unlikely to be dependent upon a particular genetic profile.

Comptes Rendus Biologies (2)


http://www.sciencedirect.com/science/article/B6X1F-4B5HS1M-D/2/5bd843838df0233a5ed6912d499d9ce6

We describe a high-throughput cDNA sequencing pipeline (http://www.hgsc.bcm.tmc.edu/projects/cdna) built in response to the emerging need for rapid sequencing of large cDNA collections. Using this strategy cDNA inserts are purified and joined through concatenation into large molecules. These 'pseudo-BACs' are subjected to random shotgun sequencing whereby the majority of cDNA inserts in the pool are sequenced. Using this concatenation cDNA sequencing platform, we have contributed more than 13,000 full-length cDNA sequences from human and mouse to the Mammalian Gene Collection (MGC). To cite this article: P.H. Gunaratne et al., C. R. Biologies 326 (2003).


http://www.sciencedirect.com/science/article/B6X1F-49H69XT-5/2/9f4f52a1f3108106c8198e2a8ad3b86

In order to investigate human-louse phylogeny, we partially sequenced two nuclear (18S rRNA
and EF-1[alpha]) and one mitochondrial (COI) genes from 155 Pediculus from different geographical origins. The phylogenetic analysis of 18S rRNA and EF-1[alpha] sequences showed that human lice were classified into lice from Sub-Saharan Africa and lice from other areas. In both clusters, head and body lice were clearly grouped into two separate clusters. Our results indicate that the earliest divergence within human pediculidae occurred between African lice and other lice, and the divergence between head and body lice was not the result from a single event. To cite this article: Z. Yong et al., C. R. Biologies 326 (2003).


http://www.sciencedirect.com/science/article/B6VJ5-402TP37-12/2/b13dc6d1eeb6d58d6e1af72b9b0a800

We describe here a new stop mutation at triosephosphate isomerase (TPI) position 145 in a Hungarian family for which the first mutation (240 Phe -> Leu) was published earlier. The entire genomic TPI locus (exons, introns and promoter) was sequenced and found to be identical in the two compound-heterozygote brothers. Both brothers have the same well-compensated level of non-spherocytic hemolytic anemia and very high levels of the TPI substrate dihydroxyacetonephosphate (DHAP), but only one brother manifests neurologic disorders. Differences in nonsense-mediated mRNA decay may be at the basis of the differences in phenotype expression although it cannot be excluded the interaction with a modifier gene. Based on our earlier results, the development of neurodegeneration may be decisively modulated by the cellular environment of the mutant proteins initiating the process of focal apoptosis of neurons in glycolytic, peroxisomal and prion-induced neurological diseases.

Current Biology 9


http://www.sciencedirect.com/science/article/B6VRT-4D5X1XC-6B/2/185db7f2335073beb64aad0963c0a0f7

Background: Heavy-chain diseases (HCDs) are human lymphoproliferative neoplasias that are characterized by the secretion of truncated immunoglobulin heavy chains devoid of light chains. We have previously proposed -- by analogy to the process by which mutated growth factor receptors can be oncogenic -- that because the genetic defects in HCDs result in the production of abnormal membrane-associated heavy chains lacking an antigen-binding domain, these abnormal B-cell antigen receptors might engage in ligand-independent signalling. Normal pre-B-
cell development requires the presence of the pre-B-cell receptor, formed by the association of [\(\mu\)] heavy chains with two polypeptides -- so-called surrogate light chains, Vpre-B and [\(\lambda\)]5 -- that are homologous to the variable and constant portions of immunoglobulin light chains, respectively. To assess whether amino-terminal truncation of membrane-associated heavy chains results in their constitutive activation, we have examined the ability of a HCD-associated [\(\mu\)] protein to promote pre-B-cell development in transgenic mice.

Results When the [\(\mu\)] HCD transgene is introduced into SCID mice, CD43- pre-B cells develop normally. To determine whether this pre-B-cell development requires surrogate light chains, we backcrossed mice expressing full-length or truncated [\(\mu\)] transgenes with [\(\lambda\)]5-deficient mice. Our results show that the truncated heavy chain, but not the normal chain, is able to promote pre-B-cell development in the absence of [\(\lambda\)]5. We also show that truncated [\(\mu\)] chains spontaneously aggregate at the surface of bone marrow cells.

Conclusion Expression of the truncated [\(\mu\)] heavy chain overrides a tightly controlled step of pre-B-cell development, which strongly suggests that a constitutive signal is delivered by the truncated [\(\mu\)] chain disease protein. The self-aggregation of [\(\mu\)] chain disease proteins might account for this constitutive activation. We conclude that amino-terminal truncation of heavy chains could play a role in the genesis of HCD neoplasia if it occurs at an appropriate stage of B-cell differentiation, namely in a mature B cell.


http://www.sciencedirect.com/science/article/B6VRT-4CB6PVS-6/2/9aacee2345f4b6d676e924c8a3d8878d

Background: Variants of the green fluorescent protein (GFP) with different colors would be very useful for simultaneous comparisons of multiple protein fates, developmental lineages and gene expression levels. The simplest way to shift the emission color of GFP is to substitute histidine or tryptophan for the tyrosine in the chromophore, but such blue-shifted point mutants are only dimly fluorescent. The longest wavelengths previously reported for the excitation and emission peaks of GFP mutants are 488 and 511 nm, respectively.

Results Additional substitutions, mainly in residues 145-163, have improved the brightness of the blue-shifted GFP mutants with histidine and tryptophan in place of tyrosine 66. Separate mutations have pushed the excitation and emission peaks of the most red-shifted mutant to 504 and 514 nm, respectively. At least three different colors of GFP mutants can now be cleanly distinguished from each other under the microscope, using appropriate filter sets. A fusion protein consisting of linked blue- and green-fluorescent proteins exhibits fluorescence resonance energy transfer, which is disrupted by proteolytic cleavage of the linker between the two domains.

Conclusion Our results demonstrate that the production of more and better GFP variants is possible and worthwhile. The production of such variants facilitates multicolor imaging of differential gene expression, protein localization or cell fate. Fusions between mutants of different colors may be useful substrates for the continuous in situ assay of proteases. Demonstration of energy transfer between GFP variants is an important step towards a general method for monitoring the mutual association of fusion proteins.


http://www.sciencedirect.com/science/article/B6VRT-428DKHP-1/2/763173edc9cc475e17a77f185bae97fa

Background: In the leech Helobdella robusta, an annelid worm, the early pattern of cell divisions is stereotyped. The unequal first cleavage yields cells AB and CD, which differ in size,
cytoplasmic inheritance, normal fate, and developmental potential. Results: Here we report a dynamic and transcription-independent pattern of WNT signaling in the two-cell stage of H. robusta. Surprisingly, HRO-WNT-A is first expressed in a stochastic manner, such that either AB or CD secretes the protein in each embryo. This stochastic phase is followed by a deterministic phase during which first AB, then CD expresses HRO-WNT-A. When contact between the cells is reduced or eliminated, both AB and CD express HRO-WNT-A simultaneously. Finally, bathing embryos in anti-HRO-WNT-A antibody during first cleavage reduces the adhesion between cells AB and CD. Conclusions: Our findings show that the stochastic phase of HRO-WNT-A signaling in the two-cell stage of Helobdella is negatively regulated by cell-cell contact and that this early signaling affects cell adhesion without affecting cell fate. We speculate that the primordial function of wnt class genes may have been to regulate cell-cell adhesion and that the nuclear signaling components of the wnt pathway arose later in association with the evolution of diverse cell types.


Background: Two competing hypotheses for the origins of Polynesians are the 'express-train' model, which supposes a recent and rapid expansion of Polynesian ancestors from Asia/Taiwan via coastal and island Melanesia, and the 'entangled-bank' model, which supposes a long history of cultural and genetic interactions among Southeast Asians, Melanesians and Polynesians. Most genetic data, especially analyses of mitochondrial DNA (mtDNA) variation, support the express-train model, as does linguistic and archaeological evidence. Here, we used Y-chromosome polymorphisms to investigate the origins of Polynesians. Results: We analysed eight single nucleotide polymorphisms (SNPs) and seven short tandem repeat (STR) loci on the Y chromosome in 28 Cook Islanders from Polynesia and 583 males from 17 Melanesian, Asian and Australian populations. We found that all Polynesians belong to just three Y-chromosome haplotypes, as defined by unique event polymorphisms. The major Y haplotype in Polynesians (82% frequency) was restricted to Melanesia and eastern Indonesia and most probably arose in Melanesia. Coalescence analysis of associated Y-STR haplotypes showed evidence of a population expansion in Polynesians, beginning about 2,200 years ago. The other two Polynesian Y haplotypes were widespread in Asia but were also found in Melanesia. Conclusions: All Polynesian Y chromosomes can be traced back to Melanesia, although some of these Y-chromosome types originated in Asia. Together with other genetic and cultural evidence, we propose a new model of Polynesian origins that we call the 'slow-boat' model: Polynesian ancestors did originate from Asia/Taiwan but did not move rapidly through Melanesia; rather, they interacted with and mixed extensively with Melanesians, leaving behind their genes and incorporating many Melanesian genes before colonising the Pacific.


The human head louse (Pediculus humanus capitis) and body louse (P. humanus corporis or P. h. humanus) are strict, obligate human ectoparasites that differ mainly in their habitat on the host [1 and 2]: the head louse lives and feeds exclusively on the scalp, whereas the body louse feeds on the body but lives in clothing. This ecological differentiation probably arose when humans adopted frequent use of clothing, an important event in human evolution for which there is no
direct archaeological evidence. We therefore used a molecular clock approach to date the origin of body lice, assuming that this should correspond with the frequent use of clothing. Sequences were obtained from two mtDNA and two nuclear DNA segments from a global sample of 40 head and body lice, and from a chimpanzee louse to use as an outgroup. The results indicate greater diversity in African than non-African lice, suggesting an African origin of human lice. A molecular clock analysis indicates that body lice originated not more than about 72,000 +/- 42,000 years ago; the mtDNA sequences also indicate a demographic expansion of body lice that correlates with the spread of modern humans out of Africa. These results suggest that clothing was a surprisingly recent innovation in human evolution.


http://www.sciencedirect.com/science/article/B6VRT-408JDCB-G/2/327e8acb9bb451588146370e20428cf3

Background: Human epidermis is renewed throughout life from stem cells in the basal layer of the epidermis. Signals from the surrounding keratinocytes influence the differentiation of the stem cells, but the nature of the signals is unknown. In many developing tissues, signalling mediated by the transmembrane protein Delta1 and its receptor Notch1 inhibits differentiation. Here, we investigated the role of Delta-Notch signalling in postnatal human epidermis.

Results: Notch1 expression was found in all living epidermal layers, but Delta1 expression was confined to the basal layer of the epidermis, with highest expression in those regions where stem cells reside. By overexpressing Delta1 or DeltaT, a truncated form of Delta1, in primary human keratinocytes and reconstituting epidermal sheets containing mixtures of Delta-overexpressing cells and wild-type cells, we found that cells expressing high levels of Delta1 or DeltaT failed to respond to Delta signals from their neighbours. In contrast, wild-type keratinocytes that were in contact with neighbouring cells expressing Delta1 were stimulated to leave the stem-cell compartment and initiate terminal differentiation after a few rounds of division. Delta1 promoted keratinocyte cohesiveness, whereas DeltaT did not.

Conclusions: We propose that high Delta1 expression by epidermal stem cells has three effects: a protective effect on stem cells by blocking Notch signalling; enhanced cohesiveness of stem-cell clusters, which may discourage intermingling with neighbouring cells; and signalling to cells at the edges of the clusters to differentiate. Notch signalling in epidermal stem cells thus differs from other progenitor cell populations in promoting, rather than suppressing, differentiation.


http://www.sciencedirect.com/science/article/B6VRT-4DFJW0T-8/2/2e6814f225ab1e4f2db09d62e9310da

Background: Many site-specific recombinases act by forming and resolving branched Holliday junction intermediates. Previous findings have been consistent with models involving branch migration across the 'overlap region' of obligate homology, located between the staggered sites where the two single-strand exchanges occur. We have investigated the validity of such models in the case of bacteriophage [lambda] site-specific recombination.

Results By using synthetic [lambda] att-site Holliday junctions, incorporating sequence heterologies that impose constraints on branch migration, we have found that the optimal position of the junction for either top-strand or bottom-strand resolution by [lambda] integrase (Int) is not at the ends, but close to the middle of the seven base-pair overlap region. A minor shift of the branch point around the central base pair caused a remarkable switch in resolution bias. Our findings suggest that branch migration is
limited to the central one to three base pairs of the overlap region. They lead to a new model for [lambda] site-specific recombination, in which there are two symmetrical swaps of two to three nucleotides each, linked by a central isomerization step that causes a change of the stacking interactions between the four junction arms. On the basis of isolated strand-joining reactions carried out by Int in the presence or absence of base complementarity, we propose that sequence homology is sensed during the annealing step prior to strand joining. The new model eliminates mechanistic complications associated with large helical rotations required by branch-migration models.

Conclusion

The results reported here suggest that the recognition of sequence homology in Int-dependent site-specific recombination does not rely primarily on branch migration. The property of cleaving Holliday junctions a few base pairs away from the crossover puts [lambda] Int into the same category as endonucleases that cleave Holliday junctions in homologous recombination.


http://www.sciencedirect.com/science/article/B6VRT-4909176-Y/2/0046673165b0f3b4820948641bac5830

The determination of nuclear DNA sequences from ancient remains would open many novel opportunities such as the resolution of phylogenies, the sexing of hominid and animal remains, and the characterization of genes involved in phenotypic traits. However, to date, single-copy nuclear DNA sequences from fossils have been determined only from bones and teeth of woolly mammoths preserved in the permafrost [1]. Since the best preserved ancient nucleic acids tend to stem from cold environments [2 and 3], this has led to the assumption that nuclear DNA would be retrievable only from frozen remains. We have previously shown that Pleistocene coprolites stemming from the extinct Shasta sloth (Nothrotheriops shastensis, Megatheriidae) contain mitochondrial (mt) DNA from the animal that produced them as well as chloroplast (cp) DNA from the ingested plants [4]. Recent attempts to resolve the phylogeny of two families of extinct sloths by using strictly mitochondrial DNA has been inconclusive [5]. We have prepared DNA extracts from a ground sloth coprolite from Gypsum Cave, Nevada, and quantitated the number of mtDNA copies for three different fragment lengths by using real-time PCR. We amplified one multicopy and three single-copy nuclear gene fragments and used the concatenated sequence to resolve the phylogeny. These results show that ancient single-copy nuclear DNA can be recovered from warm, arid climates. Thus, nuclear DNA preservation is not restricted to cold climates.


http://www.sciencedirect.com/science/article/B6VRT-4DG5TDG-T/2/7a5488015b4f7ae56ea164a705b6abd44

Recent inquiries have revealed a surprisingly large number (>2500) of naturally occurring antisense transcripts [1, 2, 3 and 4], but their function remains largely undiscovered. A better understanding of antisense mechanisms is clearly needed because of their potentially diverse roles in gene regulation and disease [5, 6, 7 and 8]. A well-documented case occurs in X inactivation, the mechanism by which X-linked gene expression is equalized between XX females and XY males [9]. The antisense gene Tsix [6] determines X chromosome choice and represses the noncoding silencer, Xist [10, 11 and 12]. In principle, Tsix action may involve RNA, the act of transcription, or local chromatin. Here, we create novel Tsix alleles to distinguish transcription-versus RNA-based mechanisms. When Tsix transcription is terminated before Xist (TsixTRAP), Tsix cannot block Xist upregulation, suggesting the importance of overlapping antisense
transcription. To separate the act of transcription from RNA, we knocked in Tsix cDNA in the reverse orientation (TsixcDNA) to restore RNA levels in cis without concurrent transcription across Xist. However, TsixcDNA cannot complement TsixTRAP. Surprisingly, both mutations disrupt choice, indicating that this epigenetic step requires transcription. We conclude that the processed antisense RNA does not act alone and that Tsix function specifically requires antiparallel transcription through Xist. A mechanism of transcription-based feedback regulation is proposed.

Current Opinion in Biotechnology  (2)


http://www.sciencedirect.com/science/article/B6VRV-45478C2-8S/2/bb9cb140b6add63b96d22780cfa3cd5

The development of 5’ nuclease assays represents a significant advance in nucleic acid quantitation. This approach utilizes the 5’-3’ exonuclease activity of Thermus aquaticus (Taq) polymerase to cleave a dual-labelled probe annealed to a target sequence during amplification. The release of a fluorogenic tag from the 5’ end of the probe is proportional to the target sequence concentration (copy number), and can be measured either at endpoint (post-amplification), or in ‘real time’, where the increase in emission intensity is followed on a per-cycle basis.


http://www.sciencedirect.com/science/article/B6VRV-45478C2-8R/2/d7858accd0abb73dc5f7cef7fa3111a

After a decade of intensive use as an in vitro alternative to cloning DNA, PCR is now well established as the default method for DNA and RNA analysis. Recent developments have consolidated this position by the introduction of more robust formats, improvements in thermal cyclers and labelling and detection methods. The trend is towards increasing automation, although comparatively few diagnostic kits based on PCR are in wide use. At the same time the applications of PCR are being extended with modifications such as long, accurate PCR and arrayed oligonucleotides or expressed sequences.

Current Opinion in Immunology  (1)

Liang, P. and A. B. Pardee (1995). "Recent advances in differential display." *Current Opinion in
Differential display and RNA arbitrary primed polymerase chain reaction are methods recently designed to identify and isolate differentially expressed genes. Methodological modifications have since been introduced to streamline the techniques. The major efforts have centered on how to eliminate false positives as approached from a variety of angles, ranging from RNA sample preparation, northern blot confirmation, primer length variation, to better experimental design.

**Current Therapeutic Research (1)**


Background: We previously reported that the carotid artery intima-media thickness (IMT) increased with age and that patients with type 2 diabetes mellitus (DM) had a significantly larger IMT than did age-matched nondiabetic subjects with normal glucose tolerance. Although the exact mechanism behind the increase in IMT in diabetic patients has not been determined, data obtained from in vivo and in vitro studies suggest that hyperglycemia-induced oxidative stress may lead to atherogenesis. Objective: The aim of this single-center study was to determine whether long-term oxidative stress and the carotid IMT are influenced by differences of the angiotensin-converting enzyme insertion/deletion (ACE I/D) and NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) oxidase p22phox C242T genotypes. Methods: Eligible subjects were Japanese patients with type 2 DM. Polymorphism of the ACE I/D and p22phox gene was investigated using polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism, respectively. The rate of an acquired mutation of mitochondrial DNA—that is, A-to-G substitution at position 3243 (mtDNA A3243G)—was determined by real-time PCR. As a marker of early atherosclerosis, the carotid artery IMT was measured using high-resolution B-mode ultrasonography. Results: A total of 262 Japanese patients (173 men, 89 women; mean [SEM] age, 58 [0.6] years [range, 18-80 years]) were recruited and enrolled for study. An ACE D-positive (DD or DI) and p22phox 242T-negative genotype (CC) was associated with a significantly higher mtDNA A3243G mutation rate than the other 3 possible genotypes (0.0219% [0.0028%] vs 0.0097% [0.0012%]; P < 0.05). Conclusion: In this study, the ACE D-positive and p22phox 242T-negative genotype showed higher rates of somatic mtDNA mutation (mtDNA A3243G) and higher carotid mean and maximum IMT levels.

**Cytokine (13)**

http://www.sciencedirect.com/science/article/B6WDF-4D10K4Y-1/2/70addbf5e498510ccce88ca22fd5b334

We have characterized the expression of six cytokine mRNAs in highly purified B cells from bovine leukemia virus (BLV)-infected cows with persistent lymphocytosis. Selected cytokine mRNAs included those encoding tumor necrosis factor (TNF), lymphotixin-\([\alpha]\) (LT-\([\alpha]\)), transforming growth factor-\([\beta]\)1 (TGF-\([\beta]\)1), interleukin-1\([\beta]\) (IL-1\([\beta]\)), interleukin-6 (IL-6) and interleukin-10 (IL-10). Fresh B cells from cows with persistent lymphocytosis constitutively transcribed TNF, LT-\([\alpha]\) and TGF-\([\beta]\)1 mRNAs. Although IL-1\([\beta]\), IL-6 and IL-10 mRNAs were barely detectable in fresh B cells from cows with persistent lymphocytosis, transcripts encoding these cytokines were strongly and rapidly upregulated in B cells after cell culture. Results from this study provide the first evidence that B cells infected with BLV express specific cytokine mRNAs in vivo.


http://www.sciencedirect.com/science/article/B6WDF-4CXTVR8-2/2/f4501a989f087038749c33d6a031de1

In clinical practice, diagnosis and risk prediction are usually based on the analysis of serum or plasma proteins whereas gene expression analysis is not used on a routine basis. In order to compare the diagnostic and predictive relevance of serum protein and peripheral blood mRNA levels, we determined cytokine levels of end-stage renal failure patients undergoing hemodialysis. These patients face a high mortality mainly due to acceleration of atherosclerosis and subsequent severe vascular events. mRNA expression of the pro-inflammatory cytokine TNF\([\alpha]\) was significantly elevated in hemodialysis patients and further increased after 2 h of dialysis treatment. In contrast, gene expression of the anti-inflammatory cytokine TGF\([\beta]\) was significantly decreased. Patients who died during the observation period of 36 months had significantly increased mRNA levels of TNF\([\alpha]\) and decreased TGF\([\beta]\) mRNA expression at baseline. Survival analysis indicated that increased TNF\([\alpha]\) mRNA levels (PP<0.001) predict mortality. The corresponding cytokines in serum showed some association with disease, but serum concentrations neither changed during hemodialysis nor predicted mortality. This study shows that gene expression patterns of circulating leukocytes may present an important new diagnostic tool to predict clinical outcome in patients with inflammatory vascular diseases.


Sequence data for type I interferons (IFNs) have previously only been available for birds and eutherian ('placental') mammals, but not for the other two groups of extant mammals, the marsupials and monotremes. This has left a large gap in our knowledge of the evolutionary and functional relationships of what is a complex gene family in eutherians. In this study, a PCR-based survey of type I IFN genes from a marsupial, the tammar wallaby (Macropus eugenii), and
a monotreme, the short-beaked echidna (Tachyglossus aculeatus), was conducted. Along with Southern blot and phylogenetic analysis, this revealed a large number of type I IFN genes for the wallaby, rivalling that of eutherians, but relatively few type I IFN genes in the echidna. The wallaby genes include both IFNA and IFNB orthologues, indicating that the gene duplication leading to these subtypes occurred prior to the divergence of marsupials and eutherians some 130 million years ago. Results from this study support the idea that the expansion of type I IFN gene complexity in mammals coincides with a concomitant expansion in the functionality of these molecules. For example, this expansion in complexity may have, at least partially, facilitated the evolution of viviparity in marsupials and eutherians. Other evolutionary aspects of these sequences are also discussed.


http://www.sciencedirect.com/science/article/B6WDF-4C6J6YT-S4/2/a08bca4d3a8da566f10bfce1bdde4964

Recombinant tumor necrosis factor alpha (TNF-[alpha]) administration significantly delayed the development of lupus nephritis in the New Zealand black x New Zealand white (NZB x NZW)F1 and to a lesser extent in the MRL-lpr/lpr model systems. TNF-[alpha] treatment was effective when treatment was initiated at 2, 3, or 4 months of age but was ineffective if initiated as late as 6.5 months of age. Treatment of (NZB x NZW)F1 mice for 3 months was more effective than treatment continued for 6 months. Anti-TNF-[alpha] antibodies did not develop in these mice. Flow microfluorometry analysis showed no major effects on B, T, or monocyte cell population in cells from the peritoneum, spleen, lymph node, and thymus. A decrease in class II la expression on macrophages in the peritoneum of TNF-[alpha]-treated mice was noticed. A correlation between the level of TNF-[alpha] inducibility in vitro and the effect of TNF-[alpha] administration in vivo could be shown. Although a limited polymorphism could be shown by restriction fragment length polymorphism, using an amplified (AC)n microsatellite located in the 5' regulatory region of TNF-[alpha], a much more extensive interallelic polymorphism was found. The AC microsatellite allele found in NZW mice was unique and different from other lupus strains and nonautoimmune strains. These results have possible implications to the pathogenesis of systemic lupus erythematosus.


http://www.sciencedirect.com/science/article/B6WDF-4CXTVR8-4/2/73902d8513acd22224ac6e5fb5d766a2

Single nucleotide polymorphisms (SNPs), particularly those within regulatory regions of genes that code for cytokines often impact expression levels and can be disease modifiers. Investigating associations between cytokine genotype and disease outcome provides valuable insight into disease etiology and potential therapeutic intervention. Traditionally, genotyping for cytokine SNPs has been conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a low throughput technique not amenable for use in large-scale cytokine SNP association studies. Recently, Taqman(R) real-time PCR chemistry has been adapted for use in allelic discrimination assays. The present study validated the accuracy and utility of real-time PCR technology for a number of commonly studied cytokine polymorphisms known to influence chronic inflammatory diseases. We show that this technique is amenable to high-throughput genotyping and overcomes many of the problematic features associated with
PCR-RFLP including post-PCR manipulation, non-standardized assay conditions, manual allelic identification and false allelic identification due to incomplete enzyme digestion. The real-time PCR assays are highly accurate with an error rate in the present study of <1% and concordance rate with PCR-RFLP genotyping of 99.4%. The public databases of cytokine polymorphisms and validated genotyping assays highlighted in the present study will greatly benefit this important field of research.

Knerr, K., R. Futh, et al. "Chronic inflammation and hemodialysis reduce immune competence of peripheral blood leukocytes in end-stage renal failure patients." Cytokine In Press, Corrected Proof http://www.sciencedirect.com/science/article/B6WDF-4FH0W7K-4/2/765a75a9776ed2a5e44cc9a7d6a568ea

Immunoincompetence is a profound problem in end-stage renal failure patients undergoing hemodialysis, and chronic inflammation with altered serum levels of inflammation markers has been reported. Gene expression patterns have had little relevance for leukocyte research so far because of limitations in transcript levels and stability. Using a new stimulation system we induced the expression of immune-relevant transcripts in whole blood preparations ex vivo and stabilized transcript levels by preventing RNA degradation and uncontrolled gene induction. Using quantitative real-time PCR we could show that basal TGF-β mRNA expression is about 2-fold decreased in end-stage renal failure patients, while expression of TNF-α becomes 2-fold increased, further doubling during hemodialysis. By short term stimulation with phytohemagglutinin (PHA) for 2 h we tested for the immune competence of peripheral blood leukocytes and demonstrated that hemodialysis decreases TNF-α-mediated immune responsiveness more than 3-fold. This study shows by induction and stabilization of immune-relevant transcripts that chronic inflammation and hemodialysis are crucial factors for disturbed immune competence of end-stage renal failure patients.


http://www.sciencedirect.com/science/article/B6WDF-4B427DW-3/2/0fb2d0cfa819dc84d282355a563b383

Background/AimsIn hepatitis C virus infection an inappropriate ratio of pro-inflammatory and anti-inflammatory cytokines may either determine different outcomes of the infection or affect the benefit of antiviral treatment. Given that polymorphisms in regulatory regions of cytokine genes influence cytokine production, we determined frequency of polymorphisms of IL-10, IFN[gamma], and TNF[alpha] genes in HCV-infected patients and healthy controls, and investigated their association with either ongoing or cleared HCV infection, or with response to treatment.MethodsGenomic DNA from 270 patients and 145 controls sharing the same ethnic background was studied by polymerase chain reaction, restriction enzyme digestion, direct sequencing, and microsatellite analysis. ResultsThe IL-10 ATA haplotype was more frequent in patients with spontaneous HCV RNA clearance (36.0%) than in patients with persistent infection (23%) (p=0.009, p CORRECTED = 0.036). Neither TNF -308 and -238 polymorphisms nor IFN[gamma] alleles variability were associated with different HCV outcome. However, the combination of ATA homozygous state and IFN[gamma] 119 allele was more frequent in patients with spontaneous HCV clearance than in patients with ongoing disease (p=0.012; p CORRECTED = 0.048). We could not confirm the reported effect of genetic influence on the response to treatment.ConclusionsOur findings indicate that heterogeneity in the promoter region of the IL-10 gene has a role in determining a spontaneous favourable outcome of HCV infection.

http://www.sciencedirect.com/science/article/B6WDF-4CF5GNX-89/2/6ace5033dad5d248d9414156f798fc05

Molecular cloning of canine interleukin-8 (IL-8) was performed to establish a basis for its investigation in the canine immune system. From a cDNA pool constructed from LPS-stimulated popliteal lymph node cells, canine IL-8 cDNA covering the whole coding region was amplified by polymerase chain reaction. The nucleotide sequence of a canine IL-8 clone, designated pcIL-8#38, was highly similar to those of human, rabbit and porcine IL-8, and comprised 353 bp with an open reading frame that encoded 101 amino acids. Analysis of the deduced amino acid sequence of insert DNA in pcIL-8#38 showed 76.5, 80.2, and 87.0% similarities with human, rabbit and porcine IL-8 proteins, respectively. Insert DNA of pcIL-8#38 was transferred to a mammalian expression vector, pcDL-SR[alpha]296, and transfected into Cos7 cells. The supernatant of the transfectant had neutrophil chemotactic activity when it was examined by the neutrophil migration assay, suggesting that our cloned cDNA was biologically active. The cloned canine IL-8 cDNA will be useful for canine inflammatory disease and comparative immunology research.


http://www.sciencedirect.com/science/article/B6WDF-4CP68M7-4/2/8607489d33c1768c0b8ad812e40474d8

Aotus spp. monkeys are considered the ideal model for studying the progress of malarial infection and the immune response it elicits. We describe the use of a recently developed technique, real-time quantitative RT-PCR, to quantify several Aotus monkey cytokine mRNAs involved in Th1/Th2 responses (IL-4, IL-10, TNF-[beta] and IFN-[gamma]). Specific primers were designed for each cytokine and standard curves were constructed using serial dilutions of pDNA containing each target sequence. Results were normalized to GAPDH housekeeping gene expression levels. Standard curves showed high correlation coefficients and were linear over a wide range of copy numbers. Quantification of Aotus samples showed little intra- and inter-experiment variation, thus, the technique has proven to be highly reproducible and sensitive allowing us to detect as little as 25 copies/[mu]l of target DNA. This technique will allow studying Th1 and Th2 cytokine patterns elicited in response to infection for prospectively evaluating the efficacy of malarial vaccines.


http://www.sciencedirect.com/science/article/B6WDF-4CF5G7F-4G/2/ff10a5e47ecf632fee69eb65392500d0

Kit ligand, or stem cell factor, is a recently identified growth factor, which binds to and activates the c-kit proto-oncogene, and which has been shown to act synergistically with other haematopoietic growth factors in the bone marrow. We have previously shown that several isoforms of kit ligand, which arise due to alternative splicing, are expressed in human placenta. In order to elucidate the role of c-kit and its ligand during human placental development we have investigated the expression of c-kit and kit ligand in human first trimester and term placenta as
well as in pregnant and non-pregnant endometrium, by immunocytochemistry and flow cytometric analysis. In non-pregnant endometrium no expression of kit ligand was seen. By contrast, in first trimester decidua, kit ligand was strongly expressed by the arterial media of maternal blood vessels. Kit ligand was also expressed throughout pregnancy by invasive fetal extravillous trophoblast, and by fetal fibroblasts within the placental villi. c-kit was found to be expressed on Hofbauer cells within the chorionic villi, and by decidual macrophages at all stages in pregnancy. c-kit was also detected on the small CD56dim subset of uterine large granular lymphocytes which form the major leukocyte population in human first trimester decidua. Our results suggest that kit ligand may be involved in the regulation of fetal macrophages, and in particular in signalling between invading extravillous trophoblast which expresses kit ligand, and maternal leukocytes bearing the c-kit receptor.


http://www.sciencedirect.com/science/article/B6WDF-49M0KT1-2/2/31a33f1a1a5d527995b053d8164f6ed5

The goal of this research was to determine whether differential pulmonary IL-12 gene expression controls susceptibility to Sendai virus-induced chronic airway inflammation and fibrosis in inbred rat strains. Sendai virus-resistant F344 rats and susceptible BN rats were studied from 1 to 14 days following virus inoculation. F344 rats had 3.4-fold higher IL-12 mRNA levels detected by real-time PCR in lung than BN rats as early as two days following inoculation. This increase in mRNA was associated at two days with increased total IL-12 protein and with a 2-fold increase in numbers of bronchiolar, OX-6-positive dendritic cells and an increased number of IL-12 p40-positive, bronchiolar macrophages and dendritic cells (pp<0.05). The results demonstrate that there is differential pulmonary IL-12 gene expression between virus-susceptible and resistant rat strains and that IL-12 treatment can provide significant protection from virus-induced chronic airway inflammation and remodeling during early life.


http://www.sciencedirect.com/science/article/B6WDF-49505VD-3/2/4f7d2f594558a577f1c340fbe5da7a56

The chemokine, mob-1, is involved in inflammatory and immune responses and may be an important mediator of the inflammatory response in the liver. Here, we investigated the upstream signal pathways that could be involved in the regulation of mob-1 expression. We have found that in primary rat hepatocytes the isolation and subsequent culture of these cells induced mob-1 expression. A similar induction of mob-1 mRNA was observed when the hepatocytes were stimulated with interferon-[gamma] (IFN-[gamma]). When hepatocytes were stimulated with IFN-[gamma] or cytokine mixture (IFN-[gamma], interleukin-1[beta] and tumour necrosis factor-[alpha]), c-Jun N-terminal kinase (JNK), p38 and extracellular-regulated kinase (ERK) were phosphorylated, suggesting an involvement of the mitogen-activated protein kinases (MAPK) in the induction of mob-1 expression. The p38 kinase inhibitor, SB 203580, and the NF-[kappa]B inhibitor, MG-132, inhibited the induction of mob-1 mRNA and the effects were not additive. These results demonstrate that in primary rat hepatocytes the transient induction of mob-1 expression was regulated by p38 kinase and NF-[kappa]B through a common regulatory pathway.

http://www.sciencedirect.com/science/article/B6WDF-4F02GVT-3/2/2df4efaa4b5d97c92c740397f5b92444

The aim of this study was to evaluate whether there was any correlation between Helicobacter pylori-associated diseases and (1) H. pylori virulence genes or (2) IL-1B, IL-1RN, IFN-G, TNF-A, IL-10 genetic polymorphisms. Patients with non-cardia gastric cancer (NCGC, n = 129) or benign gastroduodenal diseases (n = 792) were studied. IL-1RN intron 2 VNTR polymorphism (PCR), IL-1B -31 C/T (RFLP), the SNPs of IFN-G (+874 A/T), TNF-A (-1031 C/T, -857 C/T, -376 A/G, -308 A/G, -238 A/G), IL-10 (-1082 A/G, -819 C/T, -592 A/C) (Taqman chemistry) were studied. cagA, s1 and m1 vacA, were PCR amplified. Duodenal ulcer was more frequent in TNF-A -857 TT and in IL-1RN 1,2 subjects. TNF-A -857 TT genotype was also correlated with gastric ulcer. IL-10 -819 TT genotype was associated with intestinal metaplasia and NCGC. Antral inflammation was associated with TNF-A -1031 TT, while corpus activity with IL-10 -819 CC. H. pylori infection was associated with TNF-A -308 AG genotype, while IFN-G +874 AA genotype was associated with cagA. In conclusion, among host genetic factors contributing to H. pylori disease outcome, IFN-G +874 AA genotype favors cagA positive infections, TNF-A -857 TT duodenal ulcer while IL-10 -819 TT intestinal metaplasia and NCGC.

*Developmental & Comparative Immunology* **(17)**


http://www.sciencedirect.com/science/article/B6T5X-4899NJJ-1/2/97c5da167287f8ebe4c8f759b54bc86

We have cloned and characterized a cDNA encoding Cg-tal in the Pacific oyster Crassostrea gigas. The isolated cDNA encodes a 219 amino acids protein that contains the basic helix-loop-helix (bHLH) domain homologous to that of vertebrate and invertebrate Tal1/SCL. Phylogenetic analyses sustained that Cg-Tal belongs to this family of bHLH transcription factors. Northern blot analysis of Cg-tal mRNA expression in adult oyster tissues indicated that Cg-tal was specifically expressed in hemocytes, in a constitutive manner. In vertebrates, activation of Tal1/SCL expression is essential for the initiation of hematopoiesis and the formation of hematopoietic stem cells. Considering Tal1/SCL function in vertebrates, Cg-Tal is likely to constitute a promising tool for studying hematopoiesis in oyster.


http://www.sciencedirect.com/science/article/B6T5X-449T5DD-
That the plasma concentration of certain divalent cations change during an inflammatory insult provides a major host defense response in vertebrate animals. This study was designed to investigate the involvement of iron sequestration in invertebrate immune responses. A ferritin molecule was cloned from an echinoderm coelomocyte cDNA library. The amino acid sequence showed sequence homology with vertebrate ferritin. The cDNA contained a conserved iron responsive element sequence. Studies showed that stimulated coelomocytes released iron into in vitro culture supernatants. The amount of iron in the supernatants decreased over time when the amebocytes were stimulated with LPS or PMA. Coelomocytes increased expression of ferritin mRNA after stimulation. In vertebrates, cytokines can cause changes in iron levels in macrophages. Similarly, echinoderm macrokines produced decreases in iron levels in coelomocyte supernatant fluids. These results suggest that echinoderm ferritin is an acute phase protein and suggest that sequestration of iron is an ancient host defense response in animals.


Alignment of the predicted polypeptide sequences shows a conserved hydrophobic signal peptide of 22 amino acids followed by 25 amino acids that are identical (WF2) or homologous to the amino acid sequence of pleurocidin, followed by a conserved acidic portion. Southern hybridisation analysis indicates that related peptides are encoded in the genomes of other flatfish species. Northern and RT-PCR analyses of RNA from multiple tissues show that two of the pleurocidin genes are expressed predominantly in the skin whereas two other genes are expressed mainly in the intestine. RT-PCR assays of total RNA from larvae of different ages provide the first evidence of developmental expression of antimicrobial peptides in fish and indicate that the pleurocidin gene is first expressed at 13 days post-hatch in winter flounder.


http://www.sciencedirect.com/science/article/B6T5X-46SNYY5-1/2/2bfc66c364686b4d4daf598b7cd27171

In the course of suppressive subtractive hybridisation between sodium alginate-induced peritoneal cells (SA-PC) and normal head kidney cDNAs in common carp (Cyprinus carpio), a cytokine-like cDNA clone was found. The clone, named M17, contains a 1600 bp nucleotide sequence that encodes a 215 amino acid putative protein that would have a pl of 9.01 and would include a 33 amino acid signal peptide. The 3' untranslated region has seven ATTTA mRNA destabilising motifs that are common in cytokines and oncogenes. In a BLASTP search, M17 was most similar to chicken ciliary neurotrophic factor (CNTF) with 25% amino acid identity, followed by mammalian CNTF, cardiotoxin-1 and leukemia inhibitory factor (LIF) all of which belong to the IL-6 subfamily. However, M17 has some differences with CNTF in that CNTF has no signal sequence, the gene organisation of M17 is three exons and two introns, whereas that of CNTF is two exons and one intron, M17 has seven cysteines while CNTF has one cysteine, and M17 mRNA is detected in peripheral blood leukocytes as well as brain, whereas CNTF is expressed only in the nervous system. Compared to other members in the IL-6 subfamily cytokines, M17's cysteine positions and gene organisation are similar to those of oncostatin M and LIF, although amino acid identities are only 15-17%. Southern hybridisation suggested that M17 is a single copy gene. SA-PC showed significantly higher M17 mRNA levels than normal head kidney cells, which are considered to be a source of the SA-PC, indicating that M17 is inducible by inflammatory stimulation.


http://www.sciencedirect.com/science/article/B6T5X-40962SK-8/2/14df458749dbc6fe900e5060aff4db69

In order to characterize the Major histocompatibility complex (MHC) class II A genes of the channel catfish (Ictalurus punctatus) a cDNA library was screened and PCR was performed. Four different full-length cDNA sequences for MHC class II A genes were obtained from a clonal B cell line derived from an outbred fish. Two different genomic sequences and corresponding cDNAs were obtained from a presumably homozygous gynogenetic catfish. The A genes have five exons and four phase one introns. The first exon encodes the 5' untranslated region (UTR) and leader peptide; the second and third exons encode the [alpha]1 and [alpha]2 domains, respectively. The connecting peptide, transmembrane and cytoplasmic domains, as well as part of the 3' UTR, are encoded by the fourth exon and the rest of the 3' UTR is encoded by the fifth exon. Southern blot analyses using an exon three probe revealed two to four hybridizing fragments with considerable
restriction fragment length polymorphisms evident among randomly selected outbred channel catfish. These findings are consistent with the presence of at least two functional polymorphic MHC class II A gene loci. An unusual aspect of the channel catfish MHC class II [alpha] chain is its lack of N-linked glycosylation sites.


http://www.sciencedirect.com/science/article/B6T5X-3W321NP-5/2/92a32e2cddb606216ef0e48ef932cae7

A full-length cDNA clone (Onmy-UA-C32) encoding a major histocompatibility complex (MHC) class I heavy chain was isolated from a rainbow trout thymus cDNA library. Onmy-UA-C32 alpha I and III extracellular domains were most similar to other salmonids (92 and 86% at the nucleotide and amino acid level) but interestingly the alpha II domain is closer to that of the carp (74 and 73%) and zebrafish (75 and 70%). In addition, Onmy-UA-C32 displays conservation of residues known to be essential for the function and structure of MHC class Ia molecules. Northern blot hybridization with alpha 2 or 2-3 domain probes of Onmy-UA-C32 detected high expression (2.6 kb) of this gene in the spleen, thymus, kidney, heart and intestine with lower levels being observed in the brain and liver. No tissues were found to be negative indicating a ubiquitous pattern of expression for Onmy-UA-C32. Onmy-UA-C32 may therefore represent a MHC class Ia gene in trout as well as providing new insights regarding the evolution of the MHC within teleost species. Copyright


http://www.sciencedirect.com/science/article/B6T5X-4DD87HK-1/2/ebb24fcd2857e875d07a489e3c48f428

Fibrinogen-related proteins (FREPs) are hypothesized to function in non-self-recognition in the snail Biomphalaria glabrata. To investigate this assumption, the expression of four members of the FREP gene family was studied using quantitative PCR at 0.5-16 days following exposure of M line and BS-90 strain B. glabrata to Echinostoma paraensei and Schistosoma mansoni. Both strains react to, but fail to eliminate E. paraensei. Only the BS-90 strain is immunologically resistant to S. mansoni. Both snail strains responded to E. paraensei with significantly elevated expression of FREP 2 and 4. Following exposure to S. mansoni, resistant BS-90 snails showed an increase in expression of FREP 2 and 4 (57-fold and 4.5-fold increase, respectively), susceptible M line snails did not display a FREP response. Expression of FREP 3 and 7 was not significantly elevated in any snail/trematode combination. These expression profiles support the hypothesis that some FREPs play a role in the anti-trematode responses in B. glabrata.


http://www.sciencedirect.com/science/article/B6T5X-4DD87HK-1/2/ebb24fcd2857e875d07a489e3c48f428
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Avian thrombocytes are nucleated blood cells homologous in function to mammalian platelets. In the present study, we obtained a cDNA from chicken thrombocyte polyadenylated RNA [Poly(A)+RNA], which coded for the chicken PDGF-B chain. The sequence was 1083-bp long and had an open reading frame (ORF) of 753-bp. At the amino acid level, the predicted mature protein showed 69% homology with the processed coding region of human PDGF-B. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis showed that PDGF-B mRNA was expressed at high levels in thrombocytes and in the lung. The expression of PDGF-B chain mRNA in thrombocytes reached its maximum level 12 h following type 1 collagen treatment. These results suggest that chicken PDGF-B chain may play an important role in the vascular system and in healing wounded tissue.


Despite considerable advances in our understanding of teleost immunity, relatively few cytokine genes, including those for interferon (IFN), have been identified at the molecular level. In contrast, numerous studies have shown that following virus infection or exposure to double-stranded RNA, fish or fish cells produce a soluble factor that is functionally similar to mammalian IFN. A putative catfish (CF) IFN cDNA was identified by BLASTX screening of a catfish EST library generated from a mixed lymphocyte culture enriched for NK-like cells. Consistent with its designation as a putative cytokine cDNA, the 3' non-translated region contained multiple copies of an RNA instability motif. Analysis of the deduced amino acid sequence of CF IFN showed low levels of identity/similarity to a panel of mammalian and avian IFN proteins, and markedly higher similarity to a recently identified zebrafish IFN. To determine if the identified cDNA encoded CF IFN, expression was monitored following infection of channel catfish ovary (CCO) cells with UV-inactivated catfish reovirus or exposure to double-stranded RNA, treatments which induce IFN or IFN-like activity in catfish and other species. In both cases, upregulation of putative CF IFN mRNA was detected. Moreover, upregulation of CF IFN mRNA was accompanied by the appearance of an antiviral factor in the culture medium. To confirm these results, recombinant CF IFN was synthesized in COS-7 cells and shown to have antiviral activity in CCO cells. Collectively, these results argue strongly that the identified catfish cDNA is an IFN homolog.
To further characterize genes of immunological importance from non-placental mammals, cDNAs encoding [beta]2-microglobulin ([beta]2m) were isolated from two prototherians, the platypus and an echidna, and one metatherian, a grey short-tailed opossum. In addition, a second allele of [beta]2m was identified in another metatherian species, the brushtail possum. Analysis of the deduced translations revealed conservation of key residues in these molecules over a long evolutionary history. The types of nucleotide substitutions present among the various taxa are also consistent with purifying selection at this conserved locus. An evolutionary tree of [beta]2m was constructed that supports the classic view of evolution with prototherians as the basal mammalian group.

Tumor necrosis factor receptor (TNFR) superfamily regulates diverse biologic functions, including cell proliferation, differentiation, and survival, in addition to providing costimulatory signals for programmed cell death or apoptosis. In this study, cDNA fragments for two distinct TNFR homologues were obtained from a Japanese flounder, Paralichthys olivaceus, cDNA library. Full-length cDNAs of TNFR-1 and TNFR-2 homologues were obtained by using these cDNA fragments as probes. The cDNA for the Japanese flounder TNFR-1 homologue predicts a peptide of 395 amino acids that is 35% identical to the extracellular region of mouse TNFR-1, whereas the cDNA of the Japanese flounder TNFR-2 homologue predicts a peptide of 483 amino acids that is 40% identical to the extracellular region of human TNFR-2. The cytoplasmic domain contains a sequence that has the consensus motif of the death domain of the Japanese flounder TNFR-1 homologue. In a healthy fish, the mRNAs of both TNFR homologues were predominantly expressed in leukocytes, kidney, gill, and spleen. Expression of the Japanese flounder TNFR-1 homologue was induced in peripheral blood lymphocytes (PBLs) after stimulation with LPS (500 [mu]g/ml) for 1 h, and TNFR-2 homologue was strongly induced in PBLs after stimulation with Con A (50 [mu]g/ml) and PMA (0.35 [mu]g/ml) for 3 h. The different expression patterns of the two distinct TNFR homologues may be critical in determining whether binding with TNF-[alpha] or TNF-[beta] have activating, proliferative, or apoptotic effects on target cells.
The MAGE (Melanoma Associated Antigen) family tumor-specific antigens are shared by a number of histologically different tumors. Till date, only human and mouse MAGE genes have been characterized. Our study describes the first non-mammalian member of MAGE superfamily, DMAGE from D. melanogaster. A conceptual translation of the cDNA of DMAGE identifies a putative protein that contains a motif that shares eight out of nine amino acids with the previously identified promiscuous, HLA-A2 restricted antigenic epitope in the C-terminus of human MAGE-B1 and -B2. Similarly, this motif of DMAGE shares seven out of nine amino acids with the same antigenic epitope of human MAGE-A3 and -A12. Thus, the phylogeny of proteins that activate tumor specific T-cells in mammals as unmutated self-proteins began at least 100 million years earlier in evolution than the emergence of the adaptive immune system of higher vertebrates. Northern analysis revealed that DMAGE is a developmentally regulated gene highly expressed in adult fruit fly and in the embryo of D. melanogaster. In contrast, the expression level of the mRNA of DMAGE in fruit fly larva is substantially lower than in embryo and adult fly. We propose that studies of DMAGE on D. melanogaster may help define the function(s) of MAGE super-family genes.


http://www.sciencedirect.com/science/article/B6T5X-3RYCJTD-3/2/8aaa24a42ca3daa7c897fee86282fffb5

To further elucidate the cellular mechanisms that mediate programmed cell death in avian immune cells, differential display analysis was employed to identify differentially expressed genes in chicken thymocytes undergoing apoptosis. Primary cultures of thymocytes were treated with dexamethasone to activate apoptosis and RNA was isolated for differential display analysis. A differential display product designated A1 (479 bp) was identified. This display product was subcloned and induced expression of the genes was confirmed by ribonuclease protection analysis. Nucleotide sequence analysis of A1 revealed a putative 82 amino acid open reading frame that demonstrated limited homology with Bad, an apoptotic regulatory protein. Thus, A1 may represent the avian homolog of Bad.


http://www.sciencedirect.com/science/article/B6T5X-4C9YYD0-7/2/cd1a0cdfc00b347726f35ba16c4a923

The major histocompatibility complex (MHC) class II B locus of the striped bass (Morone saxatilis) was found to contain multiple forms of the class II B gene. Seven complete MHC class II B cDNA clones were isolated and sequenced, identifying five unique allelic forms of a MHC class II B gene. Among three specimens, each representing a geographically distinct population (Chesapeake Bay, MD; Roanoke River, NC; and Santee-Cooper Reservoir, SC) extensive variability was detected in the [beta]1 encoding domain, which corresponds with the functional peptide-binding region (PBR) of known MHC class II molecules. The location of variable amino acid residues in the [beta]1 domains corresponds with polymorphic sites observed in other teleosts and higher vertebrates. The amino acid translated [beta]2 domain encoding regions, transmembrane regions, and cytoplasmic regions of the five clones correlated well with those of known vertebrate MHC class II proteins. Seventy-one percent of the variability found within the presumed PBR encoded at the MHCMosa class II B locus corresponded with that of the PBR of a human MHC class II B gene. Overall, the Mosa sequences showed greatest similarity to the MHC class II B genes of cichlid fishes, as expected from phylogenetic relationships.

http://www.sciencedirect.com/science/article/B6T5X-48JSX1T-1/2/21b740de4452ae3a57ace6866da2e31d

A tumor necrosis factor (TNF) [alpha]-like gene, encoding a propeptide of 230 amino acids and a mature (soluble) peptide of 162 amino acids, was identified in channel catfish (Ictalurus punctatus). While the catfish protein shared features in common with both mammalian TNF[alpha] and TNF[beta] homologs, overall sequence identity/similarity was slightly higher vs. TNF[alpha] genes when mature TNF sequences were compared. Phylogenetic analysis placed catfish and other fish TNF sequences within their own cluster apart from mammalian TNF[alpha] and [beta] genes, and supported the suggestion that TNF[alpha] and [beta] genes separated after the divergence of mammals and teleosts. In contrast to trout and carp, but similar to flounder, catfish TNF was present as a single copy gene. Expression studies demonstrated that catfish TNF[alpha] mRNA was present in all tested tissues (i.e. liver, spleen, head kidney, mesonephros, gill, thymus, and PBLs) from an unstimulated fish. Moreover, catfish TNF was constitutively expressed in actively proliferating, but otherwise unstimulated, macrophage (42TA) and T cell (G14D; TS32.17) lines, but not in B cell (1G8 or 3B11) or fibroblast lines. TNF expression was upregulated in PBLs, and in G14D and 42TA cells, but not in 3B11 cells, by PMA/calcium ionophore treatment. These results demonstrate that a catfish homolog of TNF[alpha] has been identified, and indicate that catfish TNF[alpha] is expressed in catfish in a manner similar to that seen in mammals.

Developmental Biology  (15)


During vertebrate embryogenesis, the somites form by segmentation of the trunk mesoderm, lateral to the neural tube, in an anterior to posterior direction. Analysis of differential gene expression during somitogenesis has been problematic due to the limited amount of tissue available from early mouse embryos. To circumvent these problems, we developed a modified differential display PCR technique that is highly sensitive and yields products that can be used directly as in situ hybridisation probes. Using this technique, we isolated NLRR-1 as a gene expressed in the myotome of developing somites but not in the presomitic mesoderm. Detailed expression analysis showed that this gene was expressed in the skeletal muscle precursors of the myotome, branchial arches and limbs as well as in the developing nervous system. Somitic expression occurs in the earliest myoblasts that originate from the dorsal lip in a pattern reminiscent of the muscle determination gene Myf5, but not at the ventral lip, indicating that NLRR-1 is expressed in a subset of myotome cells. The NLRR genes comprise a three-gene family encoding glycosylated transmembrane proteins with external leucine-rich repeats, a fibronectin domain, an immunoglobulin domain and short intracellular tails capable of mediating protein-protein interaction. Analysis of NLRR-3 expression revealed regulated expression in the
neural system in developing ganglia and motor neurons. NLRR-2 expression appears to be predominately confined to the adult. The regulated embryonic expression and cellular location of these proteins suggest important roles during mouse development in the control of cell adhesion, movement or signalling.


http://www.sciencedirect.com/science/article/B6WDG-4DJB6T-1/2/47adc2c79e907615c0b8b87c90af4132

This study presents functional and molecular evidence for acquisition of multidrug transporter-mediated efflux activity as a consequence of fertilization in the sea urchin. Sea urchin eggs and embryos express low levels of efflux transporter genes with homology to the multidrug resistance associated protein (mrp) and permeability glycoprotein (p-gp) families of ABC transporters. The corresponding efflux activity is low in unfertilized eggs but is dramatically upregulated within 25 min of fertilization; the expression of this activity does not involve de novo gene expression and is insensitive to inhibitors of transcription and translation indicating activation of pre-existing transporter protein. Our study, using specific inhibitors of efflux transporters, indicates that the major activity is from one or more mrp-like transporters. The expression of activity at fertilization requires microfilaments, suggesting that the transporters are in vesicles and moved to the surface after fertilization. Pharmacological inhibition of mrp-mediated efflux activity with MK571 sensitizes embryos to the toxic compound vinblastine, confirming that one role for the efflux transport activity is embryo protection from xenobiotics. In addition, inhibition of mrp activity with MK571 alone retards mitosis indicating that mrp-like activity may also be required for early cell divisions.


http://www.sciencedirect.com/science/article/B6WDG-4B516430-5/2/06a20c041b38d200ac34819bbaa

We isolated a full-length cDNA clone of amphioxus AmphiNk2-tin, an NK2 gene similar in sequence to vertebrate NK2 cardiac genes, suggesting a potentially similar function to Drosophila tinman and to vertebrate NK2 cardiac genes during heart development. During the neurula stage of amphioxus, AmphiNk2-tin is expressed first within the foregut endoderm, then transiently in muscle precursor cells in the somites, and finally in some mesoderm cells of the visceral peritoneum arranged in an approximately midventral row running beneath the midgut and hindgut. The peritoneal cells that express AmphiNk2-tin are evidently precursors of the myocardium of the heart, which subsequently becomes morphologically detectable ventral to the gut. The amphioxus heart is a rostrocaudally extended tube consisting entirely of myocardial cells (at both the larval and adult stages); there are no chambers, valves, endocardium, epicardium, or other differentiated features of vertebrate hearts. Phylogenetic analysis of the AmphiNk2-tin sequence documents its close relationship to vertebrate NK2 class cardiac genes, and ancillary evidence suggests a relationship with the Drosophila NK2 gene tinman. Apparently, an amphioxus-like heart, and the developmental program directing its development, was the foundation upon which the vertebrate heart evolved by progressive modular innovations at the genetic and morphological levels of organization.
The Drosophila CNS midline cells constitute a specialized set of interneurons, motorneurons, and glia. The utility of the CNS midline cells as a neurogenomic system to study CNS development derives from the ability to easily identify CNS midline-expressed genes. For this study, we used a variety of sources to identify 281 putative midline-expressed genes, including enhancer trap lines, microarray data, published accounts, and the Berkeley Drosophila Genome Project (BDGP) gene expression data. For each gene, we analyzed expression at all stages of embryonic CNS development and categorized expression patterns with regard to specific midline cell types. Of the 281 candidates, we identified 224 midline-expressed genes, which include transcription factors, signaling proteins, and transposable elements. We find that 58 genes are expressed in mesectodermal precursor cells, 138 in midline primordium cells, and 143 in mature midline cells—50 in midline glia, 106 in midline neurons. Additionally, we identified 27 genes expressed in glial and mesodermal cells associated with the midline cells. This work provides the basis for future research that will generate a complete cellular and molecular map of CNS midline development, thus allowing for detailed genetic and molecular studies of neuronal and glial development and function.

In Drosophila, the RNA helicase VASA (VAS) is required for both germ line formation and oocyte differentiation. While the murine VAS homologue is required for spermatogenesis, it is dispensable for germ line formation. The molecular basis for this apparently dual role of VAS in germ line ontogeny is, however, unclear. Recent evidence indicates that fish, like flies, employs VAS both in early and late stages of the germ line development and that there is a sex-linked differential expression of splice variants. We show here that the longer of two splice variants of zebrafish vas is transiently downregulated in the germ line around the time when the germ cells reach the developing gonad. Using transgenic vas:EGFP fish lines, which allow us to distinguish between male and female individuals, we show that the long splice variant reappears in both sexes at around day 25 and is subsequently downregulated during male gonadal development. Our data further suggest that there is a switch from maternal to zygotic expression of the long splice variant of vas as sexual dimorphic development commences.

Telomerase is critical for the protection of germ line and stem cell chromosomes from fatal shortening during replication. In most organisms, telomerase activity is suppressed in
progressively committed cells and falls to basal rates in terminally differentiated lineages. The colonial ascidian Botryllus schlosseri propagates asexually and sexually, presumably from pools of stem cells that self-renew throughout the 2- to 5-year colony life span. Asexual budding takes place continuously from the parental body wall. When the colony reaches a critical size, sexual reproduction commences with the generation of gonads. Here, we establish the existence of 6-15 kb telomeres on the ends of Botryllus chromosomes. We develop a real-time quantitative PCR telomeric repeat amplification protocol (TRAP) assay that reliably detects 0.2-100 TPG units in cells and tissues. We find highest levels of enzymatic activity in the gonads, developing embryos, and tissues containing the earliest asexual buds. Telomerase activity appears to be suppressed in later buds during organogenesis and falls to basal rates in mature zooids. We postulate that this pattern reflects maximum telomere restoration in somatic stem cells of early buds and suppression of telomerase activity in progenitors and terminally differentiated cells, indicative of an alternate role for stem cells as repeated body regenerators in colonial life histories.


http://www.sciencedirect.com/science/article/B6WDG-4C76C1G-1/2/457ba64104db88cb6e178a3c56ed6276

The establishment of neural circuits in the spinal cord depends on the differentiation of functionally distinct types of neurons in the embryonic neural tube. A number of genes have recently been shown to control the generation of dorsal interneurons through inductive signals provided by the roof plate. The roof plate is a transient signaling center on the dorsal midline of the neural tube that coordinates dorsal CNS development through the action of local peptide signals, primarily the bone morphogenic proteins (BMPs) and the Wingless-related genes (Wnts). The role of the roof plate has become evident through studies of mutations of genes in these gene families, and through several spontaneously occurring mouse mutants, including dreherJ (drJ), all of which cause dorsal neural tube defects. We previously demonstrated that the roof plate is missing in the dreher mouse. Positional cloning of the dreher locus demonstrated that an inactivating point mutation in the LIM homeodomain (HD) transcription factor encoded by the Lmx1a gene, is responsible for the dreherJ phenotype [Nature, 403 (2000) 764]. Here we report that Lmx1a is first expressed at E8.5 in a small number of cells in the lateral neural plate. As the neural tube closes, Lmx1a expression is restricted to the roof plate. In drJ/drJ, although non-functional Lmx1a is correctly expressed at E8.5-E9.5, its expression is lost in the spinal cord roof plate by E10.5. Coincident with the loss of Lmx1a expression, Bmp expression fails, and the generation and differentiation of the dorsal-most spinal cord neurons, the dl1 interneurons, is abnormal. In drJ/drJ embryos, defects are evident in the number of dl1 progenitors, as well as in their migration to form the lateral and medial nuclei, and axon patterning, through mechanisms that apparently involve defects in early steps of neuronal polarity. Consistent with the general hypothesis that a failure of roof plate formation and function results in deficits in dorsal patterning of the neural tube, the dreher affects the generation and differentiation of the dl1 interneuron population.


http://www.sciencedirect.com/science/article/B6WDG-4CP68N2-3/2/202818a0aabb712f8e60d714ca77ba5f

The pha-2 mutant was isolated in 1993 by Leon Avery in a screen for worms with visible defects
in pharyngeal feeding behavior. In pha-2 mutant worms, the pharyngeal isthmus is abnormally thick and short and, in contrast to wild-type worms, harbors several cell nuclei. We show here that pha-2 encodes a homeodomain protein and is homologous to the vertebrate homeobox gene, Hex (also known as Prh). Consistent with a function in pharyngeal development, the pha-2 gene is expressed in the pharyngeal primordium of Caenorhabditis elegans embryos, particularly in pm5 cells that form the bulk of the isthmus. We show that in the pha-2 mutant there is a failure of the pm5 cells to elongate anteriorly while keeping their nuclei within the nascent posterior bulb to form the isthmus during the 3-fold embryonic stage. We also present evidence that pha-2 regulates itself positively in pm5 cells, that it is a downstream target of the forkhead gene pha-4, and that it may also act in the isthmus as an inhibitor of the ceh-22 gene, an Nkx2.5 homolog. Finally, we have begun characterizing the regulation of the pha-2 gene and find that intronic sequences are essential for the complete pha-2 expression profile. The present report is the first to examine the expression and function of an invertebrate Hex homolog, that is, the C. elegans pha-2 gene.


Mitogen-activated protein kinase (MAPK) pathways mediate some important cellular processes and are likely to also regulate preimplantation development. The role of p38 MAP kinase signaling during murine preimplantation development was investigated in the present study. p38 MAPK, p38-regulated or activated kinase (PRAK; MK5), map kinase-activated protein kinase 2 (MK2), and heat shock protein 25 (hsp25) mRNAs and proteins were detected throughout preimplantation development. Two-cell stage embryos cultured in the presence of SB220025 and SB203580 (specific inhibitors of p38 MAPK [alpha]/[beta]), progressed to the eight-cell stage with the same frequency as controls; however, treated embryos halted their development at the 8- to 16-cell stage. In addition, embryos treated with p38 MAPK inhibitors displayed a complete loss of MK2 and hsp25 phosphorylation and also a complete loss of filamentous actin as indicated by the absence of rhodamine-phalloidin staining. In these inhibitor-treated groups, the embryos were composed of a mixture of compacting and noncompacting cells, and the embryos were one to two cell divisions behind controls. Treated embryos remained viable as the developmental blockade was rescued by removing embryos from the drug treatment and placing them in drug-free medium until they progressed to the blastocyst stage. This study demonstrates that p38 MAPK activity is required to support development through the murine preimplantation interval.


Double-strand RNA (dsRNA)-mediated posttranscriptional gene silencing, also known as RNA interference (RNAi), is a powerful tool to inhibit gene expression in several experimental model systems, including Arabidopsis, Caenorhabditis, and Drosophila. We previously described that the microinjection of Mos dsRNA into fully grown mouse oocytes results in the specific degradation of Mos mRNA in a time- and concentration-dependent manner. We report here a transgenic RNAi approach that is suitable to study gene function during mouse oocyte development and differentiation. The oocyte-specific Zp3 promoter was used to drive the expression of a long hairpin dsRNA (~500 bp) targeting Mos mRNA. Transgenic founder animals
appeared healthy, but while males were fertile, females were not, in accordance with the known Mos null phenotype. The amount of Mos mRNA in the transgenic F1 females was reduced by >90%, whereas there was no decrease in the nontargeted tissue plasminogen activator (Plat) mRNA. Moreover, the maturation-associated increase in mitogen-activated protein (MAP) kinase activity was not observed, and the metaphase II eggs underwent spontaneous parthenogenetic activation, thus recapitulating the Mos null phenotype. This approach provides a powerful method to study the functions of any oocyte-synthesized gene during oocyte development and early embryogenesis.


http://www.sciencedirect.com/science/article/B6WDG-4DKTPB0-6/2/9dda2a5a1e9d832ac72202a187dd23f7

Calpactin I, one of the EDTA-extractable proteins of the lens membrane, binds phospholipid and actin in a calcium-dependent manner. It is also a known substrate of the pp60src kinase. Analysis of embryonic chicken lens RNA with a bovine calpactin I-specific cDNA probe revealed the presence of a ~1.8 Kb calpactin mRNA in the lens cells. Six-day embryonic chicken lenses were microdissected into central epithelium, equatorial epithelium, and fiber cells. Total cytoplasmic RNA was isolated from these samples and calpactin I mRNA levels were determined by the polymerase chain reaction (PCR) following reverse transcription (RT). Quantitative PCR indicates that the calpactin I mRNA levels in the equatorial epithelium are greater than in the central epithelium by a factor of 12.7 +/- 2.7. Calpactin I mRNA in fiber cells is an additional 3.5 +/- 1.5 times greater than in the equatorial epithelium. Whole mounts of embryonic chicken lens epithelia and histological sections of whole lenses were also examined with an antibody directed against chicken calpactin I. Calpactin I was predominantly localized in a punctate distribution in equatorial epithelial cells and near the plasma membrane of elongate fiber cells. The elevated levels of calpactin I mRNA observed in the equatorial epithelium and fiber cells and the immunological localization of the protein suggest a possible role of calpactin I in the elongation of fiber cells during lens differentiation.


http://www.sciencedirect.com/science/article/B6WDG-4CJXT1T-1/2/c599ac524c87adea7acc1f10f709b3

In ascidian eggs, the existence of several localized maternal cytoplasmic determinants has been proposed and the importance of localized mRNAs for tissue differentiation has been demonstrated. We previously identified the ascidian Y-box proteins (CiYB1, 2 and 3), homologues of which are known to be involved in the storage of maternal mRNA in oocytes of other organisms. In this study, we found that CiYB1 protein is abundant in the gonad, egg, and embryo. Purification of messenger ribonucleoprotein (mRNP) particles from the gonad revealed that CiYB1 was one of their major components. A significant change in the distribution of CiYB1 protein from stored mRNP particles in the gonad to the ribosome fraction in eggs and embryos was observed. This change correlates most likely with the shift of stored maternal mRNAs to polyribosomes. Moreover, we found that CiYB1 colocalized with Cipem and Ci-macho1 mRNAs, which are localized at the posterior end of the embryo at the cleavage stage. Cipem and Ci-macho1 mRNAs were co-immunoprecipitated with CiYB1 in the oocyte and embryo lysates. The formation of a complex between Cipem mRNA and CiYB1 protein resulted in translational
repression in the in vitro translation system. Our results indicate that associating with CiYB1 protein contributes to the translational control of the localized mRNA in eggs and embryos.


http://www.sciencedirect.com/science/article/B6WDG-4F030CR-7T/2/df76d6bd3c1e6be20bc5a3ae29764cf0

A major unsolved problem in developmental biology is to determine when and how time- and position-restricted instructions are signaled and received during secondary embryonic inductions such as branching morphogenesis. The mouse embryonic lung rudiment was used to test the hypothesis that endogenous peptide growth factors, specifically epidermal growth factor (EGF), serve as instructive epigenetic signals for morphogenesis. The presence of EGF precursor mRNA transcripts was detected using the reverse-transcriptase-coupled polymerase chain reaction both in E11-E17-day mouse embryo lung tissues in vivo and in E11-day lung cultured for up to 7 days in vitro under chemically defined, serum-free conditions. Immunolocalization identified a position-restricted distribution of EGF in and around the primitive airways both during in vivo lung morphogenesis and in culture. EGF receptors (EGFR) coimmunolocalized with EGF in the primitive airways. Addition of exogenous EGF to lungs in culture resulted in significant concentration-dependent stimulation of branching morphogenesis, DNA, RNA, and protein content, and in [3H]thymidine incorporation into DNA. Conversely, the addition of tyrphostin (specific EGF receptor kinase antagonist) to lungs in culture resulted in concentration-dependent inhibition of branching morphogenesis, DNA, RNA, and protein content, and in [3H]thymidine incorporation into DNA without apparent cytotoxicity. The inhibition of the EGF signal by tyrphostin was confirmed by immunoprecipitation of tyrosine phosphoproteins. We conclude that early mouse embryo lungs express EGF transcripts and corresponding EGF peptides in a specific position-restricted distribution which coimmunolocalizes with EGFR in the primitive airways, while stimulatory and inhibitory studies indicate a functional role for the transduced EGF signal in the epigenetic regulation of lung branching morphogenesis. We speculate that the peptide growth factor EGF serves a function in secondary embryonic morphogenetic inductions, which may be modulated by interaction with other growth factors.


http://www.sciencedirect.com/science/article/B6WDG-47X6RT9-42/b6849f3d3c1d9e483dd3318c46280c56

Selected for its high relative abundance, a protein spot of MW ~75 kDa, pI 5.5 was cored from a Coomassie-stained two-dimensional gel of proteins from 2850 zona-free metaphase II mouse eggs and analyzed by tandem mass spectrometry (TMS), and novel microsequences were identified that indicated a previously uncharacterized egg protein. A 2.4-kb cDNA was then amplified from a mouse ovarian adapter-ligated cDNA library by RACE-PCR, and a unique 2043-bp open reading frame was defined encoding a 681-amino-acid protein. Comparison of the deduced amino acid sequence with the nonredundant database demonstrated that the protein was ~40% identical to the calcium-dependent peptidylarginine deiminase (PAD) enzyme family. Northern blotting, RT-PCR, and in situ hybridization analyses indicated that the protein was abundantly expressed in the ovary, weakly expressed in the testis, and absent from other tissues. Based on the homology with PADs and its oocyte-abundant expression pattern, the protein was designated ePAD, for egg and embryo-abundant peptidylarginine deiminase-like protein. Anti-
recombinant ePAD monospecific antibodies localized the molecule to the cytoplasm of oocytes in primordial, primary, secondary, and Graafian follicles in ovarian sections, while no other ovarian cell type was stained. ePAD was also expressed in the immature oocyte, mature egg, and through the blastocyst stage of embryonic development, where expression levels began to decrease. Immunoelectron microscopy localized ePAD to egg cytoplasmic sheets, a unique keratin-containing intermediate filament structure found only in mammalian eggs and in early embryos, and known to undergo reorganization at critical stages of development. Previous reports that PAD-mediated deimination of epithelial cell keratin results in cytoskeletal remodeling suggest a possible role for ePAD in cytoskeletal reorganization in the egg and early embryo.


http://www.sciencedirect.com/science/article/B6WDG-4C4WXCW-2/2/54f3d1692748ceebfd2a41ecd45b5136

The subventricular zone (SVZ) of the developing mammalian forebrain gives rise to astrocytes and oligodendrocytes in the neocortex and white matter, and neurons in the olfactory bulb in perinatal life. We have examined the developmental fates and spatial distributions of the descendants of single SVZ cells by infecting them in vivo at postnatal day 0-1 (P0-1) with a retroviral "library". In most cases, individual SVZ cells gave rise to either oligodendrocytes or astrocytes, but some generated both types of glia. Members of glial clones can disperse widely through the gray and white matter. Progenitors continued to divide after stopping migration, generating clusters of related cells. However, the progeny of a single SVZ cell does not differentiate synchronously: individual clones contained both mature and less mature glia after short or long intervals. For example, progenitors that settled in the white matter generated three types of clonal oligodendrocyte clusters: those composed of only myelinating oligodendrocytes, of both myelinating oligodendrocytes and non-myelinating oligodendrocytes, or of only non-myelinating cells of the oligodendrocyte lineage. Thus, some progenitors do not fully differentiate, but remain immature and may continue to cycle well into adult life.

Developmental Brain Research (8)


http://www.sciencedirect.com/science/article/B6SYW-3T46VPV-6/2/b559a1455e84b92eb2338ac68a5b384e

The present study documents the steady-state levels for the mRNAs encoding acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD2) and brain long-chain acyl-CoA synthase (BLACS) during mouse development. It is shown that ACC and FAS mRNA levels are at a maximum 5 days after birth, a time when cell proliferation is intense in the mouse brain, and then decrease steadily to reach 20% of those maximal values at day 20. The ACC transcript isoforms, which were detected in the central nervous system (CNS), originated
from promoter P2 of the ACC gene. They encode ACC enzymes which cannot be phosphorylated at the Ser-1200 locus, thus indicating that brain ACC is highly sensitive to citrate activation. The developmental pattern for the SCD2 mRNA level is different from that of true myelin genes, such as CGT. Indeed, the steady-state levels for SCD2 and CGT in 5-day-old brain represent 85% and 5% of their maximal values, respectively. BLACS expression rose during the developmental period studied, but a slow decrease in the mRNA levels was not observed after postnatal day 20, unlike in 'myelin-specific' genes. Therefore, it appears that the expression of the genes involved in fatty acid biosynthesis is independent of the myelinating signal in the mouse CNS.


http://www.sciencedirect.com/science/article/B6SYW-3R37WNP-1/2/cf43206befbb6ffdf975407cd1903a65

Previous studies have demonstrated the critical role glutamate plays in the hypothalamus, both in the developing and adult brain. The expression of metabotropic glutamate receptor (mGluR) mRNA (mGluR1-8) was studied in the suprachiasmatic (SCN) and arcuate (ARC) nuclei. Using reverse Northern blots and cDNA-PCR, we found that all eight cloned mGluRs were expressed in these brain regions. Most had not previously been detected here. Surprisingly, this included mGluRs that had previously been thought to be restricted to the retina, such as mGluR6. We also detected, cloned, and sequenced a splice variant of mGluR7 (mGluR7b). Developmentally, the age of maximal expression of mGluRs was dependent on the region. For instance, mGluR5 was more strongly expressed in neonatal ARC than in adult, whereas the opposite was true in the SCN. Compared with P10 neonates, mGluR1, R3, R6, R7a, R7b, and R8 showed a greater expression in adult SCN and ARC.


http://www.sciencedirect.com/science/article/B6SYW-3TT60NV-5/2/a39ad79f62b55c7afbb1e2d4636723697

Using degenerate primers designed to amplify genes containing homeodomains, we have used reverse transcription and polymerase chain reaction to amplify and clone a rat homeobox gene. Based on the nucleotide and predicted amino acid sequences, the rat cDNA clone contains a high degree of sequence similarity to murine genes which are members of the paired-like class of homeobox genes (Ptx2, Ottx2, solurshin and Ptx1). Considering the high degree of sequence similarity and similar restricted expression patterns, we have named the cloned rat gene rPtx2 (rat Ptx2 homolog). Northern analysis revealed two rPtx2 transcripts expressed in the developing rat brain. Yet, only a single gene was detected by Southern blot hybridization, suggesting that multiple messages are the result of alternative transcriptional initiation, splicing or processing of a common message. The expression pattern of rPtx2 was further delineated by in situ hybridization to rat embryos. Within the brain, tissue specific expression was observed in the differentiating neural cells of the posterior hypothalamus, tegmentum, and rhombomere r1. Expression was also observed in the developing pituitary, maxilla, mandible, tongue and umbilical cord. To further study the control of Ptx2 gene expression, we used an in vitro model for neural differentiation by treating mouse embryonic stem cells with retinoic acid. Within 24 h and prior to detection of a neural phenotype in the culture, murine Ptx transcripts were induced and remained elevated for at least 6 days. This suggests that retinoic acid may be an important inductive signal which regulates the developmental and tissue-specific expression of Ptx2.

http://www.sciencedirect.com/science/article/B6SYW-485P97H-42/2/3a35235cde58fa0902dce1d485089879

We have examined the central nervous system (CNS) of developing and adult transgenic mice carrying sequences upstream of the histone H1[deg] gene fused to the E. coli [beta]-galactosidase gene (lac Z). The transgene is induced in a subset of the neuronal population during postnatal development, coinciding with neuronal terminal differentiation. At postnatal day 9, the earliest time at which the transgene product can be detected, positive neurons are observed in the granular layer of the cerebellar cortex and in the pyramidal fields of the hippocampus. The transgene is then induced in other areas of the CNS, such as the neocortex, thalamus, hypothalamus, olfactory bulb, globus pallidus superior and inferior colliculus, substantia nigra, pontine nuclei and brain stem. Induction is unrelated with determination and quiescence, which are essentially prenatal. The overlapping of the temporal and regional patterns of transgene activity with those of the endogenous protein shows that the accumulation of H1[deg] in differentiating neurons is at least in part under transcriptional control. In the light of these results, the H1[deg] gene appears as the only mammalian histone gene that specifically responds to terminal differentiation. However, not all terminally differentiated neurons express H1[deg] at detectable levels. For instance, Purkinje cells are negative. In neurons, terminal differentiation appears thus as a necessary, but not a sufficient condition for increased H1[deg] expression.


http://www.sciencedirect.com/science/article/B6SYW-3YCM5D2-D/2/8919325158b8f31b4a87c46a52402fd3

Gap junction coupling between neurons is important for the temporal and spatial co-ordination of neocortical development and can be visualised by dye-coupling. Neuronal dye-coupling in the rat neocortex is extensive during the first 2 postnatal weeks and diminishes rapidly thereafter. We used RT (reverse transcriptase)-PCR to investigate the time-related changes in mRNA expression for the connexins (Cx) Cx 26, Cx 30, Cx 32, Cx 36, Cx 37, Cx 40, Cx 43, Cx 45 and Cx 46 as well as for [beta]-actin and GAPDH in rat neocortex during the first 6 postnatal weeks. The time courses for mRNA expression for GAPDH, Cx 30, Cx 36 and Cx 43 were also investigated by northern blotting. Cx 30 and Cx 45 mRNA abundance showed no time-dependent changes during the early postnatal period. The relative abundance of Cx 32, Cx 43 and Cx 46 mRNA increased significantly during the first 2-3 weeks and then remained relatively constant during weeks 3-6. The relative abundance of Cx 26, Cx 36, Cx 37 and Cx 40 mRNA also increased significantly during the first 10-15 postnatal days but then declined significantly from their peak values during weeks 3-6. [beta]-actin mRNA expression showed no time-related changes but GAPDH mRNA expression increased significantly during the first postnatal week, then remained constant. The time-dependent changes in mRNA relative abundance for GAPDH, Cx 36 and Cx 43 determined by northern blotting corroborate the results from the RT-PCR study. None of the Cx exhibited time-dependent changes in mRNA expression in homogenates of rat neocortex which parallel the changes in neuronal dye-coupling during postnatal development.

The expression of the amyloid precursor protein (APP) gene has been examined in the basal forebrain of rats from birth to adulthood. Levels of total APP mRNA are highest at birth and at postnatal day 15 (P15). The most abundant transcript in rat brain in APP-695, whose expression has previously been found to be largely restricted to the central nervous system. Comparison of the developmental profiles of APP-695 mRNA with that of Kunitz-protease inhibitor (KPI)-containing APP mRNA shows that the greatest difference in expression occurs at P15, when APP-695 message levels are over 6-fold higher than KPI-containing APP mRNA (APP-751, APP-770). This is the largest difference in the APP-695/KPI-APP ratio observed during postnatal development and coincides with the period of maximal neurotrophic responsiveness in the basal forebrain. These results suggest that the APP gene is alternatively spliced during postnatal development and that regulated expression of APP-695 may be influenced by neurotrophic factors in vivo.


To test the hypothesis whether a failure to express neurotrophins or a neurotrophin receptor might underlie the pathology observed in mutant mice with degeneration of regionally distinct subpopulations of neurons, the expression of BDNF, NT-3, TrkB, TrkC and synaptophysin mRNA was examined in the cerebellum of mutant lurcher (lc/*) and weaver (wv/*)(wv/wv) mice. To identify the expression patterns of individual neurons, we used in situ hybridization with digoxigenin labeled ribonucleotide probes. RT-PCR of cerebellar mRNA for BDNF, NT-3, TrkB and TrkC (GAPDH as internal standard) was performed in parallel. Although especially in homozygous (wv/wv) weaver mice the normal anatomical order and number of the cerebellar neurons is grossly disturbed, residual Purkinje and granule neurons of both mutants displayed a normal expression pattern of the neurotrophins examined. Thus, the affected animals showed no significant signal decrease compared to healthy littermates or C3H mice. Our results suggest that the loss of specific neuron populations in the cerebellum of either mutant occurs via mechanisms either independent or downstream of the neurotrophins examined in this study.


Fetal alcohol exposure is the most common nonhereditary cause of mental retardation in the western world. Rats prenatally treated with ethanol liquid diet exhibit extensive defects in the brain that accurately model those observed in humans. To analyze the ethanol effects on gene expression during brain development, we performed mRNA differential display and two-dimensional electrophoresis on gestational day (G) 13 and G16 brain from rats treated with
ethanol liquid diet. Using mRNA differential display followed by a variety of quantitative analyses, three genes were confirmed to be ethanol-responsive. Among them was Neuroendocrine-Specific Protein-A (NSP-A), which is known to be affected by thyroid hormone in the cortex at this developmental time. However, two additional genes known to be thyroid hormone-responsive were unaffected by ethanol, indicating that interference with thyroid hormone action may not be a predominant pathway by which alcohol induces damage in the fetal brain. The observation that interferon-inducible protein-10 (IP-10) is up-regulated in ethanol-treated fetal brain may indicate the presence of a disease process recruiting CD8+ T-cells capable of interfering with myelination. The result of two-dimensional (2D) electrophoresis and Western analyses demonstrated that few changes in the abundance of individual proteins or the phosphorylation of proteins at threonine and tyrosine were induced by prenatal ethanol exposure. A critical analysis of the approaches used in the present study may be important for future studies in this field.

Developmental Cell  (1)


http://www.sciencedirect.com/science/article/B6WW3-4FVYW18-N/2/136ede20e52cbd1fa31721ba7ee6cc6d

Summary Inductive interactions between gut endoderm and the underlying mesenchyme pattern the developing digestive tract into regions with specific morphology and functions. The molecular mechanisms behind these interactions are largely unknown. Expression of the conserved homeobox gene Barx1 is restricted to the stomach mesenchyme during gut organogenesis. Using recombinant tissue cultures, we show that Barx1 loss in the mesenchyme prevents stomach epithelial differentiation of overlying endoderm and induces intestine-specific genes instead. Additionally, Barx1 null mouse embryos show visceral homeosis, with intestinal gene expression within a highly disorganized gastric epithelium. Barx1 directs mesenchymal cell expression of two secreted Wnt antagonists, sFRP1 and sFRP2, and these factors are sufficient replacements for Barx1 function. Canonical Wnt signaling is prominent in the prospective gastric endoderm prior to epithelial differentiation, and its inhibition by Barx1-dependent signaling permits development of stomach-specific epithelium. These results define a transcriptional and signaling pathway of inductive cell interactions in vertebrate organogenesis.

Diabetes  (48)


http://diabetes.diabetesjournals.org/cgi/content/abstract/53/7/1857
To ascertain whether distinct chromosomal loci existed that were linked to severe obesity, as well as to utilize the increased heritability of this excessive phenotype, we performed a genome-wide scan in severely obese French Caucasians. The 109 selected pedigrees, totaling 447 individuals, required both the proband and a sibling to be severely obese (BMI \( \geq 35 \text{ kg/m}^2 \)), and 84.8% of the nuclear families possessed \( \geq 1 \) morbidly obese sibling (BMI \( \geq 40 \)). Severe and morbid obesity are still relatively rare in France, with rates of 2.5 and 0.6%, respectively. The initial genome scan consisted of 395 evenly spaced microsatellite markers. Six regions were found to have suggestive linkage on 4q, 6cen-q, 17q, and 19q for a BMI \( \geq 35 \) phenotypic subset, and 5q and 10q for an inclusive BMI \( \geq 27 \) group. The highest peak on chromosome 19q (logarithm of odds [LOD] = 3.59) was significant by genome scan simulation testing (P = 0.042). These regions then underwent second-stage mapping with an additional set of 42 markers. BMI \( \geq 35 \) analysis defined regions on 17q23.3-25.1 and 19q13.33-13.43 with a maximum likelihood score LOD of 3.16 and 3.21, respectively. Subsequent pooled data analysis with an additional previous population of 66 BMI \( \geq 35 \) sib-pairs led to a significant LOD score of 3.8 at the 19q locus (empirical P = 0.023). For more moderate obesity and overweight susceptibility loci, BMI \( \geq 27 \) analysis confirmed suggestive linkage to chromosome regions 5q14.3-q21.3 (LOD = 2.68) and 10q24.32-26.2 (LOD = 2.47). Plausible positional candidate genes include NR1H2 and TULP2.


http://diabetes.diabetesjournals.org/cgi/content/abstract/54/5/1392

Thiazolidinediones such as pioglitazone improve insulin sensitivity in diabetic patients by several mechanisms, including increased uptake and metabolism of free fatty acids in adipose tissue. The purpose of the present study was to determine the effect of pioglitazone on mitochondrial biogenesis and expression of genes involved in fatty acid oxidation in subcutaneous fat. Patients with type 2 diabetes were randomly divided into two groups and treated with placebo or pioglitazone (45 mg/day) for 12 weeks. Mitochondrial DNA copy number and expression of genes involved in mitochondrial biogenesis were quantified by real-time PCR. Pioglitazone treatment significantly increased mitochondrial copy number and expression of factors involved in mitochondrial biogenesis, including peroxisome proliferator-activated receptor (PPAR)-(gamma) coactivator-1[alpha] and mitochondrial transcription factor A. Treatment with pioglitazone stimulated the expression of genes in the fatty acid oxidation pathway, including carnitine palmitoyltransferase-1, malonyl-CoA decarboxylase, and medium-chain acyl-CoA dehydrogenase. The expression of PPAR-(alpha), a transcriptional regulator of genes encoding mitochondrial enzymes involved in fatty acid oxidation, was higher after pioglitazone treatment. Finally, the increased mitochondrial copy number and the higher expression of genes involved in fatty acid oxidation in human adipocytes may contribute to the hypolipidemic effects of pioglitazone.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/7/2317

For any mitochondrial DNA (mtDNA) mutation, the ratio of mutant to wild-type mtDNA (% heteroplasmy) varies across tissues, with low levels in leukocytes and high levels in postmitotic tissues (e.g., skeletal muscle). Direct sequencing is the gold-standard method used to detect novel mutations, but can only reliably detect % heteroplasmy >25%, which is rare in leukocytes. Therefore, we investigated the role of mtDNA defects in maternally inherited diabetes by first
screening for the A3243G tRNALeu(UUR) mutation by restriction assay, followed by sequencing of the entire mitochondrial genome using skeletal muscle derived mtDNA. A total of 28 patients had maternally inherited diabetes either alone (group 1, n = 17) or with one or more additional features of mitochondrial disease, including bilateral sensori-neural deafness and neuromuscular disease (group 2, n = 11). Three patients (all from group 2) carried the A3243G mutation. Skeletal muscle mtDNA from eight group 1 patients and six more group 2 patients was sequenced. No pathogenic mutations were found in the group 1 patients, while two patients from group 2 had mutations at positions 12258 and 14709 in the tRNA serine and glutamic acid genes, respectively. We conclude, therefore, that screening for mtDNA mutations should be considered in patients with maternally inherited diabetes, but only when additional features of mitochondrial disease are present.


http://diabetes.diabetesjournals.org/cgi/content/abstract/53/12/3258

Metformin reduces the incidence of progression to type 2 diabetes in humans with obesity or impaired glucose tolerance. We used an animal model to investigate whether metformin could prevent acute lipid-induced insulin resistance and the mechanisms involved. Metformin or vehicle was administered to rats daily for 1 week. Rats were studied basally, after 3.75 h of intralipid-heparin or glycerol infusion, or after 5 h of infusion with a hyperinsulinemic-euglycemic clamp between 3 and 5 h. Metformin had no effect on plasma triacylglycerol or nonesterified fatty acid concentrations and did not alter glucose turnover or gluconeogenic enzyme mRNA after lipid infusion. However, metformin normalized hepatic glucose output and increased liver glycogen during lipid infusion and clamp. Basal liver (but not muscle or fat) AMP-activated protein kinase activity was increased by metformin (by 310%; P < 0.01), associated with increased phosphorylation of acetyl CoA carboxylase. Postclamp liver but not muscle phosphorylated/total Akt protein was increased, whereas basal c-Jun NH2-terminal kinase-1 and -2 protein expression were reduced (by 39 and 53%, respectively; P < 0.05). Metformin also increased hepatic basal I{kappa}B{alpha} levels (by 260%; P < 0.001) but had no effect on tyrosine phosphorylation or expression of insulin receptor substrate-1 (IRS-1). In summary, metformin opposes the development of acute lipid-induced insulin resistance in the liver through alterations in multiple signaling pathways.


http://diabetes.diabetesjournals.org/cgi/content/abstract/53/4/1162

Dysfunction of the actin cytoskeleton is a key event in the pathogenesis of diabetic nephropathy. We previously reported that certain cytoskeletal genes are upregulated in mesangial cells exposed to a high extracellular glucose concentration. One such gene, caldesmon, lies on chromosome 7q35, a region linked to nephropathy in family studies, making it a candidate susceptibility gene for diabetic nephropathy. We screened all exons, untranslated regions, and a 5-kb region upstream of the gene for variation using denaturing high-performance liquid chromatography technology. An A>G single nucleotide polymorphism (SNP) at position -579 in the promoter region was associated with nephropathy in a case-control study using 393 type 1 diabetic patients from Northern Ireland (odds ratio [OR] 1.38, 95% CI 1.02-1.86, P = 0.03). A similar trend was found in an independent sample from a second center. When the sample groups were combined (n = 606), the association between the -579G allele and nephropathy
remained significant (OR 1.35, 1.07-1.70, P = 0.01). The haplotype structure in the surrounding 7-kb region was determined. No single haplotype was more strongly associated with nephropathy than the -579A>G SNP. These results suggest a role for the caldesmon gene in susceptibility to diabetic nephropathy in type 1 diabetes.


http://diabetes.diabetesjournals.org/cgi/content/abstract/53/7/1714

Pancreatic muscarinic acetylcholine receptors play an important role in stimulating insulin and glucagon secretion from islet cells. To study the potential role of the M3 muscarinic receptor subtype in cholinergic stimulation of insulin release, we initially examined the effect of the muscarinic agonist, oxotremorine-M (Oxo-M), on insulin secretion from isolated pancreatic islets prepared from wild-type (WT) and M3 receptor-deficient mice (M3+/- and M3-/- mice). At a stimulatory glucose level (16.7 mmol/l), Oxo-M strongly potentiated insulin output from islets of WT mice. Strikingly, this effect was completely abolished in islets from M3-/- mice and significantly reduced in islets from M3+/- mice. Additional in vitro studies showed that Oxo-M-mediated glucagon release was also virtually abolished in islets from M3-/- mice. Consistent with the in vitro data, in vivo studies showed that M3-/- mice displayed reduced serum insulin and plasma glucagon levels and a significantly blunted increase in serum insulin after an oral glucose load. Despite the observed impairments in insulin release, M3-/- mice showed significantly reduced blood glucose levels and even improved glucose tolerance, probably due to the reduction in plasma glucagon levels and the fact that M3-/- mice are hypophagic and lean. These findings provide important new insights into the metabolic roles of the M3 muscarinic receptor subtype.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/4/1281

Peroxisome proliferator-activated receptor-γ (PPARGC1) is a transcriptional coactivator that has been implicated in the regulation of genes involved in energy metabolism. We studied associations of two polymorphisms identified in PPARGC1 transcripts with obesity indices in 591 middle-aged men and 467 middle-aged women of a cross-sectional Austrian population. Because neither polymorphic site was likely to be a functional site, we analyzed sex-specific associations of two loci haplotype combinations with obesity indices. Significant associations with BMI (P = 0.006), waist (P = 0.01) and hip circumference (P = 0.03), and total body fat (P = 0.005) and borderline significant associations with abdominal visceral and subcutaneous fat were observed in women but not men. In women, plasma triglycerides, HDL cholesterol, and glucose significantly differed by haplotype combinations, but these associations were not maintained after statistical consideration of BMI. The haplotype combination of the double-variant allele with the double-wild-type allele was associated with the lowest obesity indices, whereas homozygosity for the double-variant allele was not discriminatory among haplotype combinations. These studies suggest functional differences of PPARGC1 haplotypes in human energy metabolism and support a role of PPARGC1 in obesity.
The Arg972 insulin receptor substrate-1 (IRS-1) variant has been hypothesized to play a role in pancreatic (beta)-cell stimulus-coupled insulin secretion and survival. We analyzed the relations between type 1 diabetes and the Arg972 IRS-1 variant. The frequency of the IRS-1 Arg972 variant was investigated in two independent sets of unrelated patients: a case-control study and a collection of type 1 diabetes simplex families. In the former group, frequency of the IRS-1 Arg972 variant was significantly increased in the patients ($P = 0.0008$), conferring an OR of 2.5. Transmission disequilibrium analysis of data obtained from the family set revealed that the Arg972 IRS-1 variant was transmitted from heterozygous parents to affected probands at a frequency of 70.2% ($P < 0.02$). Arg972 IRS-1 frequency showed no significant correlation with HLA genotypic risk for type 1 diabetes. Arg972 IRS-1 type 1 diabetic patients also had lower fasting plasma concentrations of C-peptide at the time of diagnosis with respect to patients carrying the wild-type IRS-1 ($0.49 \pm 0.058$, $n = 34$, and $0.76 \pm 0.066$, $n = 134$, respectively [means $\pm$ SE]; $P = 0.051$). Our findings suggest a role for Arg972 IRS-1 in conferring risk for the development of type 1 diabetes.


The molecular mechanisms mediating acute regulation of insulin release by glucose are partially known. The process involves at least two pathways that can be discriminated on basis of their (in)dependence of closure of ATP-sensitive potassium (K+ATP) channels. The mechanism of the K+ATP channel-independent pathway was proposed to involve cataplerosis, the export of mitochondrial intermediates into the cytosol and in the induction of fatty acid-derived signaling molecules. In the present article, we have explored in fluorescence-activated cell sorter (FACS)-purified rat (beta)-cells the molecular steps involved in chronic glucose regulation of the insulin secretory response. When compared with culture in 10 mmol/l glucose, 24 h culture in 3 mmol/l glucose shifts the phenotype of the cells into a state with low further secretory responsiveness to glucose, lower rates of glucose oxidation, and lower rates of cataplerosis. Microarray mRNA analysis indicates that this shift can be attributed to differences in expression of genes involved in the K+ATP channel-dependent pathway, in cataplerosis and in fatty acid/cholesterol biosynthesis. This response was paralleled by glucose upregulation of the transcription factor sterol regulatory element binding protein 1c (SREBP1c) (ADD1) and downregulation of peroxisome proliferator--activated receptor--alpha and PPAR-(beta) (PPAR(delta)). The functional importance of cataplerosis via citrate for glucose-induced insulin release was further supported by the observation that two ATP-citrate lyase inhibitors, radicicol and (-)-hydroxycitrate, block part of glucose-stimulated release in (beta)-cells. In conclusion, chronic glucose regulation of the glucose-responsive secretory phenotype is associated with coordinated changes in gene expression involved in the K+ATP channel-dependent pathway, in cataplerosis via citrate and in acyl CoA/cholesterol biosynthesis.

IGF-I has a critical role in growth and metabolism. A microsatellite polymorphism 1 kb upstream to the IGF-I gene has recently been associated with several adult phenotypes. In a large Dutch cohort, the absence of the commonest allele (Z) was associated with reduced serum IGF-I levels, reduced height, and an increased risk of type 2 diabetes and myocardial infarction. This result has not been replicated, and the role of this polymorphism in these traits in U.K. subjects is not known. We sought further evidence for the involvement of this variant in type 2 diabetes using a case-control study and IGF-I and diabetes-related traits in a population cohort of 640 U.K. individuals aged 25 years. Absence of the common allele was not associated with type 2 diabetes (odds ratio 0.70, 95% CI 0.47-1.04 for X/X versus Z/Z genotype, \( \chi^2 \) test for trend across genotypes, \( P = 0.018 \)). In the population cohort, the common allele (Z) was associated with decreased IGF-I levels (\( P = 0.01 \)), contrary to the Dutch study, but not with adult height (\( P = 0.23 \)), glucose tolerance (\( P = 0.84 \)), oral glucose tolerance test-derived values of \( \beta \)-cell function (\( P = 0.90 \)), or insulin resistance (\( P = 0.66 \)). There was no association with measures of fetal growth, including birth weight (\( P = 0.17 \)). Our results do not support the previous associations and suggest that the promoter microsatellite is unlikely to be functionally important.


Recently, an A-to-G variant in intron 3 (SNP43) of the calcium-activated neutral protease 10 gene (CAPN10) was identified as a possible type 2 diabetes susceptibility gene through positional cloning in Mexican-Americans. We conducted cross-sectional and prospective studies to evaluate the relation between SNP43 and type 2 diabetes and related traits in middle-aged African-American participants of the Atherosclerosis Risk in Communities Study, a population-based longitudinal study. At baseline, 269 prevalent diabetes cases and 1,159 nondiabetic control subjects were studied. Those with the G/G genotype were more likely to have diabetes than those with the A/G or A/A genotype (odds ratio [OR] 1.41, 95% CI 1.00-1.99, \( P = 0.05 \)). In the prospective study, 166 of the control subjects developed incident diabetes over 9 years of follow-up. The incidence of diabetes for individuals with the G/G genotype did not differ significantly from those with at least one copy of the A allele (23.3 vs. 19.5 per 1,000 person years, \( P = 0.29 \)). Pooling prevalent and incident diabetic cases together, individuals with the G/G genotype were \( \sim \)40% more likely to have diabetes than those without (OR 1.38, 95% CI 1.04-1.83, \( P = 0.03 \)). Because of the high frequency of the G allele (0.88), \( \sim \)25% of the susceptibility to type 2 diabetes in African-Americans may be attributed to the G/G genotype at SNP43 of CAPN10, although most of the subjects with the G/G genotype did not develop diabetes over the 9 years of follow-up. We conclude from this large prospective study that the G allele of SNP43 of CAPN10 or another allele or gene that is in linkage disequilibrium with it increases susceptibility to type 2 diabetes in African-Americans.

embryonic pancreata with dexamethasone, a glucocorticoid agonist, induced a decrease of insulin-expressing cell numbers and a doubling of acinar cell area, indicating that glucocorticoids favored acinar differentiation; in line with this, expression of Pdx-1, Pax-6, and Nkx6.1 was downregulated, whereas the mRNA levels of Ptf1-p48 and Hes-1 were increased. The selective inactivation of the GR gene in insulin-expressing \((\beta)\)-cells in mice (using a RIP-Cre transgene) had no measurable consequences on \((\beta)\)- or \((\alpha)\)-cell mass, whereas the absence of GR in the expression domain of Pdx-1 (Pdx-Cre transgene) led to a twofold increased \((\beta)\)-cell mass, with increased islet numbers and size but normal \((\alpha)\)-cell mass in adults. These results demonstrate that glucocorticoids play an important role in pancreatic \((\beta)\)-cell lineage, acting before hormone gene expression onset and possibly also modulating the balance between endocrine and exocrine cell differentiation.


http://diabetes.diabetesjournals.org/cgi/content/abstract/53/9/2455

Atherosclerosis in type 2 diabetic patients has been linked to increased oxidative stress. Glutathione peroxidase-1 (GPx-1) plays an important role in the antioxidant defense of the vascular wall. To assess the association between variants in the GPx-1 gene and atherosclerosis, we screened the gene in 184 Japanese type 2 diabetic patients and identified four polymorphisms (-602A/G, +2C/T, Ala5/Ala6, and Pro198Leu). Among these polymorphisms, -602G, +2T, Ala6, and 198Leu were in strong linkage disequilibrium with each other. The patients were divided into two groups on the basis of the codon 198 polymorphism, Pro/Pro \((n = 151)\) and Pro/Leu \((n = 33)\), to analyze clinical characteristics. The mean intima-media thickness (IMT) of common carotid arteries \((P = 0.0028)\) and the prevalence of cardiovascular disease \((P = 0.035)\) and peripheral vascular disease \((P = 0.027)\) were significantly higher in the Pro/Leu group than in the Pro/Pro group. In vitro functional analyses indicated that the combination of polymorphisms \((\text{Ala6/198Leu})\) of the GPx-1 gene had a 40% decrease in enzyme activity, and the combination of polymorphisms \((-602G/+2T)\) had a 25% decrease in transcriptional activity. These results suggest that functional variants in the GPx-1 gene are associated with increased IMT of carotid arteries and risk of cardiovascular and peripheral vascular diseases in type 2 diabetic patients.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/5/1477

In humans and animal models, increased lipid content of skeletal muscle is strongly associated with insulin resistance. However, it is unclear whether this accumulation is due to increased uptake or reduced utilization of fatty acids (FAs). We used 3H-R-bromopalmitate tracer to assess the contribution of tissue-specific changes in FA uptake to the lipid accumulation observed in tissues of insulin-resistant, high fat-fed rats (HFF) compared with control rats (CON) fed a standard diet. To study FA metabolism under different metabolic states, tracer was infused under basal conditions, during hyperinsulinemic-euglycemic clamp (low FA availability) or during the infusion of intralipid and heparin (high FA availability). FA clearance was significantly increased in the red gastrocnemius muscle of HFF under conditions of low \((HFF = 10.4 \pm 1.1; \ CON = 7.4 \pm 1.0)\) min \(-1\) (middle dot) g \(-1\); \(P < 0.05)\), basal \((HFF = 8.3 \pm 1.4; \ CON = 4.5 \pm 0.7)\) min \(-1\) (middle dot) g \(-1\); \(P < 0.01)\), and high \((HFF = 7.0 \pm 0.8; \ CON = 4.3 \pm 0.5)\) min \(-1\) (middle dot) g \(-1\); \(P < 0.05)\) FA levels. This indicates
an adaptation by muscle for more efficient uptake of lipid. Associated with the enhanced efficiency of FA uptake, we observed increases in CD36/FA translocase mRNA expression (P < 0.01) and acyl-CoA synthetase activity (P < 0.02) in the same muscle. FA clearance into white adipose tissue was also increased in HFF when circulating FA were elevated, but there was little effect of the high-fat diet on hepatic FA uptake. In conclusion, insulin resistance induced by feeding rats a high-fat diet is associated with tissue-specific adaptations that enhance utilization of increased dietary lipid but could also contribute to the accumulation of intramuscular lipid with a detrimental effect on insulin action.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/12/3577

Transforming growth factor-β (TGF-β) may be critical in the development of diabetic nephropathy (DN), and genetic predisposition is an important determinant of DN risk. We evaluated mRNA expression levels of TGF-β system components in cultured skin fibroblasts (SFs) from type 1 diabetic patients with fast versus slow development of DN. A total of 125 long-standing type 1 diabetic patients were ranked by renal mesangial expansion score (MES) based on renal biopsy findings and diabetes duration. Patients in the highest quintile of MES who were also microalbuminuric or proteinuric (n = 16) were classified as "fast-track" for DN, while those in the lowest quintile who were also normoalbuminuric (n = 23) were classified as "slow-track" for DN. Twenty-five normal subjects served as control subjects. SFs were cultured in medium with 25 mmol/l glucose for 36 h. SF mRNA expression levels for TGF-β1, TGF-β type II receptor (TGF-β RII), thrombospondin-1, and latent TGF-β binding protein-1 (LTBP-1) were measured by real-time RT-PCR. LTBP-1 mRNA expression was reduced in slow-track (0.99 +/- 0.38) versus fast-track patients (1.65 +/- 0.52, P = 0.001) and control subjects (1.41 +/- 0.7, P = 0.025). mRNA levels for TGF-β1, TGF-β RII, and thrombospondin-1 were similar in the three groups. Reduced LTBP-1 mRNA expression in SFs from slow-track patients may reflect genetically determined DN protection and suggests that LTBP-1 may be involved in the pathogenesis of DN through the regulation of TGF-β activity.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/9/2773

In the mouse β-cell, glucose generates large amplitude oscillations of the cytosolic-free Ca2+ concentration ([Ca2+]i) that are synchronous to insulin release oscillations. To examine the role played by [Ca2+]i oscillations in the process of insulin release, we examined the effect of plasma membrane Ca2+-ATPase (PMCA) overexpression on glucose-induced Ca2+ oscillations and insulin release in BRIN-BD11 cells. BRIN-BD11 cells were stably transfected with PMCA2wb. Overexpression could be assessed at the mRNA and protein level, with appropriate targeting to the plasma membrane assessed by immunofluorescence and the increase in PMCA activity. In response to K+, overexpressing cells showed a markedly reduced rise in [Ca2+]i. In response to glucose, control cells showed large amplitude [Ca2+]i oscillations, whereas overexpressing cells showed markedly reduced increases in [Ca2+]i without such large oscillations. Suppression of [Ca2+]i oscillations was accompanied by an increase in glucose metabolism and insulin release that remained oscillatory despite having a lower periodicity. Hence, [Ca2+]i oscillations appear unnecessary for glucose-induced insulin release and may even be less favorable than a stable
increase in [Ca2+]i for optimal hormone secretion. [Ca2+]i oscillations do not directly drive insulin release oscillations but may nevertheless intervene in the fine regulation of such oscillations.


http://diabetes.diabetesjournals.org/cgi/content/abstract/54/4/1009

Diabetes is the most prevalent and serious metabolic disease, and the number of diabetic patients worldwide is increasing. The reduction of insulin biosynthesis in pancreatic (beta)-cells is closely associated with the onset and progression of diabetes, and thus it is important to search for ways to induce insulin-producing cells in non-(beta)-cells. In this study, we showed that a modified form of the pancreatic and duodenal homeobox factor 1 (PDX-1) carrying the VP16 transcriptional activation domain (PDX-1/VP16) markedly increases insulin biosynthesis and induces various pancreas-related factors in the liver, especially in the presence of NeuroD or neurogenin 3 (Ngn3). Furthermore, in streptozotocin-induced diabetic mice, PDX-1/VP16 overexpression, together with NeuroD or Ngn3, drastically ameliorated glucose tolerance. Thus PDX-1/VP16 expression, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. This approach warrants further investigation and may have utility in the treatment of diabetes.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/3/871

Cholesteryl ester transfer protein (CETP) is a key regulating factor of lipid metabolism, and the polymorphism of its gene may therefore be a candidate for modulating the lipid parameters, altering the susceptibility to atherosclerosis in type 2 diabetic subjects. In a group of 443 unrelated Japanese patients with type 2 diabetes, we studied the B1B2 polymorphism at the CETP locus, which is detectable with the restriction enzyme TaqI. Patients were separated into three groups according to genotype and compared based on their clinical characteristics, lipid parameters, and macrovascular complications. The B2 allele was associated in a dose-dependent fashion with higher HDL cholesterol and apolipoprotein AI levels, together with lower CETP concentrations. Furthermore, the prevalence of macrovascular complications, such as coronary heart disease, arteriosclerosis obliterans, and cerebral vascular disease, was significantly higher in subjects with the B1B1 genotype. Multiple logistic regression analysis also showed that the B1 allele of CETP genotype was associated with the incidence of these three complications independently of other risk factors. Thus, in type 2 diabetic patients, the B1B2 polymorphism of CETP gene is likely to be a strong genetic predictor of macrovascular complications.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/1/79
Previous studies using semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) have demonstrated that islet xenograft rejection in mice is dominated by Th2-associated cytokines, i.e., interleukin (IL)-4 and IL-10. However, immunohistochemical stainings show that the morphological pattern in this model is more reminiscent of a delayed-type hypersensitivity (DTH) reaction, which is associated with a Th1 response. This study was designed to resolve the mechanisms of acute cellular xenograft rejection in rats transplanted with fetal porcine islet-like cell clusters (ICCs). Real-time quantitative RT-PCR was used to quantify the mRNA expression of cytokines in the grafts and lymph nodes, and the findings were related to the immunopathology of the rejecting grafts. By day 1, mRNA expression levels of IL-1{beta}, IL-2, IL-12p40, interferon-{gamma}, and tumor necrosis factor-{alpha} were already induced in the lymph nodes. From days 3 to 12, an increasing amount of activated macrophages was seen in the grafts, whereas T- and NK-cells were fewer and mainly accumulated in the periphery of the grafts. Most of the ICCs were rejected by day 5. Transcripts of Th1-associated cytokines were dominant in both regional lymph nodes and in the grafts, with peak levels on days 3 and 5, respectively. The mRNA expression of IL-4 was increased on day 12, and it correlated with the infiltration of eosinophils and an increased level of xenoreactive IgG. The data presented indicate that an islet xenograft triggers a sequential activation of 1) a Th1-associated response characterized by graft destruction in a DTH-like reaction and then 2) a subsequent Th2-associated response characterized by increased levels of xenoreactive antibodies.


Reducing fibroblast activation in the kidney is important in the development of diabetic nephropathy. Various renal cell types, including fibroblasts, contribute to the excessive matrix deposition in the kidney. Although transforming growth factor-{beta} (TGF-{beta}) has been thought to play a major role during fibrosis, other growth factors are also involved. Here we examined the effects of connective tissue growth factor (CTGF) and IGF-I on collagen type I and III production by human renal fibroblasts and their involvement in glucose-induced matrix accumulation. We have demonstrated that both CTGF and IGF-I expressions were increased in renal fibroblasts under hyperglycemic conditions, also in the absence of TGF-{beta} signaling. Although CTGF alone had no effect on collagen secretion, combined stimulation with IGF-I enhanced collagen accumulation. Furthermore, IGF-I also had a synergistic effect with glucose on the induction of collagens. Moreover, we observed a partial inhibition of glucose-induced collagen secretion with neutralizing anti-CTGF antibodies, thereby demonstrating for the first time the involvement of endogenous CTGF in glucose-induced effects in human renal fibroblasts. Therefore, the cooperation between CTGF and IGF-I might be involved in glucose-induced matrix accumulation in tubulointerstitial fibrosis and might contribute to the pathogenesis of diabetic nephropathy.


The transcription factor sterol regulatory element binding protein (SREBP)-1c is intimately involved in the regulation of lipid and glucose metabolism. To investigate whether mutations in this gene might contribute to insulin resistance, we screened the exons encoding the aminoterminal transcriptional activation domain in a cohort of 85 unrelated human subjects with...
severe insulin resistance. Two missense mutations (P87L and P416A) were found in single affected patients but not in 47 control subjects. However, these variants were indistinguishable from the wild-type in their ability to bind DNA or to transactivate an SREBP-1 responsive promoter construct. We also identified a common intronic single nucleotide polymorphism (C/T) located between exon 18c and 19c. In a case-control study of 517 U.K. Caucasian case subjects and 517 age- and sex-matched control subjects, the T-allele at this locus was significantly associated with type 2 diabetes in men (odds ratio = 1.42 [1.11-1.82], P = 0.005) but not women. In a separate population-based study of 1,100 Caucasians, carriers of the T-allele showed significantly higher levels of total and LDL cholesterol (P < 0.05) compared with wild-type individuals. In summary, we have conducted the first study of the SREBP-1c gene as a candidate for human insulin resistance. Although the rare mutations identified were functionally silent in the assays used, we obtained some evidence, which requires conformation in other populations, that a common variant in the SREBP-1c gene might influence diabetes risk and plasma cholesterol level.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/2/413

Hypertrophy is one mechanism of pancreatic \{beta\}-cell growth and is seen as an important compensatory response to insulin resistance. We hypothesized that the induction of protective genes contributes to the survival of enlarged (hypertrophied) \{beta\}-cells. Here, we evaluated changes in stress gene expression that accompany \{beta\}-cell hypertrophy in islets from hyperglycemic rats 4 weeks after partial pancreatectomy (Px). A variety of protective genes were upregulated, with markedly increased expression of the antioxidant genes heme oxygenase-1 and glutathione peroxidase and the antiapoptotic gene A20. Cu/Zn-superoxide dismutase (SOD) and Mn-SOD were modestly induced, and Bcl-2 was modestly reduced; however, several other stress genes (catalase, heat shock protein 70, and p53) were unaltered. The increases in mRNA levels corresponded to the degree of hyperglycemia and were reversed in Px rats by 2-week treatment with phlorizin (treatment that normalized hyperglycemia), strongly suggesting the specificity of hyperglycemia in eliciting the response. Hyperglycemia in Px rats also led to activation of nuclear factor-\{kappa\}B in islets. The profound change in \{beta\}-cell phenotype of hyperglycemic Px rats resulted in a reduced sensitivity to the \{beta\}-cell toxin streptozotocin. Sensitivity to the toxin was restored, along with the \{beta\}-cell phenotype, in islets from phlorizin-treated Px rats. Furthermore, \{beta\}-cells of Px rats were not vulnerable to apoptosis when further challenged in vivo with dexamethasone, which increases insulin resistance. In conclusion, \{beta\}-cell adaptation to chronic hyperglycemia and, hence, increased insulin demand is accompanied by the induction of protective stress genes that may contribute to the survival of hypertrophied \{beta\}-cells.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/6/1793

To test the hypothesis that c-Myc plays an important role in \{beta\}-cell growth and differentiation, we generated transgenic mice overexpressing c-Myc in \{beta\}-cells under control of the rat insulin II promoter. F1 transgenic mice from two founders developed neonatal diabetes (associated with reduced plasma insulin levels) and died of hyperglycemia 3 days after birth. In pancreata of
transgenic mice, marked hyperplasia of cells with an altered phenotype and amorphous islet organization was displayed: islet volume was increased threefold versus wild-type littermates. Apoptotic nuclei were increased fourfold in transgenic versus wild-type mice, suggesting an increased turnover of \( \beta \)-cells. Very few cells immunostained for insulin; pancreatic insulin mRNA and content were markedly reduced. GLUT2 mRNA was decreased, but other \( \beta \)-cell-associated genes (IAPP [islet amyloid pancreatic polypeptide], PDX-1 [pancreatic and duodenal homeobox-1], and BETA2/NeuroD) were expressed at near-normal levels. Immunostaining for both GLUT2 and Nkx6.1 was mainly cytoplasmic. The defect in \( \beta \)-cell phenotype in transgenic embryos (embryonic days 17-18) and neonates (days 1-2) was similar and, therefore, was not secondary to overt hyperglycemia. When pancreata were transplanted under the kidney capsules of athymic mice to analyze the long-term effects of c-Myc activation, \( \beta \)-cell depletion was found, suggesting that, ultimately, apoptosis predominates over proliferation. In conclusion, these studies demonstrate that activation of c-Myc in \( \beta \)-cells leads to 1) increased proliferation and apoptosis, 2) initial hyperplasia with amorphous islet organization, and 3) selective downregulation of insulin gene expression and the development of overt diabetes.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/5/1265

The peroxisome proliferator-activated receptor (PPAR)-(gamma)2 gene polymorphism Pro12Ala has been associated with increased insulin sensitivity in some but not all studies. Little is known about its effect on the tracking of insulin resistance status over time. These aspects were examined in a community-based sample of 686 white young adults, aged 20-38 years, and 426 white children, aged 4-17 years, and a subsample of a cohort (n = 362) who participated both as children and adults, with an average follow-up period of 13.4 years. Insulin resistance was measured by the homeostasis model assessment of insulin resistance (HOMA-IR) using fasting insulin and glucose. The frequency of the variant Ala12 allele was 0.104 in whites vs. 0.017 in blacks. After adjusting for sex, age, and BMI, adult subjects with the genotype Pro/Pro, Pro/Ala, and Ala/Ala, respectively, showed significant decreasing trends in fasting insulin (11.7, 10.3, and 8.8 (micro)U/ml; \( P = 0.002 \)) and HOMA-IR (2.4, 2.1, and 1.7; \( P = 0.006 \)). Similar but nonsignificant trends were noted in childhood. A significant genotype-BMI interaction effect on insulin \( (P = 0.020) \), glucose \( (P = 0.007) \), and HOMA-IR \( (P = 0.001) \) was found in adulthood, with carriers versus noncarriers showing attenuated association with BMI. The genotype-BMI interaction effect on these variables tended to be similar in childhood. With respect to tracking over time, of individuals in the top age- and sex-specific quartile of HOMA-IR in childhood, 48.7% (38/78) of noncarriers vs. 16.7% (2/12) of the carriers \( (P = 0.035) \) remained in the same quartile in adulthood. A similar trend was observed for insulin \( (2/13 \text{ vs. } 35/77, P = 0.037) \). In conclusion, the Pro12Ala polymorphism of the PPAR-(gamma)2 gene beneficially influences insulin resistance and its tracking from childhood to adulthood. Further, the Ala12 allele attenuates the adverse association between adiposity and insulin resistance measures.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/5/1609

In an attempt to identify novel susceptibility genes predisposing to early-onset diabetes (EOD), we performed a genome-wide scan using 433 markers in 222 individuals (119 with diabetes) from 29 Scandinavian families with \( \geq 2 \) members with onset of diabetes \( \leq 45 \text{ years} \). The
The highest nonparametric linkage (NPL) score, 2.7 (P < 0.01), was observed on chromosome 1p (D1S473/D1S438). Six other regions on chromosomes 3p, 7q, 11q, 18q, 20q, and 21q showed a nominal P value <0.05. Of the EOD subjects in these 29 families, 20% were GAD antibody positive and 68% displayed type 1 diabetes HLA risk alleles (DQB*02 or 0302). Mutations in maturity-onset diabetes of the young (MODY) 1-5 genes and the A3243G mitochondrial DNA mutation were detected by single-strand conformation polymorphism and direct sequencing. To increase homogeneity, we analyzed a subsample of five families with autosomal dominant inheritance of EOD (greater than or equal to two members with age at diagnosis \(\geq 35\) years). The highest NPL scores were found on chromosome 1p (D1S438-D1S1665; NPL 3.0; P < 0.01) and 16q (D16S419; NPL 2.9; P < 0.01). After exclusion of three families with MODY1, MODY3, and mitochondrial mutations, the highest NPL scores were observed on chromosomes 1p (D1S438; NPL 2.6; P < 0.01), 3p (D3S1620; NPL 2.2; P < 0.03), 5q (D5S1465; NPL 2.1; P < 0.03), 7q (D7S820; NPL 2.0; P < 0.03), 18q (D18S535; NPL 1.9; P < 0.04), 20q (D20S195; NPL 2.5; P < 0.02), and 21q (D21S1446; NPL 2.2; P < 0.03). We conclude that considerable heterogeneity exists in Scandinavian subjects with EOD; 24% had MODY or maternally inherited diabetes and deafness, and 60% were GAD antibody positive or had type 1 diabetes-associated HLA genotypes. Our data also point at putative chromosomal regions, which could harbor novel genes that contribute to EOD.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/5/1523

Polymorphism of \(\alpha2\) integrin (C807T) is shown to be associated with an increased incidence of thrombotic cardiovascular events. However, it is not clear whether this polymorphism is associated with atherosclerotic arterial wall thickening. In this study, we examined the association of C807T polymorphism with arterial wall thickness in 265 control subjects and 272 patients with type 2 diabetes. In all subjects, intima-media thickness of the right carotid artery in the 807TT group (0.649 +/- 0.028 mm [SE]) was significantly (P = 0.0228, Scheffe's F test) less than in the 807CC group (0.767 +/- 0.033). This effect of polymorphism is gene dose dependent (P = 0.0227, ANOVA). The similar association was also observed in patients with diabetes but not in control subjects. Multiple regression analysis in all subjects revealed that the T allele was inversely \((\beta = -0.095, P = 0.021)\) associated with intima-media thickness independent of age, HbA1c, and HDL cholesterol. Finally, an inverse relation between the occurrence of carotid plaque and the T allele was observed in patients with diabetes with an adjusted odds ratio of 0.487 (P = 0.031) in multiple logistic regression analyses. These results suggest that the number of 807T alleles in \(\alpha2\) integrin is protective against atherosclerotic arterial wall thickening and the occurrence of plaque in patients with type 2 diabetes.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/11/3336

We have investigated, in 282 multiplex Caucasian families (the Human Biological Data Interchange Repository), the association of type 1 diabetes with polymorphisms in the IL4R gene. IL4R encodes a subunit of the interleukin-4 receptor, a molecule critical to T-helper cell development. By genotyping eight different IL4R single-nucleotide polymorphisms (SNPs) and identifying haplotypes (complex alleles) in the multiplex type 1 diabetic families who were stratified for HLA genotype, we have observed significant evidence of linkage and association of
the IL4R gene to type 1 diabetes. In particular, we have identified a specific haplotype that appears to be protective and observed that this protective effect is strongest among individuals not carrying the HLA DR3/DR4 genotype (which confers the strongest genetic risk for type 1 diabetes). These findings suggest an important role for the IL4R gene in immune-related disease susceptibility and illustrate the value of using multi-SNP haplotype information in association studies.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/5/1276

The orphan receptor small heterodimer partner (SHP, NR0B2) modulates the transcription activity of the MODY1 gene HNF4a. Mutations in SHP were found in 7% of Japanese obese young-onset type 2 diabetic patients and were associated with moderate obesity and increased birth weight. We investigated SHP in 1927 U.K. subjects, examining relationships with type 2 diabetes, obesity, and birth weight. Sequencing of the coding region of SHP in 122 obese, young-onset type 2 diabetic patients detected the polymorphism G171A. The polymorphism was not associated with diabetes in case control or familial association studies. The A allele (frequency 0.07) was not associated with obesity in type 2 diabetic subjects (n = 348), their parents (n = 272), or young nondiabetic adults (n = 925). However, the rare (<1%) AA homozygotes had a raised BMI in each cohort; this was significant when all cohorts were combined (Z score = 0.67 AA vs. -0.05 G/x, P = 0.02). There was no association with corrected birth weight in 382 normal babies, but the only AA baby was 4,069 g. Our study suggests that genetic variation in SHP is unlikely to be common in the predisposition to diabetes, obesity, or increased birth weight in U.K. Caucasians.

The E23K polymorphism of the pancreatic (β)-cell ATP-sensitive K+ (KATP) channel subunit Kir6.2 (KCNJ11) is associated with type 2 diabetes in whites, and a recent in vitro study of the E23K variant suggests that the association to diabetes might be explained by a slight inhibition of serum insulin release. In a study comprising 519 unrelated glucose-tolerant subjects, we addressed the question as to whether the E23K variant was related to reduced serum insulin release during an oral glucose tolerance test (OGTT). Furthermore, the polymorphism was examined in a case-control study comprising 803 type 2 diabetic patients and 862 glucose-tolerant control subjects. The E23K variant was associated with significant reductions in the insulinogenic index (P = 0.022) and serum insulin levels under the response curve during an OGTT (0-120 min) (P = 0.014) as well as with an increase in BMI (P = 0.013). In the present study, the association of the E23K polymorphism with type 2 diabetes was not significant (P = 0.26). However, the K23K genotype significantly associated with type 2 diabetes in a meta-analysis of white case and control subjects (n = 2,824, odds ratio [OR] 1.49, P = 0.00022). In conclusion, the widespread E23K polymorphism may have a diabetogenic effect by impairing glucose-induced insulin release and increasing BMI.


Type 1 diabetes is an autoimmune disease with a Th1 phenotype in which insulin-producing (β)-cells in the pancreas are destroyed. The T-cell-specific transcription factor TCF7 activates genes involved in immune regulation and is a candidate locus for genetic susceptibility to type 1 diabetes. A nonsynonymous single nucleotide polymorphism (SNP) (Pro to Thr) in the TCF7 gene, C883A, was examined in samples from 282 Caucasian multiplex type 1 diabetic families. HLA-DRB1 and -DQB1 genotypes were previously determined for these samples, allowing data stratification based on HLA-associated risk. The transmission disequilibrium test showed significant overtransmission of the A allele from fathers (64.1%, P < 0.007) and nonsignificant overtransmission (57.4%, P < 0.06) of the A allele to patients who do not carry the highest-risk HLA-DR3/DR4 genotype. Elliptical sib pair analysis showed significant associations of the A allele with type 1 diabetes in paternal transmissions (P < 0.03), transmissions to male children (P < 0.04), and in the non-DR3/DR4 group (P < 0.04). These data also suggest that TCF7 C883A may affect age of disease onset. Analysis of genotype data from surrounding SNPs suggests that this TCF7 polymorphism may itself represent a risk factor for type 1 diabetes.


The pancreatic and duodenal homeobox factor-1 (PDX-1), also known as IDX-1/STF-1/IPF1, is a homeodomain-containing transcription factor, plays a central role in regulating pancreatic development and insulin gene transcription. Furthermore, even in adults, PDX-1 is associated with islet neogenesis and differentiation of insulin-producing cells from progenitor cells. Here, we report for the first time that PDX-1 protein can permeate cells due to an Antennapedia-like protein...
transduction domain sequence in its structure and that transduced PDX-1 functions similarly to endogenous PDX-1; it binds to the insulin promoter and activates its expression. PDX-1 protein can also permeate into isolated pancreatic islets, which leads to stimulation of insulin gene expression. Moreover, PDX-1 protein transduced into cultures of pancreatic ducts, thought to be islet progenitor cells, induces insulin gene expression. These data suggest that PDX-1 protein transduction could be a safe and valuable strategy for enhancing insulin gene transcription and for facilitating differentiation of ductal progenitor cells into insulin-producing cells without requiring gene transfer technology.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/1/199

We have identified patients in whom strenuous physical exercise leads to hypoglycemia caused by inappropriate insulin release (exercise-induced hyperinsulinism [EIHI]). The aim of the present study was to test the hypothesis that the increased levels of lactate and/or pyruvate during anaerobic exercise would trigger the aberrant insulin secretion in these patients. A total of 12 patients (8 women and 4 men from two families) were diagnosed with EIHI, based on hypoglycemia and a more than threefold increase in plasma insulin induced by a 10-min bicycle exercise test. The mode of inheritance was autosomal dominant in these families. The acute response of insulin release to a bolus of intravenous pyruvate (13.9 mmol/1.73 m2) was studied in the patients and eight healthy control subjects. Insulin secretion did not respond to the pyruvate bolus in healthy control subjects. However, all EIHI patients responded to pyruvate, displaying a brisk increase in plasma insulin. The 1 + 3-min peak response was 5.6-fold in the patients and 0.9-fold in the control subjects (P < 0.001). To test the hypothesis that the pathogenesis of EIHI would involve monocarboxylate transport or metabolism in the β-cell, we sequenced the genes encoding the known monocarboxylate transporter proteins and tested the transport of pyruvate into patient fibroblasts. The results revealed normal coding sequences and pyruvate transport. In conclusion, EIHI represents a new autosomal-dominant hyperinsulinemia syndrome that may be more common than has been realized. The pyruvate test provides a simple, safe, and specific diagnostic test for this condition.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/suppl_3/S308

Genome scans in families with type 2 diabetes identified a putative locus on chromosome 20q. For this study, linkage disequilibrium mapping was used in an effort to narrow a 7.3-Mb region in an Ashkenazi type 2 diabetic population. The region encompassed a 1-logarithm of odds (LOD) interval around the microsatellite marker D20S107, which gave a LOD score of >3 in linkage analysis of a combined Caucasian population. This 7.3-Mb region contained 25 known and 99 predicted genes. Predicted single nucleotide polymorphisms (SNPs) were chosen from public databases and validated. Two SNPs were unique to the Ashkenazi. Here, 91 SNPs with a minor allele frequency of [≥]10% were genotyped in pooled DNA from 150 case subjects and 150 control subjects of Ashkenazi Jewish descent. The SNP association study showed that SNP rs2664537 in the TIX1 gene had a significant P value of 0.035, but the finding did not replicate in an additional case pool. In addition, HNF4a and Mybl2 were screened for mutations and new polymorphisms. No mutations were identified, and a new nonsynonymous SNP (R687C in exon 14 of Mybl2) was found. The limits to this type of association study are discussed.

http://diabetes.diabetesjournals.org/cgi/content/abstract/53/2/486

In a panel of large Caucasian pedigrees, we genotyped markers in eight chromosomal regions previously reported as supporting linkage with type 2 diabetes. We previously reported significant linkage on chromosome 20q (maximum logarithm of odds score [MLS] = 2.79) in this panel. In the present analysis, candidate regions on 1q, 2q, 3q, 5q, 9q, and 10q yielded little evidence for linkage; a region on 2p (MLS = 1.64, P = 0.01 at position 9.0 cM) gave suggestive evidence of linkage; and a region on 8p (MLS = 3.67, P = 2.8 x 10^-5, at position 7.6 cM) gave significant evidence of linkage. Conditional analyses were performed for both 2p and 8p regions and the region reported on 20q. The MLS for 2p increased from 1.64 to 1.79 (empirical P = 0.142) when conditioned for heterogeneity on 20q. The case was similar for 8p, where the MLS increased from 3.67 to 4.51 (empirical P = 0.023) when conditioned on families without evidence of linkage at 20q. In conclusion, our data support a type 2 diabetes susceptibility locus on chromosome 8p that appears to be independent from other susceptibility loci. Although we were able to replicate linkage in our pedigrees on chromosome 2p, we did not find evidence of linkage for regions on 1q, 2q, 3q, 5q, 9q, or 10q.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/3/657

During short-term fasting, substrate utilization in skeletal muscle shifts from predominantly carbohydrate to fat as a means of conserving glucose. To examine the potential influence of short-term fasting and refeeding on transcriptional regulation in skeletal muscle, muscle biopsies were obtained from nine male subjects at rest, after 20 h of fasting, and 1 h after consuming either a high-carbohydrate (CHO trial) or a low-carbohydrate (FAT trial) meal. Fasting induced an increase in transcription of the pyruvate dehydrogenase kinase 4 (PDK4) (10-fold), lipoprotein lipase (LPL) (~2-fold), uncoupling protein 3 (UCP3) (~5-fold), and carnitine palmitoyltransferase I (CPT I) (~2.5-fold) genes. Surprisingly, transcription of PDK4 and LPL increased further in response to refeeding (both trials) to more than 50-fold and 6- to 10-fold, respectively, over prefasting levels. However, responses varied among subjects with two subjects in particular displaying far greater activation of PDK4 (>100-fold) and LPL (>20-fold) than the other subjects (mean ~8-fold and ~2-fold, respectively). Transcription of UCP3 decreased to basal levels after the CHO meal but remained elevated after the FAT meal, whereas CPT I remained elevated after both refeeding meals. The present findings demonstrate that short-term fasting/refeeding in humans alters the transcription of several genes in skeletal muscle related to lipid metabolism. Marked heterogeneity in the transcriptional response to the fasting/refeeding protocol suggests that individual differences in genetic profile may play an important role in adaptive molecular responses to metabolic challenges.

The Pro12Ala polymorphism in the PPAR(\text{gamma})2 gene has been associated with reduced risk of type 2 diabetes and insulin resistance. Recently, an association between dizygotic twinning and PPAR(\text{gamma}) gene polymorphisms has been proposed. We investigated the phenotypic appearance of the two polymorphisms (Pro12Ala and exon 6 C\rightarrow T) in PPAR(\text{gamma}) among elderly twins (207 monozygotic [MZ] and 342 dizygotic [DZ]) and evaluated whether they could explain previously reported differences in plasma glucose and insulin profiles among MZ and DZ twins. We demonstrated a significant impact of the Pro12Ala polymorphism on glucose tolerance, diabetic status, homeostasis model assessment for insulin resistance, and plasma insulin profiles in twins. No impact of the silent exon 6 polymorphism on glucose homeostasis or plasma insulin profiles was found. Independent of the polymorphisms, we observed a significant impact of zygosity status per se on the plasma insulin profile after oral glucose ingestion, with the MZ twins being more hyperinsulinemic, indicating insulin resistance, than the DZ twins. Nonsignificantly higher glucose concentrations were observed among MZ compared with DZ twins. We demonstrated an association between the Ala allele and reduced risk of diabetes and insulin resistance in twins. However, the differences in metabolic profiles among MZ and DZ twins were not explained by differences in frequencies of the genetic variants and may be due to intrauterine environmental factors operating in twins independent of genotype. Accordingly, our study simultaneously supports a role for both the intrauterine environment (thrifty phenotype) and for genetics (thrifty genotype) in the etiology of insulin resistance and perhaps glucose intolerance in twins.


The embryonic pancreas is thought to develop from pluripotent endodermal cells that give rise to endocrine and exocrine cells. A key guidance mechanism for pancreatic development has previously been found to be epithelial-mesenchymal interaction. Interactions within the epithelium, however, have not been well studied. Glucagon is the earliest peptide hormone present at appreciable levels in the developing pancreatic epithelium (embryonic day [E]-9.5 in mouse). Insulin accumulation begins slightly later (E11 in mouse), followed by a rapid accumulation during the "second wave" of insulin differentiation (\text{\sim}E15). Here we found that blocking early expression and function of glucagon, but not GLP-1, an alternate gene product of preproglucagon mRNA, prevented insulin-positive differentiation in early embryonic (E11) pancreas. These results suggest a novel concept and a key role for glucagon in the paracrine induction of differentiation of other pancreatic components in the early embryonic pancreas.


Impairment of retinal vascular homeostasis is associated with the development and progression of diabetic retinopathy involving gap junction intercellular communication (GJIC) activity. The principal gap junction protein of intercellular communication, connexin, was investigated to determine the effects of high glucose concentrations on the expression of endothelial-specific connexins (Cx37, Cx40, and Cx43), connexin phosphorylation pattern, and GJIC activity. Rat microvascular endothelial (RME) cells grown in high (30 mmol/l)-glucose medium for 9 days had
reduced Cx43 expression: Cx43 mRNA (68 +/- 13% of control; P = 0.019, n = 5) and protein (55.6 +/- 16% of control; P = 0.003, n = 5) levels were reduced; however, Cx37 and Cx40 expression was not affected. Using alkaline phosphatase and Western blot analyses, we identified three forms of Cx43: a nonphosphorylated form (P0) and two phosphorylated forms (P1 and P2). Expression of all three forms was decreased in cells grown in high-glucose medium: PO, 73 +/- 15% of control (P = 0.04); P1, 57 +/- 16% of control (P = 0.01); and P2, 42 +/- 22% of control (P = 0.006). Using immunofluorescence microscopy, we observed Cx43 localization at specific sites of contact (plaques) between adjacent cells. In cells grown in high-glucose medium, we observed reduced plaque counts (63 +/- 6% of control; P = 0.009) and decreased intensity of Cx43 immunofluorescence compared with cells grown in normal medium. Furthermore, using scrape load dye transfer (SLDT) technique, we found that these cells exhibited reduced GJIC activity (60% of control; P = 0.01, n = 5). The reduction in GJIC activity correlated with the decreased Cx43 protein levels (r = 0.9). These results indicate that high glucose concentrations inhibited GJIC activity by reducing Cx43 synthesis in RME cells. Impaired intercellular communication may contribute to breakdown of homeostatic balance in diabetic microangiopathy.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/5/1111

A new murine model of porcine islet-like cell cluster (ICC) xenograft rejection, avoiding interference of unspecific inflammation, was introduced and used to investigate rejection mechanisms. Athymic (nu/nu) mice were transplanted with syngeneic, allogeneic, or xenogeneic islets under the kidney capsule. After the original transplantation, immune cells in porcine ICC xenografts undergoing rejection in native immunocompetent mice were transferred to the peritoneal cavity of the athymic mice. At defined time points after transfer, the primary grafts were evaluated by immunohistochemistry and real-time quantitative RT-PCR to estimate cytokine and chemokine mRNA expression. Transfer of immunocompetent cells enabled athymic (nu/nu) mice to reject a previously tolerated ICC xenograft only when donor and recipient were matched for major histocompatibility complex (MHC). In contrast, allogeneic and syngeneic islets were not rejected. The ICC xenograft rejection was mediated by transferred T-cells. The main effector cells, macrophages, were shown to be part of a specific immune response. By day 4 after transplantation, there was an upregulation of both Th1- and Th2-associated cytokine transcripts. The transferred T-cells were xenospecific and required MHC compatibility to induce rejection. Interaction between the TCR of transferred T-cells and MHC on host endothelial cells and/or macrophages seems necessary for inducing ICC xenograft rejection.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/3/784

Paternal mutation of ATP-sensitive K+ (KATP) channel genes and loss of heterozygosity (LOH) of the 11p15 region including the maternal alleles of ABCC8, IGF2, and CDKN1C characterize the focal form of persistent hyperinsulinemic hypoglycemia of infancy (FoPHHI). We aimed to understand the actual nature of FoPHHI in comparison with insulinoma. In FoPHHI, the lesion consists in clusters of (beta)-cells surrounded by non-(beta)-cells. Compared with adjacent islets, proinsulin mRNA is similar and proinsulin production higher (P <= 0.02), indicating regulation at a translational level, with slightly lower insulin stock and lower ABCC8 peptide labeling (P<0.05).
Insulinomas, composed of (beta)-cell nests or cords, have similar proinsulin mRNA compared with adjacent islets, highly variable proinsulin production, lower insulin stock ($P <= 0.02$), and higher ABCC8 peptide labeling ($P<0.05$). Proinsulin mRNA is lower than in FoPHHI ($P<0.001$). Islets adjacent to FoPHHI appear to be resting, in contrast to those adjacent to insulinomas, evidencing intrapancreatic regulation of islet (beta)-cell activity. IGF2 peptide is present inside and outside both lesions, but IGF2 mRNA is restricted to the lesions. The 11p15 LOH and absence of CDKN1C peptide staining are demonstrated in all FoPHHI but also in three of eight insulinomas. Despite some molecular similarities, FoPHHI is thus fundamentally different from insulinoma.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/7/1611

Insulin resistance is a component of type 2 diabetes and often precedes pancreatic (beta)-cell failure. Contributing factors include obesity and a central pattern of fat accumulation with a strong genetic component. The adipocyte secreted hormone resistin has been proposed as a link between the adipocyte and insulin resistance by inhibition of insulin-stimulated glucose uptake and/or blocking adipocyte differentiation. Here we report that the G/G genotype of a single nucleotide polymorphism (SNP) in the promoter of the human resistin gene, -180C>G, had significantly increased basal promoter activity in adipocytes. These data were recapitulated in vivo, where G/G homozygotes had significantly higher resistin mRNA levels in human abdominal subcutaneous fat. A significant interaction was also found between the -180C>G SNP, a marker of oxidative stress (NAD[P]H quinone oxidoreductase mRNA) and homeostasis model assessment of insulin resistance. In addition, resistin mRNA was positively and independently correlated with insulin resistance and hepatic fat as measured by liver X-ray attenuation. These data implicate resistin in the pathophysiology of the human insulin resistance syndrome, an effect mediated by the -180C>G promoter SNP and potentially cellular oxidative stress.


http://diabetes.diabetesjournals.org/cgi/content/abstract/52/12/2914

It is well recognized that the agouti/melanocortin system is an important regulator of body weight homeostasis. Given that agouti is expressed in human adipose tissue and that the ectopic expression of agouti in adipose tissue results in moderately obese mice, the link between agouti expression in human adipose tissue and obesity/type 2 diabetes was investigated. Although there was no apparent relationship between agouti mRNA levels and BMI, agouti mRNA levels were significantly elevated in subjects with type 2 diabetes. The regulation of agouti in cultured human adipocytes revealed that insulin did not regulate agouti mRNA, whereas dexamethasone treatment potently increased the levels of agouti mRNA. Experiments with cultured human preadipocytes and with cells obtained from transgenic mice that overexpress agouti demonstrated that melanocortin receptor (MCR) signaling in adipose tissue can regulate both preadipocyte proliferation and differentiation. Taken together, these results reveal that agouti can regulate adipogenesis at several levels and suggest that there are functional consequences of elevated agouti levels in human adipose tissue. The influence of MCR signaling on adipogenesis combined with the well-established role of MCR signaling in the hypothalamus suggest that adipogenesis is coordinately regulated with food intake and energy expenditure.

http://diabetes.diabetesjournals.org/cgi/content/abstract/52/1/214

Polycystic ovary syndrome (PCOS) is a leading cause of anovulatory infertility and affects [~]4-7% of reproductive age women in the U.S. It is characterized by hyperandrogenemia and chronic anovulation and is associated with insulin resistance, obesity, and increased risk for type 2 diabetes. In a screen of candidate genes, a region on chromosome 19p13.3 was identified that shows significant evidence for both linkage and association with PCOS. A promising candidate gene for PCOS, resistin, maps to exactly this region. Resistin is a protein hormone thought to modulate glucose tolerance and insulin action. We tested for association between a single nucleotide polymorphism in the promoter region of the resistin gene and three phenotypes: PCOS, obesity, and insulin resistance. We did not find evidence for association with any of the phenotypes. It is therefore unlikely that variation in the resistin gene accounts for the strong association that we observe between chromosome 19p13.3 and PCOS. Instead, this association is most likely due to a gene or genetic element in this region that has not been identified.


http://diabetes.diabetesjournals.org/cgi/content/abstract/54/5/1400

Treatment of animals or certain cells with carbon monoxide (CO), a product of heme degradation by heme oxygenase-1 (HO-1), has potent anti-inflammatory and antiapoptotic effects that contribute to the survival of transplanted organs. We report here that inducing HO-1 in, or administering CO to, only the donor can be used in a therapeutic manner to sustain the survival of transplanted allogeneic islets. Similar treatments of only the islets or only the recipient are also salutary. Administering CO only to the donor frequently leads to long-term survival of those islets in untreated allogeneic recipients, which are then antigen-specifically tolerant. Several proinflammatory and proapoptotic genes that are strongly induced in islets after transplantation in the untreated situation were significantly suppressed after administering CO to the donor without further treatment. These included tumor necrosis factor-α, inducible nitric oxide synthase, monocyte chemoattractant protein-1, granzyme B, and Fas/Fas ligand, all of which contribute to the pathogenesis of the rejection of transplanted islets. This correlated with a lesser infiltration of recipient macrophages into the transplanted islets. Our present findings show that induction of HO-1 in, or administration of CO to, only the donor, islets, or the recipient or combinations of such treatments improve allogeneic islet survival.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/9/2709

Protein kinase C (PKC) (beta) isofom activity is increased in myocardium of diabetic rodents and heart failure patients. Transgenic mice overexpressing PKC(beta)2 (PKC(beta)2Tg) in the myocardium exhibit cardiomyopathy and cardiac fibrosis. In this study, we characterized the expression of connective tissue growth factor (CTGF) and transforming growth factor (beta)
(TGF{beta}) with the development of fibrosis in heart from PKC{beta}2Tg mice at 4-16 weeks of age. Heart-to-body weight ratios of transgenic mice increased at 8 and 12 weeks, indicating hypertrophy, and ratios did not differ at 16 weeks. Collagen VI and fibronectin mRNA expression increased in PKC{beta}2Tg hearts at 4-12 weeks. Histological examination revealed myocyte hypertrophy and fibrosis in 4- to 16-week PKC{beta}2Tg hearts. CTGF expression increased in PKC{beta}2Tg hearts at all ages, whereas TGF{beta} increased only at 8 and 12 weeks. In 8-week diabetic mouse heart, CTGF and TGF{beta} expression increased two- and fourfold, respectively. Similarly, CTGF expression increased in rat hearts at 2-8 weeks of diabetes. This is the first report of increased CTGF expression in myocardium of diabetic rodents suggesting that cardiac injury associated with PKC{beta}2 activation, diabetes, or heart failure is marked by increased CTGF expression. CTGF could act independently or together with other cytokines to induce cardiac fibrosis and dysfunction.


http://diabetes.diabetesjournals.org/cgi/content/abstract/51/6/1964

We have studied the occurrence of enterovirus (EV)-RNA at the onset of childhood type 1 diabetes in all 24 new cases of childhood type 1 diabetes during 1 year in Uppsala county, Sweden. We also studied 24 matched control subjects and 20 siblings of the patients. RNA was isolated from peripheral blood mononuclear cells and EV-RNA detected by RT-PCR. Primers (groups A and B) corresponding to conserved regions in the 5' noncoding region (NCR) of EV were used in the PCRs, and the amplicons were sequenced. By the use of group A primers, EV-RNA was found in 12 (50%) of the 24 type 1 diabetic children, 5 (26%) of 19 siblings, and none of the control subjects. Both patients and siblings showed a higher frequency of EV-RNA compared with the control subjects. The group B primers detected EV-RNA in all three groups but did not show statistically significant differences between the groups. The EV-RNA positivity with the group B primers was 11 (46%) of 24 in the type 1 diabetic children, 11 (58%) of 19 in the siblings, and 7 (29%) of 24 in the control subjects. The significant difference between groups seen with the group A primers but not with the group B primers might indicate the existence of diabetogenic EV strains. The phylogenetic analysis of the PCR products revealed clustering of the sequences from patients and siblings into five major branches when the group A PCR primers were used. With the group B primers, the sequences from patients, siblings, and control subjects formed three major branches in the phylogenetic tree, where 6 of the 7 control subjects clustered together in a sub-branch of CBV-4/VD2921. Seven of the type 1 diabetic children clustered together in another sub-branch of CBV-4/VD2921. Five of the type 1 diabetic children formed a branch together with the CBV-4/E2 strain, four clustered together with CBV-5, and one formed a branch with echovirus serotype. The presence of EV-RNA in the blood cells of most newly diagnosed type 1 diabetic children supports the hypothesis that a viral infection acts as an exogenous factor. In addition, sequencing of the PCR amplicons from the type 1 diabetic children, their siblings, and matched control subjects might reveal differences related to diabetogenic properties of such a virus.


http://diabetes.diabetesjournals.org/cgi/content/abstract/53/5/1271

Dietary carbohydrate activates the sympathetic nervous system (SNS). As the mechanisms underlying this response are not fully characterized, studies were undertaken to compare SNS responses to ingestion of glucose, fructose, and galactose. SNS activity was examined using techniques of [3H]norepinephrine ([3H]NE) turnover in brown and white fat. In addition, gene...
expression for several sympathetically related proteins was also analyzed in these tissues. [3H]NE turnover in interscapular brown adipose tissue (IBAT) and retroperitoneal fat increased in response to glucose and fructose in the diet, whereas [3H]NE turnover in epididymal fat did not respond to either monosaccharide. Galactose feeding, by contrast, decreased [3H]NE turnover in IBAT, but increased it in epididymal, though not retroperitoneal, fat. Expression of GLUT4 was more abundant in IBAT and retroperitoneal fat from glucose- and fructose-fed animals than from diet- or galactose-fed rats. Chemical sympathectomy abolished the GLUT4 response in retroperitoneal fat, but was without effect on GLUT4 in epididymal fat. These studies are consistent with activation of a neural pathway by oral glucose or fructose, leading to SNS activation in IBAT and retroperitoneal fat and enhanced GLUT4 expression.

Diabetes Care (2)


http://care.diabetesjournals.org/cgi/content/abstract/27/7/1660

OBJECTIVE--Pioglitazone is a member of the thiazolidinediones (TZDs), insulin-sensitizing agents used to treat type 2 diabetes. The aim of this study was to define the effect of pioglitazone on the expression of genes related to carbohydrate and lipid metabolism in subcutaneous fat obtained from type 2 diabetic patients. RESEARCH DESIGN AND METHODS--Forty-eight volunteers with type 2 diabetes were divided into two groups treated for 12 weeks with placebo or pioglitazone (30 mg/day). The expression of several genes was quantified by real-time RT-PCR. RESULTS--Pioglitazone treatment increased the expression of genes involved in glycerol-3-phosphate synthesis. The mRNA expression of PEPCK-C and glycerol-3-phosphate dehydrogenase (GPDH) increased (P < 0.01) in patients treated with pioglitazone. There was no difference in glycerol kinase (GyK) mRNA levels. The expression of genes that regulate fatty acid availability in adipocytes, including lipoprotein lipase (LPL) and acetyl-CoA synthetase (ACS), was higher (P < 0.01) in pioglitazone-treated patients. Pioglitazone stimulated (P < 0.0001) expression of c-Cbl-associated protein (CAP), whereas tumor necrosis factor-(alpha), leptin, resistin, angiopoietin like-4, and 11-{beta}-hydroxysteroid dehydrogenase type 1 (11{beta} HSD 1) were not affected by pioglitazone. The baseline peroxisome proliferator-activated receptor (PPAR)-{gamma}1 mRNA was significantly correlated with mRNA for LPL, CAP, ACS, 11{beta} HSD 1, GyK, fatty acid synthase, leptin, and GPDH, whereas PPAR-{gamma}2 mRNA was correlated with CAP, PEPCK-C, leptin, and GPDH. CONCLUSIONS--Treatment with pioglitazone increased body weight, and this is associated with upregulation of some, but not all, genes previously demonstrated as "TZD responsive" in subcutaneous fat. The results suggest that TZDs might increase body weight through the upregulation of genes facilitating adipocyte lipid storage in vivo.


http://care.diabetesjournals.org/cgi/content/abstract/26/3/843
OBJECTIVE--To clarify the role of the T-lymphocyte-associated-4 (CTLA-4) polymorphism in the susceptibility to child-onset type 1 diabetes with regard to its clinical characteristics and complications with autoimmune thyroid disease (AITD) in the Japanese population. RESEARCH DESIGN AND METHODS--The CTLA-4 49 A/G polymorphism was detected by the PCR-restriction fragment-length polymorphism (RFLP) method in 97 type 1 diabetic subjects and 20 patients with Graves' disease, a cohort which included 4 patients who also had type 1 diabetes. RESULTS--The genotypes and allele frequencies of this polymorphism did not differ between the type 1 diabetic subjects and the control subjects. The G allele frequency was 63.9% in the type 1 diabetic subjects. The G allele frequency in the subgroup of patients with a high titer of autoantibodies to the GAD antibody (Ab) was 72.9% (P = 0.0499 vs. control subjects); in the subgroup of patients without HLA DRB1*0405, it was 72.6% (P = 0.0271 vs. control subjects); and in the subgroup of patients with a residual \{beta\}-cell function, it was 78.6% (P = 0.0391 vs. control subjects). The G allele frequency in the patients with Graves' disease was also significantly higher at 78.1% (P = 0.0405 vs. control subjects). Furthermore, the frequency in our diabetic subjects complicated with Graves' disease was even higher (87.5%). CONCLUSIONS--We have demonstrated that a distinct association exists between the G allele of CTLA-4 and high values of GAD Ab, residual \{beta\}-cell function, and the absence of HLA-DRB1*0405.

Diabetes Research and Clinical Practice (8)


The aim of this study was to investigate whether an association exists between the angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism and microvascular complications of type 2 diabetes mellitus in Turkish patients. A total of 239 type 2 diabetic patients and 138 sex and age matched control subjects were included into the study. The I/D polymorphism was determined by polymerase chain reaction (PCR). Nephropathy status was determined according to urinary albumin/creatinine ratio ([mu]g/mg) (300 macroalbuminuria) and retinopathy was evaluated by fundoscopic examination and by fluorescein fundus angiography. The distribution of ACE I/D polymorphism and allele frequencies in diabetic patients were not significantly different from controls, DD genotype 32.2 versus 37.2%; ID genotype 50.6 versus 47.1%; and II 17.2 versus 15.2%; D allele 57.5 versus 61.2%; I allele 42.5 versus 38.8%. Genotype distribution between normo-, micro- and macroalbuminuric patients did not differ significantly (DD:ID:II (%), normoalbuminuria, 35:46:19; microalbuminuria, 28:55:17; macroalbuminuria, 31:55:14). There was also no difference in genotype distribution between patients with and without retinopathy (DD:ID:II (%), retinopathy positive, 32:51:17; retinopathy negative, 33:49:18). In conclusion, the ACE I/D polymorphism does not seem to be associated with diabetic nephropathy and retinopathy in Turkish type 2 diabetic patients.

Oxidative stress and the gene expression at the transcriptional level of antioxidant enzymes were investigated in two models of diabetes in mice. We used KKAy mice as a model of obese insulin-resistant diabetes, and streptozotocin-induced diabetic mice (STZ mice) as a model of insulin-deficient diabetes. C57BL mice and saline-injected ICR mice were used as the respective non-diabetic controls. To assess oxidative damage, plasma malondialdehyde (MDA), urine 8-isoprostane and 8-hydroxy deoxyguanosine (8-OHdG) were measured. The mRNA expression of antioxidant enzymes, superoxide dismutase 1 (SOD-1) and glutathione peroxidase 1 (GPx-1) in the kidney and heart were quantified using a real-time polymerase chain reaction. The KKAy mice demonstrated moderate hyperglycemia and hyperlipidemia, and the STZ mice showed severe hyperglycemia and hypolipidemia. The KKAy mice, but not the STZ mice, showed elevated plasma MDA relative to the non-diabetic controls. Urine 8-isoprostane and 8-OHdG in both diabetic mouse groups increased significantly. The urine oxidative stress markers in the severely hyperglycemic STZ mice were higher than those in the moderately hyperglycemic KKAy mice. Although GPx-1 and SOD-1 showed elevated mRNA expression in the KKAy mice in the kidney and heart, in the STZ mice they did not increase compared to the controls. The compensatory up-regulation of the mRNA expression of antioxidant enzymes may be impaired in the insulin-deficient severely hyperglycemic state.


http://www.sciencedirect.com/science/article/B6T5Y-41TN55C-5/2/4979b84e7c2f9ab9b5eacc2a87464a3

We investigated the relationship between advanced diabetic retinopathy (ADR) and an angiotensin-converting enzyme (ACE) gene polymorphism in subjects with type 2 diabetes and ADR, pre-proliferative (PrePDR) or proliferative diabetic retinopathy (PDR) without overt nephropathy. Polymerase chain reactions were used to detect insertion/deletion (I/D) polymorphisms of the ACE gene. There was no difference in the frequency of II, ID, or DD genotypes, or of I and D alleles among subjects with type 2 diabetes without diabetic retinopathy (NDR) or with simple diabetic retinopathy (SDR) and non-diabetic controls. There was also no difference in the frequency of ACE genotypes among subjects with type 2 diabetes with NDR, or SDR and ADR. However, the frequency of the ACE DD genotype in ADR was significantly higher than that in controls ([chi]2=6.64, P=0.036). On the other hand, the frequency of the D allele in ADR was significantly higher than that in controls ([chi]2=6.33, P=0.012), NDR ([chi]2=4.18, P=0.041) and SDR ([chi]2=4.89, P=0.027), respectively. These results indicate a significant relationship between the presence of the D allele polymorphism in the ACE gene and ADR in Japanese subjects with type 2 diabetes and no overt nephropathy.


http://www.sciencedirect.com/science/article/B6T5Y-44PK68P-2/2/9174a313bd7d0bceba2222edc5c7d133

Recently Iwata et al. reported that the polymorphism in NeuroD exon 2(Ala45Thr) was associated with adult-onset Type 1 diabetes in Japanese. Furthermore, the mutations in the NeuroD as a regulator of insulin transcription have been reported to result in Type 2 diabetes. We, therefore,
aimed to clarify the role of this Ala45Thr polymorphism in the susceptibility to Type 1a, immune-mediated, diabetes of child-onset Japanese patients. Eighty patients with child-onset Type 1 diabetes were examined along with 121 non-diabetic subjects as the controls. The polymorphism in Ala45Thr was defined using the PCR-RFLP method. The GAD Ab, IA-2 Ab, HLA-DRB1 genotypes and residual [beta]-cell function at 3 years from onset were evaluated in relation to the difference in this polymorphism. The frequency of the Ala45Thr heterozygotes was significantly higher in the Type 1 diabetic patients than in the controls (21.3 versus 9.9%, P=0.0252). The frequency of loss of [beta]-cell function was higher in heterozygotes patients than in wild type homozygotes patients (P=0.0112). Type 1 diabetic patients with DRB1*0901 allele showed a significantly higher frequency, 27.9%, of the Ala45Thr variant than the controls (P=0.0041). In conclusion, the Ala45Thr polymorphism contributes to the risk of development of, and to the early deterioration of [beta]-cell function, in Type 1a diabetes among the Japanese population.


http://www.sciencedirect.com/science/article/B6T5Y-42BSNV3-3/2/1348bc5b63c6cb0cbbc24e7294fa8ba51

A case-control study to investigate whether the aldose reductase (AC)n dinucleotide polymorphism (termed 5'-ALR2 polymorphism) is useful as a genetic marker for risk of microvascular complications among Caucasians Type 1 diabetic patients in Australia is reported. This marker was amplified from patient genomic DNA and then fractionated in 5% formamide-urea gels. A total of nine alleles was observed with Z, Z-2 and Z+2 being the major alleles. The distribution of alleles was comparable in diabetic subjects with diabetes and microvascular complications, diabetes without complications and normal non-diabetic control subjects. Similarly, when the distribution of alleles was examined in the patients subcategorized according to the presence of diabetic nephropathy or diabetic neuropathy, no significant association was observed. While the size of the study makes it impossible to exclude a weak linkage, it is concluded that the 5'-ALR2 polymorphism is not useful as a genetic marker for susceptibility to diabetic microvascular complications in Caucasian Type 1 diabetic patients.


http://www.sciencedirect.com/science/article/B6T5Y-3XK6TFR-8/2/34a4d28d96276e8534610e0125a7a196

To clarify risk factors for the progression of microalbuminuria in Japanese type 2 diabetic patients, the longitudinal study for 10 years was conducted on 67 outpatients with type 2 diabetes, who had shown no overt proteinuria at baseline. The urinary albumin index (UAI) has been determined based on the mean of at least two random urine samples each year. Categories were defined as normoalbuminuria (UAI =300.0). Progression was defined as worsening of the category and/or more than doubling of the baseline UAI value. Multiple logistic regression analysis was performed using age, duration of diabetes, HbA1c, blood pressure, BMI, serum lipids, smoking habits, and alcohol consumption as independent variables and the progression of microalbuminuria as a dependent variable. Age and HbA1c were estimated as significant and independent variables. Furthermore, genetic polymorphisms of angiotensin I-converting enzyme (ACE) and angiotensinogen were analyzed to evaluate the genetic contribution. The D/D genotype of ACE was significantly more common in progressors than in non-progressors. These
results suggest that glycemic control and age are important risk factors and the D/D genotype of ACE acts as a risk factor for the progression of microalbuminuria in Japanese type 2 diabetic patients.


Variants of calpain-10 gene (CAPN 10) have recently been reported to be associated with type 2 diabetes (T2DM). Haplotype combination 112/121 defined by three single nucleotide polymorphisms (SNPs) (UCSNP-43, -19 and -63) of CAPN 10 conferred the highest risk for T2DM in Mexican-Americans. In this study, we aim to examine whether these genetic variants contribute to the susceptibility for T2DM in a Chinese population. The frequencies of these three SNPs were determined in 168 patients with T2DM and 104 controls. Distribution of alleles, genotypes and haplotypes at three loci were not significantly different between the two groups. No difference was observed in the 112/121 haplotype combination distribution. However, haplotype combination 112/221 was more prevalent in the control group than in T2DM group (16.35% versus 7.14%, p = 0.025). Control subjects with haplotype combination 112/121 had higher serum cholesterol level than others without haplotype combination 112/121 (5.7 [plus-or-minus sign] 1.4 versus 5.2 [plus-or-minus sign] 0.7, p = 0.011). Our results suggest that haplotype combination 112/221 associated with reduced risk for T2DM and haplotype combination 112/121 might be a risk factor for increased serum cholesterol in Chinese population.

http://www.sciencedirect.com/science/article/B6T5Y-3W26929-6/2/4f409d0afae7974147a320b56cd63c6

In order to clarify the nature of T lymphocytes infiltrating the pancreatic islets of patients with insulin-dependent diabetes mellitus (IDDM), we analysed T cell receptor (TCR) gene transcripts expressed in pancreatic biopsy specimens of patients with recent-onset IDDM. We also investigated the expression of cytokines (interferon-[gamma]; IFN-[gamma]; tumour necrosis factor-[alpha]; TNF-[alpha]; interleukin-4: IL-4; interleukin-6: IL-6) in the same specimens. The TCR V[beta] repertoire was not restricted either in the pancreas or the peripheral lymphocytes of IDDM patients. In contrast, the TCR V[alpha] repertoire was restricted in the pancreas, but not in the peripheral blood lymphocytes, of IDDM patients. The sequence analysis of the complementarity-determining region 3 (CDR3) of the TCR[alpha] revealed the presence of dominant clonality in [alpha] chains of T cells in the patients. IFN-[gamma] mRNA was highly expressed in the pancreas of IDDM patients, while IL-4 mRNA was deficient. A lower level of expression of IL-6 mRNA was detected in the IDDM pancreas than in the control tissue. These results indicate that T cells bearing a distinct TCR[alpha] chain are selectively retained and activated within the pancreas of recent-onset IDDM.

http://www.sciencedirect.com/science/article/B6T60-3RXYC61-3/2/c78a7c1620545384eb06544f0541675f

Quantitative-competitive polymerase chain reaction (QPCR) was performed on serial sputum samples from 22 consecutive cases of acid fast bacilli (AFB) smear-positive pulmonary tuberculosis. Of 94 specimens, 55, 72, and 83% were positive by culture, AFB smear, and QPCR, respectively. Of 52 culture-positive specimens, 6% were negative by PCR, and 13% were negative by AFB smear. Of 42 culture-negative specimens, AFB smear and QPCR were positive in 55 and 61%, respectively. AFB smear and QPCR results were strongly correlated (r = 0.75, p < 0.001), but each correlated less strongly with culture (r = 0.54, p < 0.005 for smear and R = 0.52, p < 0.005 for QPCR). When patients were classified by microbiologic response, responders tended to have less DNA in their sputum and shorter time to a negative PCR result compared to nonresponders. These data do not suggest a great advantage of QPCR over AFB smear for predicting culture results in patients with pulmonary tuberculosis.


http://www.sciencedirect.com/science/article/B6T60-421TKTG-6/2/5bbf4cfbc2bf174cbb651c966684b77b

The nature and frequency of mutations in the rpoB gene of rifampin-resistant clinical Mycobacterium tuberculosis isolates vary considerably according to geographical locations. There is no information on the prevalence of specific mutations in clinical M. tuberculosis strains isolated from patients in Middle-Eastern countries. In this study, 13 rifampin-resistant and 6 susceptible clinical M. tuberculosis isolates were tested for identification and characterization of mutations in the rpoB gene by INNO-LiPA Rif. TB kit and DNA sequencing of the PCR amplified target DNA. The kit identified all six susceptible strains as rifampin-sensitive and the DNA sequence of the amplified rpoB gene in the target region matched perfectly with the wild-type sequence. The kit identified 12 resistant isolates as rifampin-resistant with specific detection of mutations in 8 isolates while one of the rifampin-resistant strain was identified as rifampin-susceptible. DNA sequencing confirmed these results and, in addition, led to the specific detection of mutations in 4 rifampin-resistant isolates in which specific base changes within the target region could not be determined by the INNO-LiPA Rif. TB kit. The majority (8 of 13) of resistant isolates involved base changes at codon 531 of the rpoB gene. Mutations at codon position 531 within the rpoB gene have also been reported in majority of rifampin-resistant strains from Greece and St. Petersburg, Russia but not from other geographical locations.


http://www.sciencedirect.com/science/article/B6T60-47F4R44-6/2/602005e45f1049c15c51c27211d63888
Mutations conferring resistance to rifampin in rifampin-resistant clinical Mycobacterium tuberculosis isolates occur mostly in the 81 bp rifampin-resistance-determining region (RRDR) of the rpoB gene. In this study, 29 rifampin-resistant and 12 -susceptible clinical M. tuberculosis isolates were tested for characterization of mutations in the rpoB gene by line probe (INNO-LiPA Rif. TB) assay and the results were confirmed and extended by DNA sequencing of the PCR amplified target DNA. The line probe assay identified all 12 susceptible strains as rifampin-sensitive and the DNA sequence of RRDR in the amplified rpoB gene from two isolates matched perfectly with the wild-type sequence. The line probe assay identified 28 resistant isolates as rifampin-resistant with specific detection of mutation in 22 isolates including one isolate that exhibited hetro-resistance containing both the wild-type pattern as well as a specific mutation within RRDR while one of the rifampin-resistant strain was identified as rifampin-susceptible. DNA sequencing confirmed these results and, in addition, led to the specific detection of mutations in 5 rifampin-resistant isolates in which specific base changes within RRDR could not be determined by the line probe assay. These analyses identified 8 different mutations within RRDR of the rpoB gene including one novel mutation (S522W) that has not been reported so far. The genotyping performed on the isolates carrying similar mutations showed that majority of these isolates were unique as they exhibited varying DNA banding patterns. Correlating the ethnic origin of the infected TB patients with the occurrence of specific mutations at three main codon positions (516, 526 and 531) in the rpoB gene showed that most patients (11 of 15) from South Asian region contained mutations at codon 526 while majority of isolates from patients (6 of 11) of Middle Eastern origin contained mutations at codon 531.


http://www.sciencedirect.com/science/article/B6T60-4DS878B-6/2/3496eb8ddcaa20814e314e27bad00ff

We have developed a real-time PCR assay for detection of Trypanosoma brucei DNA in human blood samples. The PCR was conducted with newly designed primers targeting the 177-bp repeat satellite DNA in T. brucei and with Sybr Green to monitor the amplicon accumulation. DNA purification using Chelex 100(R) resin was performed on blood samples collected on Whatman FTA(R) cards and was shown to be a simple and quantitative method as revealed by real-time PCR. The detection limit of the assay was 100 trypanosomes per mL blood, corresponding to an analytical sensitivity of 0.1 genome equivalents. Trypanosome DNA was detected in all blood samples from sleeping sickness patients and, furthermore, the identity of the amplicon was confirmed in all assays by dissociation analysis. Although template DNA from blood samples was amplified with significantly lower efficiency than genomic DNA, similar efficiency between all assays ensured quantitative results. No amplicon product was obtained with samples from uninfected individuals. The results indicate that the real-time PCR assay described is a rapid and sensitive method suitable for the detection of T. brucei in human blood samples in routine clinical laboratory practice.


http://www.sciencedirect.com/science/article/B6T60-46DKTM6-6/2/a1bcf92dd81d44283d8315e61eabfd86

To optimize routine screening for cryptosporidiosis, 198 stool samples from patients at risk and from calves were examined by enzyme immunoassay (EIA), a direct fluorescent-antibody (DFA)
and a modified immunofluorescence assay. Ninety-nine samples were positive in at least one assay, whereas 99 were negative in all three assays. Sensitivity of antigen EIA and DFA were similar (94%, 95% CI: 88-98%, and 91%, 95% CI: 84-95%). The modified immunofluorescence was significantly less sensitive (64%, 95% CI: 55-74%). 149 samples were also examined by two nested PCR assays targeting either the 18S rRNA or Cryptosporidium outer wall protein (COWP) gene. A PCR product was amplified from 86 out of 89 samples being positive in at least one other assay (sensitivity 97%, 95% CI: 91-99%). None was obtained from 60 samples negative in the three other assays. PCR assays did not increase the detection rate. Antigen EIA or DFA appear sufficient for routine Cryptosporidium screening of fecal samples.


Strains of Chlamydomphila pneumoniae may be associated with respiratory disease or atherosclerosis. Two real-time quantitative PCR assays targeting the species-specific genes Cpn0278 and ArgR were developed to compare the in vitro growth of respiratory strains AR39 and K6 with that of atherosclerotic strain A03 and to quantify C. pneumoniae in clinical samples. A third real-time PCR assay was designed to assess contamination with Mycoplasma spp. The assays targeting C. pneumoniae detected DNA concentrations corresponding to 104 to 10-4 inclusion-forming units (IFU)/reaction and were highly specific. AR39 exhibited the longest lag phase and period of exponential growth; K6 augmented growth rates at higher inocula; and A03 grew at highest rates. Contamination with Mycoplasma spp. of AR39 and A03 unlikely accounted for growth differences between them. Numbers of IFU in C. pneumoniae-positive respiratory secretions varied within 4 to 5 orders of magnitude. The assays described may prove valuable for pathogenicity studies.


http://www.sciencedirect.com/science/article/B6T60-3V98FG0-5/2/28bc41712cd3e2b4ba7e491f1d43eb4a

Polymerase chain reaction and cytotoxin assays were performed to identify as Helicobacter pylori type I (cagA+/tox+) or type II (cagA-/tox-) 56 (59.6%) strains from 94 patients. Of these patients 64 were affected by nonulcer dyspepsia (NUD), 10 by gastric ulcer (GU), 19 by duodenal ulcer (DU), and 1 by both GU and DU. H. pylori strains were tested for cagA using two sets of primers; target sequences were detected in 40-42/56 (71.4-75%) depending on the set of primers used, while cytotoxin-producing strains (tox+) were 26/56 (46.4%). Tox+ strains were isolated in 13/32 (40.6%), 2/7 (28.6%), and 11/17 (64.7%) in NUD, GU, and DU patients, respectively. However, the different percentage between cagA+ strains from NUD patients (13/32; 40.6%) and patients with ulcerative diseases (13/23; 54.2%) is not statistically significant (p = 0.462). Because the two sets of primers employed for amplification of cagA target sequences give different results, we concluded that cagA alone could not be taken as predictive factor for severity of gastroduodenal disease. It has been found that H. pylori type I is associated with duodenal ulcer disease.
Several reports have evidenced geographic differences in the prevalence of vacA (vacuolating cytotoxin gene) alleles and cagA (cytotoxin-associated gene) status among Helicobacter pylori isolates. We investigated the occurrence of these virulence-associated genes status among our isolates, and their relationship with ulcer disease outcome. Besides, ureA-B polymorphism was studied. One hundred isolates, comprising 32 from patients with ulcer disease (UD) and 68 from patients with non-ulcer dyspepsia (NUD), were analyzed. Eighty-four percent of isolates were cagA-positive without statistically significant difference in prevalence between patients with UD or NUD. Genotype vacA-s1m1 was predominant, although unlike other South American regions, subtype s1am1 occurrence was higher than s1b. The multivariate model used to estimate the predictive value of cagA and vacA status for UD development disclosed infection with vacA-s1am1 isolates as the only variable that increased the risk of UD onset. ureAB fingerprinting showed considerable genetic divergence among isolates, however, confirmed that certain DNA banding profiles are conserved worldwide.

Acinetobacter spp. isolates were increasingly obtained from clinical specimens and sterility samples, and a subsequent epidemiological investigation implicated an intermittently contaminated supply of commercially acquired enrichment broths. Typing was performed with DNA amplification by the polymerase chain reaction (PCR) using enterobacterial repetitive intergenic consensus sequence primers, ERIC2 and reverse ERIC1R. The reliability of this PCR-based typing method was verified by the ability of the technique to demonstrate homology and differences among isolates from an epidemiologically well-defined pseudo-outbreak.

The structural gene encoding the 10-kD antigen from Mycobacterium tuberculosis was amplified by the polymerase chain reaction. The 297-base-pair (bp) product was detected among 45 strains representing 14 mycobacterial species, but was absent from 11 species related to the mycobacteria. The gene was localized to a ~ 2000-bp SstII restriction fragment of the organisms' chromosomes.


A multiplex real-time polymerase chain reaction (RT-PCR) targeting the mecA and nuc genes was developed for the detection of methicillin resistance and identification of Staphylococcus aureus. Novel mecA and nuc primers and fluorescence resonance energy transfer hybridization probes specific for the mecA and nuc genes were evaluated. The assay was performed using the LightCycler system (Roche Molecular Biochemicals, Mannheim, Germany) and evaluated against the traditional gel-based multiplex PCR (PCR-gel) method currently used at Royal Perth Hospital. Clinical isolates (n = 222) and isolates from a culture collection library (n = 206) were tested by both assays in parallel. The RT-PCR assay was 100% sensitive and specific for the detection of methicillin resistance and for the identification of S. aureus when compared with the PCR-gel assay. Results from the RT-PCR assay showed 5 isolates with lower efficiency fluorescence curves for the nuc gene PCR fragment. DNA sequencing showed mutations within the region of the probe-binding sites compared with the reference strain. The results of the RT-PCR assay were available within 2 h. This rapid mecA/nuc RT-PCR assay is a suitable and practical tool for the routine detection of methicillin resistance and identification of S. aureus, which can be easily incorporated into the diagnostic molecular microbiology laboratory work flow.


This study was conducted to assess the reliability of a commercial enzyme-linked viral inducible system (ELVIS) (Diagnostic Hybrids, Inc., Athens, OH) for rapid detection and typing of herpes simplex virus (HSV). Results using ELVIS were compared to those of shell vial culture (SVC) and HSV detection with monoclonal antibodies and an immunoperoxidase stain plus typing with MicroTrak direct fluorescent antibodies (Trinity Biotech PLC, Wicklow, Ireland). Specimens yielding discrepant HSV results were tested by polymerase chain reaction (PCR); those with discrepant typing results were stained with Simulfluor (Chemicon, Temecula, CA). Of the 206 samples tested, 144 were negative and 54 were HSV-positive by both methods (agreement, 96.1%). Five specimens were positive by ELVIS but negative by SVC; 3 of these were positive and 2 were negative by HSV PCR. Both of the latter were the result of mechanical problems early in the study. Three specimens were positive by SVC but negative by ELVIS; all 3 were positive by HSV PCR. After resolution of discrepancies, the sensitivity and specificity for detection of HSV were 95.0% and 100% for SVC, respectively, and 95.0% and 98.6% for ELVIS. Of the 46 HSV-positive samples that were typed, 26 were called type 2 and 18 were type 1 by both methods (agreement, 95.7%). The 2 specimens with discrepant results were called HSV-2 by SVC, staining with MicroTrak, and HSV-1 with ELVIS; both of these were type 2 when stained with the Simulfluor reagent. ELVIS is a reliable alternative to SVC for rapid detection and typing of HSV.

A small but significant proportion of blood cultures processed by the BACTEC 9000 series systems is signaled positive, while subsequent Gram's stain and culture on solid media yield no pathogens. In this study, 15 "false-positive" vials (7 aerobes, 8 anaerobes) from 15 patients were investigated for the presence of bacteria and fungi by eubacterial 16S rDNA and panfungal 18S rDNA amplification, respectively. All samples turned out negative by both methods. Most patients (7) had neutropenia, which does not support the theory that high leukocyte counts enhance the generation of false-positive results. In conclusion, the results of this study indicate that false-negative results generated by the BACTEC 9000 series are inherent to the automated detection and not due to the growth of fastidious organisms.


Six Candida dubliniensis isolates were recovered from two HIV-infected individuals in the course of a prospective study of recurrent oral candidosis among HIV-positive patients in Spain. Candida albicans strains as well as non-albicans strains were also obtained from these two patients. C. dubliniensis strains were germ-tube-positive and produced abundant chlamydospores. Fingerprinting the genomic DNAs of these six C. dubliniensis with the C. albicans-specific probe 27A as well as karyotyping was performed to confirm the identification of these isolates. Further analysis of their genomic DNAs was performed by PCR-fingerprinting with the core sequence of phage M13, and they exhibited species-specific multilocus band patterns, clearly distinct from those of C. albicans isolates analyzed in this study and in a previous one (Diaz-Guerra 1997). Intraspecies variation was also seen among PCR patterns yielded by C. dubliniensis isolates from different patients. Although few strains have been analyzed, the use of this PCR-fingerprinting procedure is a promising tool for further epidemiologic studies with C. dubliniensis. The isolation of C. dubliniensis from Spanish HIV-infected patients contributes to the idea of widespread geographic distribution of this species.


The Roche MagNA Pure automated nucleic acid extraction system was tested for its ability to extract Borrelia burgdorferi DNA from a diverse set of spiked specimen types including blood, cerebral spinal fluid, synovial fluid, urine and ticks. A method comparison between MagNA Pure automated extraction and manual extraction, using either QIAamp columns or phenol/chloroform extraction, showed equivalent detection sensitivities for all methodologies with all specimen types (except for urine, in which case QIAamp extraction was twofold less sensitive). Eighty positive clinical specimens (as determined by an independent testing method), including 76 synovial fluid, and 4 cerebral spinal fluid specimens, were found to be positive by the MagNA Pure/real-time PCR method of extraction and detection. This data shows that the MagNA Pure system can be
used to extract B. burgdorferi DNA from clinical specimens, and when combined with real-time PCR, the result is an extremely sensitive assay with limited hands on time and rapid turn around times.


http://www.sciencedirect.com/science/article/B6T60-405KCXV-1/2/157f1cef778053da98288d09aa7fe114

To improve tools for the surveillance of invasive H. influenzae in the context of the drastic decrease of type b infections following the implementation of vaccination, a two-step PCR technique was developed to detect the capsule and type specific regions of H. influenzae. The technique of Falla et al (1994) was modified to amplify in a first step the capsule and type b regions by multiplex PCR. For non-b capsulated strains, the type a, c, d, e, and f loci were afterward detected simultaneously by an optimized touch-down PCR technique. An internal control of extraction and amplification (16S rDNA) was included for both PCR techniques. Overall, this technique was shown to perform as efficiently or better than the slide agglutination without risks of interpretation errors. Of the 138 H. influenzae strains tested, seven that had given doubtful results by the agglutination technique were unequivocally typed by PCR.


http://www.sciencedirect.com/science/article/B6T60-3VH0WSC-5/2/dc876aa69dd5a2d251b8fed3bf4ed486

A set of 47 Austrian human, food, and veterinary Escherichia coli O157:H7 isolates was used to evaluate five different epidemiological typing methods. Ribotyping using an automated microbial characterization system (RiboPrinter(TM)) was not suitable for detection of epidemiological relatedness. All but one E. coli strain were typeable by phage typing. Random amplified polymorphic DNA-PCR fingerprinting was performed using primer M13 containing the sequence 5'-GAG GGT GGC GGT TCT-3' and primer 1247 (5'-AAGAGCCCGT-3'). Although both methods recognized only two clusters, both dendrograms grouped most of the EHEC O157 isolates into epidemiologically related subgroups. Pulsed-field gel electrophoresis of XbaI digested total DNA was a valuable subtyping system. We found that major differences can exist between results of multiple subtyping methods. E. coli O157 isolates should not be classified as epidemiologically related or nonrelated on the basis of a single typing method alone.


The minimum inhibitory concentrations (MICs) of 18 antibiotics were determined for 66 clinical isolates of staphylococci. Genotypes, mutations in the quinolone resistance-determining regions
(QRDRs), and effect of efflux were determined in the 18 levofloxacin-resistant isolates, for which the MICs of levofloxacin were high (\(\geq 8\) [\(\mu\)g/ml]). The increased levofloxacin resistance mainly resulted from some combinations of mutations in the QRDRs, although NorA-mediated efflux may play a minor role in resistance. A combination of mutations in GrI (Ser80Phe), GrlB (Pro451Ser), and GyrA (Ser84Leu) was found in 4 methicillin-resistant Staphylococcus aureus (MRSA) isolates that were unrelated genotypically. The mutations in grlA QRDR varied in the isolates classified as being in an identical pulsed-field gel electrophoresis (PFGE) group, although the grlB, gyrA, and gyrB QRDRs were the same. These results suggest that the patterns of amino acid mutations in the QRDRs can provide distinct epidemiologic information from PFGE genotypes in fluoroquinolone-resistant MRSA. A combination of at least three mutations in GrI, GrI, and/or GyrA is required to increase the MICs of fluoroquinolones, although all of the levofloxacin-resistant MRSA retained the MICs of sitafloxacin in the range of 1 to 2 [\(\mu\)g/ml].


http://www.sciencedirect.com/science/article/B6T60-45R56KW-2/2/1d507e5d8882d1c51c7ca27f8f23e35b

A hexon-based fluorogenic polymerase chain reaction (PCR) assay utilizing the 5'-nuclease activity of DNA Taqpolymerase was developed as a rapid and type-specific diagnostic system for adenovirus type 4 (Ad4) detection and quantification. The assay consists of a pair of flanking primers and an internal fluorescence labeled probe that allows real time amplification to quantify the Ad4 virus. One out of 12 flanking primer pairs evaluated (combinations of three forward primers and four reverse primers) was found to be optimal for Ad4 virus detection that yielded background-free operation, i.e., no fluorescent signal generated by non-template controls. The assay was employed to detect Ad4 reference virus strain RI-67, Wyeth Ad4 vaccine strain and 71 different clinical Ad4 isolates from US military recruits used in this study with consistent sensitivity (lower detection limit) of 2-4 pfu per PCR reaction. The assay showed linear Ad4 detection with a dynamic range of greater than five logs (from 2-4 pfu/assay to greater than 105 pfu/assay). This Ad4-specific assay did not crossreact with representative members of Ad subgroups A, B, C, D and F at viral concentrations greater than 108 pfu/ml. It was also demonstrated that Ad4 viruses could be efficiently detected from throat swabs (71/72 specimens or 98.6% detection sensitivity) of infected patients by the Ad4-specific PCR. In general, there was a good correlation between PCR determined viral titers in throat swabs and time required to detect viral cytopathic effects (CPE) in cell culture. Evaluation of the simple Ad4 specific assay developed in this study could be used to provide a rapid clinically relevant diagnosis of Ad4 infections in patients with acute respiratory disease (ARD).


http://www.sciencedirect.com/science/article/B6T60-43F3W7B-3/2/239af0b0f5c2432a4902584527f8d5bf

A novel ceuE-based multiplex PCR system was developed as an efficient diagnostics test to detect and differentiate C. jejuni and C. coli. There is no cross reactivity between C. jejuni and C. coli. In addition, the assay does not produce a positive signal from other enteric bacteria including Salmonella, Shigella and Escherichia coli strains. Campylobacter detection sensitivity was determined to be equivalent to previously reported PCR for other enteric bacteria. We also
noticed that silicon dioxide extraction can improve Campylobacter detection sensitivity from infected stool samples. It was demonstrated that the PCR assay developed in this study had a much better Campylobacter detection rate than the traditional culturing method (77% versus 56%). However, we also identified small numbers of culture positive stools (8%, or 16 out of 202 samples) that did not yield PCR positive results for Campylobacter. These PCR negative/culture positive stools were proven to be inhibitory to PCR amplification.


http://www.sciencedirect.com/science/article/B6T60-3XG80VC-5/2/9747befffb63fd866e099c38ae7d70f7d

We have evaluated a PCR technique using primers based on Pneumocystis carinii major surface glycoprotein (MSG) genes, a multicopy gene family, for utility in detection of P. carinii in BAL and oropharyngeal samples obtained from immunosuppressed patients. These primers were able to detect P. carinii DNA in as little as 16 fg of genomic DNA. PCR using MSG primers detected P. carinii DNA in 7 smear-positive BAL samples (100% sensitivity), and found no P. carinii DNA in 12 smear-negative BAL samples (100% specificity). Mitochondrial ribosomal RNA (mrRNA) primers, commonly used in PCR studies of PCP, detected P. carinii in six of seven positive samples (85.7% sensitivity) and none of 12 were negative samples (100% specificity). Diagnosis of PCP by amplification of 81 oropharyngeal samples using MSG primers had a 50% sensitivity (4/8) and 96% specificity (70/73). PCR with mrRNA primers was 37.5% sensitive (3/8) and 100% specific (73/73). All three false-positive MSG results showed a very low intensity on Southern hybridization. PCR using MSG gene primers should prove valuable in the diagnosis of PCP.


http://www.sciencedirect.com/science/article/B6T60-476TVR0-4C/2/fbab5916856b98935f9b8ff64c5e5b0

The polymerase chain reaction (PCR) and automated DNA sequencing were used to detect a genetic locus, rpoB, associated with rifampin resistance in Mycobacterium tuberculosis (TB) in clinical isolates and directly in clinical specimens. Primers derived from the sequence of a TB rpoB gene fragment were used to amplify DNA from bacterial and mycobacterial isolates. An rpoB-specific PCR product was obtained for five of five TB, seven of eight other mycobacterial species. Nocardia sp., Corynebacterium sp., Streptomyces sp., Actinomyces sp., and Rhodococcus sp., but not for 15 isolates (eight genera) representing usual bacterial flora. Sequence comparison of the amplified rpoB region revealed the occurrence of TB-specific "signature nucleotides" at three positions. PCR yielded amplification products for seven of 16 clinical specimens. Five of the seven contained TB-specific DNA, as well as sequences that predicted rifampin susceptibility in accord with agar dilution results. None of ten specimens that were culture negative for TB yielded TB-specific PCR products. These results with a limited number of clinical specimens demonstrate the feasibility of direct detection by PCR of rifampin-resistant TB in clinical specimens. Such testing may serve as a rapid surrogate test for multidrug-resistant TB in laboratories with PCR and automated sequencing capability.
This study evaluated a polymerase chain reaction (PCR) method for detection of methicillin-resistant Staphylococcus aureus (MRSA) in specimens referred for nosocomial surveillance. PCR was used to detect the mecA and nuc gene targets using yellow growth on mannitol salt agar containing 6 mg/liter oxacillin (MSO-6) as a source of DNA (N = 645). The diagnostic values for PCR compared with culture methods were 97% specificity, 100% sensitivity, 96% positive predictive value, and 100% negative predictive value. Total cost for PCR per test is $3.62 compared to $4.77 for culture. However, the total cost per specimen is significantly lower due to only 20% of all surveillance specimens producing yellow colonies on MSO-6. The average turnaround time for the PCR method is 48 h compared with 82 h for culture. PCR amplification of mecA and nuc genes using yellow colonies on MSO-6 is a simple, fast, accurate and cost-effective method for routine use in clinical laboratories for detecting MRSA in surveillance specimens.


A patient case report describes an Enterococcus faecium strain isolated from a blood culture that was resistant to linezolid (MIC, 8 [mu]g/mL; G2576U mutation of 23S rRNA). Co-resistances were identified for vancomycin, ampicillin, macrolides, fluoroquinolones, chloramphenicol, rifampin, gentamicin (high-level), nitrofurantoin and trimethoprim/sulfamethoxazole. Etest (AB BIODISK, Solna, Sweden) and disk diffusion results also detected the oxazolidinone resistance pattern. Laboratories should be aware of the rare possibility of these strains occurring during linezolid therapy or spontaneously (this case) in contemporary practice, and have in vitro susceptibility methods available capable of detecting oxazolidinone resistance.


We sampled commensal yeasts from three body sites of 24 healthy individuals to examine the patterns of commensal yeast species distribution and strain relatedness within and among individuals. To examine the short-term dynamics, each individual was sampled three times every 35-40 days at each of three body sites: mouth, fingernail, and toenail. The hosts included six genealogically unrelated individuals and 18 that belonged to four families. A total of 63 morphologically distinct colonies were isolated, identified, and genotyped. Nine yeast species were recovered, including 28 isolates of Candida albicans; 26 of C. parapsilosis; 2 each of C. kru sei and C. tropicalis; and 1 each of C. famata, C. glabrata, C. guilliermondii, C. lusitaniae and Trichosporon beigeli. A significant difference in total yeast recovery rate between families was
observed. However, body sites did not differ in the rates of yeast recovery. The three body sites showed different species distributions with the fingernail sample containing the highest species diversity, followed by the toenail sample. The oral sample contained the lowest species diversity with all 23 oral isolates being C. albicans. Among the 63 strains, forty-six unique genotypes were identified by PCR fingerprinting. Eleven shared-genotypes were identified, seven of which were from the same body site of the same host. The other four were from different members of the same family. Several family-specific genotypes and genotype clusters were found but the results were inconsistent with strict familial transmission of human commensal yeasts. A single host can have multiple species or multiple genotypes of the same species at the same or different body sites. Changes of species and genotypes over the sampling period for the same body site of individual hosts were also observed, including one direct observation of familial yeast transmission between two members of the same family during our sampling period. Our results indicate dynamic processes of yeast colonization, maintenance and evolution in healthy human hosts.


http://www.sciencedirect.com/science/article/B6T60-3X231S4-6i/2/6a9b64c83146838bd390a2e113f55588

A simple PCR set-up for the detection of cytomegalovirus in clinical specimen was developed. All components of the PCR master mix including Taq DNA polymerase, uracil N-glycosilase, and primers were preformulated and stored frozen in aliquots. After thawing the master mix aliquots, the PCR was immediately started after the addition of sample DNA. This method gave excellent reproducible PCR-results without loss of enzyme activities following storage at -20[deg]C for at least 4 months.


http://www.sciencedirect.com/science/article/B6T60-48TK61T-1/2/7eb253f70e65d1d6fe4d75e76171f8b3

Phenotypic identification of fungi in clinical microbiology laboratoreis is often difficult and late, especially for slow growing and rarely encountered fungi. We describe the application of 18S ribosomal RNA (rRNA) gene sequencing in the early diagnosis of a case of Exophiala peritonitis. A yeast-like fungus was isolated from the dialysate fluid of a 66-year-old man undergoing continuous ambulatory peritoneal dialysis. It grew slowly after 12 days of incubation to yield mature cultures to permit recognition of microscopic features resembling those of Exophiala, a dematiaceous mold. 18S rRNA gene sequencing provided results 12 days earlier than phenotypic identification and revealed 15 base difference (0.9%) between the isolate and Exophiala sp. strain GHP 1205 (GenBank Accession no. AJ232954), indicating that the isolate most closely resembles a strain of Exophiala species. The patient responded to 4 weeks of intravenous amphotericin B therapy. Early identification of the fungus was important for the choice of anti-fungal regimen. As opportunistic fungal infections in immunocompromised patients are globally emerging problems, the development of molecular techniques for fungal identification is crucial for early diagnosis and appropriate treatment.
The human gastrointestinal tract harbors an extremely diverse and complex microbial ecosystem. Most of the existent data about the enteric microflora have been generated using stool samples, but the collection and storage of fecal samples are often problematic. The influence of the storage of stool samples on the bacterial diversity and the degradation of bacterial DNA was analysed in this study. Stool samples from 5 healthy volunteers were exposed to different storage temperatures and durations. The bacterial diversity and the amount of intact bacterial DNA were analysed by single-stranded conformation polymorphism analysis (SSCP) and real-time polymerase chain reaction (PCR), both using a 16S rDNA approach. Additionally, biopsy specimens were taken from 3 of the 5 individuals to compare fecal and mucosal flora. The bacterial diversity of the fecal flora and the total number of bacteria were significantly reduced after 8 and 24 hours at both room temperature and 4°C. The mucosa-associated bacterial microflora showed substantial differences compared with the fecal flora. The observed alterations of fecal flora during storage point to the difficulty of the molecular analysis of the bacterial diversity and the enumeration of bacterial cells in fecal samples.

Pneumocystis carinii pneumonia (PCP) remains a major cause of morbidity and mortality in immunocompromised patients, including those infected with human immunodeficiency virus (HIV). The advent of real-time PCR technology offers the potential for rapid PCR results for the detection of P. carinii. In this report we describe the modification and evaluation of an existing PCR-based method for the detection of P. carinii DNA, into a real-time PCR assay suitable for use with the LightCycler system. Twenty eight induced sputum and bronchial washing specimens from 28 patients were tested by both a conventional PCR assay and a real-time PCR assay. Twelve specimens (42.9%) were positive in both the conventional and real-time PCR assays and sixteen (57.1%) were negative in both assays. The melting points of the amplified P. carinii DNA product obtained by melting curve analysis by the LightCycler of all P. carinii positive specimens ranged from 81.5[deg]C to 83.9[deg]C. There were no discordant results between the two assays for any of the specimens tested and results were available within 2 h for the real-time PCR assay compared to up to 11 h for the conventional PCR assay.

This study was designed to characterize H. pylori from pediatric gastric biopsy specimens in terms of several genes (vacA, cagA, cagE, iceA1, iceA2, and babA2) proposed to be involved in...
the pathogenesis of this organism. Many of these genes have been studied in adult H. pylori isolates, however, these genes have not been well characterized in H. pylori from children. Using PCR we observed that 44% of the H. pylori in our biopsies shared two common genotypes (vacA s1b m1, cagA, cagE, iceA2 +/- babA2). While 26% of the H. pylori had unique genotypes. The cag pathogenicity island associated genes, cagA and cagE, were found together in 64% or our H. pylori, while 84% were iceA2 positive. The presence of the babA2 gene has been proposed to be associated with a higher risk of H. pylori related diseases, however, we found that only 36% of our H. pylori contained this gene.


A model of acute disseminated Candida albicans infection in New Zealand rabbits was developed to determine the sensitivity and accuracy of polymerase chain reaction (PCR) assay compared with the lysis-centrifugation blood culture method. Primers used amplify a DNA fragment from the multicopy gene coding for the small subunit rRNA, highly conserved in fungi. The sensitivity of PCR achieved in rabbit blood samples spiked with Candida albicans was 10-50 CFU/100 [mu]L. A nested-PCR increased the limit of detection 10-fold. The sensitivity achieved exclusively with the lysis-centrifugation method (37.5%) was higher than that obtained with PCR (25%), but lower than nested PCR (52.5%). The combination of both techniques, lysis-centrifugation and nested PCR, increased the overall sensitivity rate to 62.5%. These results have demonstrated that, globally, the nested PCR was more sensitive than both single PCR and lysis-centrifugation culture in detecting C. albicans in blood from immunocompetent rabbits with acute disseminated candidosis. PCR could be a useful complementary technique to traditional methods in the early diagnosis of candidemia.


Reliable detection of methicillin resistance in coagulase-negative staphylococci (CNS) is required for appropriate therapy of serious infections from these pathogens. To determine the most accurate method of measuring methicillin resistance in CNS initially reported as methicillin susceptible by automated methods, we compared mecA detection by polymerase chain reaction (PCR) with phenotypic methods. One hundred eighty-eight blood culture isolates of CNS that were initially reported as susceptible to methicillin using commercial methods (Vitek or MicroScan) were tested by agar dilution, disk diffusion, oxacillin salt agar screen plate, and a multiplex PCR assay using primer sets for mecA and 16S rRNA. Sixteen isolates (8.5%) previously reported as methicillin susceptible by automated methods contained the mecA gene. MICs of these isolates ranged from 0.5 [mu]g/mL to >=128 [mu]g/mL. Ten of these isolates had MICs equal to or below the NCCLS breakpoint of 2 [mu]g/mL. Six of the 10 isolates (4 with MICs of 0.5 [mu]g/mL and 2 with MICs of 2 [mu]g/mL) did not grow on any of the oxacillin screen plates after 48 h of incubation at 30[deg]C or 35[deg]C. All six isolates were induced to grow in the presence of oxacillin at 128 [mu]g/mL by serial passaging on plates containing increasing concentrations of antibiotic. Retesting with MicroScan and Vitek detected methicillin resistance in 7 and 10 isolates, respectively. Disk diffusion testing with incubation for 48 h proved to be the
next best method after PCR for detection of methicillin resistance (15 of 16 isolates). Commercial automated methods and some methods recommended by National Committee for Clinical Laboratory Standards may not detect methicillin resistance in CNS that carry the mecA gene and have MICs just below breakpoint.


http://www.sciencedirect.com/science/article/B6T60-3RXYC61-1/2/1c3147fba2e228bc0630a144bea17125

The opportunistic pathogen Pneumocystis carinii (PC) is a frequent cause of a life-threatening pneumonia in human immunodeficiency virus (HIV)-infected individuals and in other immunocompromised hosts. Specimens obtained from 128 bronchoalveolar lavage (BAL) fluid samples from 123 HIV-positive patients with pulmonary disease and undergoing a diagnostic bronchoscopy were evaluated to detect this organism. We have developed a rapid DNA extraction procedure for nested polymerase chain reaction (PCR) using two sets of primers (pAZ102-E, pAZ102-H and P1 = 5'-CTAGGATATAGCTGGTTTTC-3' and P2 = 5'-TCGACTATCTAGCTTATCGC-3'). The results were compared using cytological techniques (direct wet mount, Giemsa, toluidine blue O) and related to the clinical follow-up of patients. The nested PCR had a 91% sensitivity and a 93% specificity. The effect of chemoprophylaxis and the evaluation of the follow-up of patients are discussed. Nested PCR may represent an important additional tool, along with current cytological methods, for the detection of P. carinii; however, at present it cannot replace routine microbiological methods more simple and less expensive.


http://www.sciencedirect.com/science/article/B6T60-3YCM0KG-C/2/f38e299856a88da140ac044e0b5d0c0f

Current laboratory diagnosis of Lyme borreliosis relies on tests for the detection of antibodies to Borrelia burgdorferi with known limitations. By using a simple extraction procedure for urine samples, B. burgdorferi DNA was amplified by a nested PCR with primers that target the specific part of the flagellin gene. To control possible inhibition of the enzyme (polymerase), a special assay using the same primers was developed. We examined 403 urine samples from 185 patients with skin manifestations of Lyme borreliosis. Before treatment, B. burgdorferi DNA was detected in 88 of 97 patients with Lyme borreliosis. After treatment, all but seven patients became nonreactive. Six of these seven persons suffered from intermittent migratory arthralgias or myalgias, and one from acrodermatitis chronica atrophicans. Two of 49 control patients with various dermatologic disorders and none out of 22 presumably healthy persons were reactive in the PCR. In addition to urine, breast milk from two lactating women with erythema migrans was tested and also found reactive. Borrelia burgdorferi DNA can be detected with high sensitivity (91%) by a nested PCR in urine of patients with Lyme borreliosis. In addition, this test can be a reliable marker for the efficacy of treatment.

Control of Bordetella pertussis in the community is hampered by slow and insensitive diagnostic tests. We therefore examined the accuracy and cost of culture, direct fluorescent antibody (DFA) staining, and PCR in a routine clinical laboratory. Six hundred thirty seven nasopharyngeal swabs and aspirates in casamino acids transport medium were cultured, stained with polyclonal (Difco), and monoclonal (BL-5 and Accu-Mab) anti-B. pertussis reagents, and amplified by an IS481-specific PCR. PCR products were detected by a hybridization-enzyme immunoassay kit (Gen-eiti-k DEIA, DiaSorin), with confirmation by a second PCR in a separate laboratory. Sensitivities and specificities of culture, polyclonal DFA, monoclonal DFA, and PCR were 36 and 100%, 11.4 and 94.6%, 8.3 and 98.4%, and 95.0 and 99.3%, respectively, with a prevalence of 15.7%. The DFA tests were the most economical, and the PCR cost was 31% higher than culture. This study suggests that with minor improvements in economy, pertussis PCR can be implemented in a clinical laboratory with marked improvement in diagnostic accuracy.


The polymerase chain reaction (PCR) offers one of the most sensitive methods for detecting Cryptosporidium parvum but its sensitivity in fecal material can be greatly reduced by a variety of poorly defined 'inhibitors'. Methods that separate the parasitic oocyst from fecal material prior to DNA extraction circumvent inhibitor interference but are problematic for frozen specimens since 'intact' oocysts are required for separation. We report here a relatively low-cost, rapid method for extracting C. parvum DNA from frozen fecal materials that can be used in a PCR assay for detection of single parasitic oocysts.


Atypical pathogens such as Chlamydia pneumoniae, Legionella pneumophila and Mycoplasma pneumoniae are an important cause of community-acquired pneumonia. The available detection methods (culture and serology) either lack sensitivity or give only a retrospective diagnosis. In order to improve their detection and quantification in respiratory samples, a real-time multiplex PCR, performed in two separate reactions, was developed for these three pathogens. The comparison of multiplex real-time and conventional PCR assay on 73 respiratory specimens showed an overall agreement of 98.3%, corresponding to 95.8%, 100% and 100% agreement for C. pneumoniae, L. pneumophila and M. pneumoniae, respectively. Clinical application of this multiplex real-time PCR was done on 40 respiratory samples from 38 patients with respiratory tract infections. Of 19 serology-positive patients, 14 were confirmed by the multiplex real-time PCR to be infected by either one of the three pathogens. All samples from serology-negative
patients were negative with the multiplex real-time PCR.


http://www.sciencedirect.com/science/article/B6T60-48XJGBR-3/2/85063859e5a060be4efe15068f953a32

A novel polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis (PRA) of the hsp65 gene was used for the routine identification of mycobacteria in a high throughput clinical laboratory. A total of 2036 clinical isolates were tested by PRA in conjunction with other methods. The PRA identification of M. tuberculosis complex was 100% sensitive and specific, and 74.5% of nontuberculous mycobacteria (NTM) were correctly identified. It gave highly consistent results for Mycobacterium avium complex (MAC) species and for most isolates of M. fortuitum, M. chelonae, and M. kansasii. It had proven to be highly robust and stable despite usage on such a large-scale and is thus particularly suitable for use in high throughput laboratories in areas with a high incidence of tuberculosis.


http://www.sciencedirect.com/science/article/B6T60-4BGHCBM-4/2/887ccaba63a49beda37e838f6039bb90

A biotinylated single-tube nested polymerase chain reaction (PCR) assay with microwell hybridization assay (bPCR-ELISA) was developed for detection of Mycobacterium tuberculosis in clinical specimens. A total of 659 specimens (601 respiratory specimens and 58 nonrespiratory specimens) were collected for evaluation using three DNA amplification techniques: newly designed bPCR-ELISA, in-house single-tube nested PCR for IS6110 gene sequence (nPCR), and commercial automated assays, the Cobas Amplicor System from Roche Diagnostic Systems (aPCR). Sixty-four (9.7%) specimens were culture-positive for M. tuberculosis. Eleven (1.7%) specimens culture-positive for nontuberculosis mycobacteria were negative by all three PCR assays. The resolved performance of bPCR-ELISA, nPCR, and aPCR was found at sensitivities of 97%, 94%, and 97%, respectively. All three PCR assays exhibited a 100% specificity. In evaluation of bPCR-ELISA, a clear distinction between PCR-positive and PCR-negative specimens when an OD405 value of 0.6 was chosen as cut-off. With serial dilutions of M. tuberculosis H37Rv DNA, the detection limit of bPCR-ELISA was found to be 0.75 cfu per reaction at OD405 value of 0.6. Our developed bPCR-ELISA provides a highly sensitive and low-costing molecular diagnosis suitable for developing countries with high prevalence of tuberculosis.


http://www.sciencedirect.com/science/article/B6T60-4FM0P1K-3/2/26a268447638d3dd846780a316900448
A retrospective study including 515 Mycobacterium tuberculosis isolates from 215 patients was conducted to investigate possible laboratory contamination with M. tuberculosis over a 1-year period in a university hospital. All cultures underwent variable-number tandem-repeat (VNTR) typing. Cultures suspected of being contaminated in the VNTR analysis and possible sources of contamination underwent mycobacterial interspersed repetitive unit (MIRU) typing further. Overall, 8 (3.7%) cases of 215 patients were considered possible false-positives. Five (2.3%) cultures might be contaminated during initial batching processing, and 1 (0.5%) and 4 (1.9%) cultures of them were further classified as presumed and possible cases, respectively, of cross-contamination on clinical grounds. Three (1.4%) cultures might be contaminated by cultures that had been processed in species identification procedures in the same laminar-flow hood. The 2-step strategy using VNTR and MIRU analyses in combination in this study appears to be a valuable means for the study of false-positive cultures.

Dig Dis Sci (1)


Alterations of the APC, K-ras, and beta-catenin genes are defined as early events in colorectal tumorigenesis. These alterations are well-known as constituents of Vogelstein's pathway, however, the relationship among them is unclear. For understanding colorectal tumorigenesis it is important to evaluate their relationship. We analyzed the relationship between beta-catenin and K-ras gene mutations in clinical colorectal samples. Sixty-four cases of colorectal cancers (44 proximal, 20 distal) without a family history of colorectal cancer were used for this study. We purified genomic DNAs from fresh surgical samples and, thus, analyzed the mutations of beta-catenin (exon 3) and K-ras (codons 12 and 13) by PCR direct sequencing method using Big Dye terminator cycle sequencing with AmpliTaq polymerase FS. We found 27% (17/64) K-ras mutations (proximal 25%, 11/44; distal 30%, 6/20). The frequency of beta-catenin mutations was 11% (7/64; proximal 9%, 4/44; distal 15%, 3/20). All cases with beta-catenin mutation had no mutation of K-ras. All sites of beta-catenin mutation have been reported previously (codons 33, 34, 41, 45). In cell lines, it has been reported previously that beta-catenin and K-ras play the same roles in activation of cyclin D1 transcription. Our results may support this report and suggest that some colorectal cancers with beta-catenin mutation will progress without K-ras mutation. Further study may disclose a new pathway or new mechanism of colorectal tumorigenesis.

Digestive and Liver Disease (10)

Background. Kupffer cells, monocytes and infiltrating T cells have been considered the major source of interleukin-1[beta] and tumour necrosis factor-alpha in the liver. Aims. To explore the expression of interleukin-1[beta] and tumour necrosis factor-alpha and to evaluate the density and the distribution of T lymphocytes and monocytes/macrophages in the liver of patients with primary and secondary tumours. Methods. Tumoural and peritumoural liver samples were examined from 21 patients with hepatocellular carcinoma, 1 D with hepatic metastases, 5 with benign focal liver lesions and 4 healthy adult livers. Interleukin-1[beta] and tumour necrosis factor-alpha mRNAs were detected by a semiquantitative comparative reverse transcriptase polymerase chain reaction. T lymphocytes and monocytes/macrophages were detected by immunohistochemistry Results. Higher levels of interleukin-1[beta], tumour necrosis factor-alpha, CD3+ and CD68+ cells were found in the tissue surrounding hepatocellular carcinoma and metastases than in the tumour itself. A strong expression of CD68+ and CD3+ cells was found mainly along the tumour-host interface but the highest expression of CD3+ cells was found at the metastasis interfaces. Interleukin-1[beta] expression, CD3+ and CD68+ cell densities were higher in peritumoural samples than in so-called "normal" liver tissue. Conclusions. An increased production of interleukin-1[beta] and, to a lesser extent, of tumour necrosis factor-alpha mRNA coincides with the presence of cancer, be it primary or secondary, both in healthy and cirrhotic livers. The presence of cancer, irrespective of the presence of underlying liver damage, appears to play the most important role.


Background. Keratin 8 is a major component of intermediate filaments in single-layered epithelia of the gastrointestinal tract. Keratin 8 deficient mice display signs of colitis and diarrhoea characteristic for inflammatory bowel disease. Very recently, two keratin 8 mutations, Y54H and G62C, were identified. Aims. We investigated if these keratin 8 missense mutations were associated with inflammatory bowel disease. Patients. In total, 217 German patients with Crohn's disease, 131 German patients with ulcerative colitis, and 560 German control subjects were enrolled in this study. Methods. Samples were analysed by PCR amplification and subsequent melting curve analysis using fluorescence resonance energy transfer probes. Results. The G62C mutation was detected in five (2.3%) patients presenting with Crohn's disease and in three (2.3%) with ulcerative colitis. In comparison, 9 (1.6%) out of 560 controls were heterozygous for this mutation. No patient or control was homozygous for this mutation. Patients carrying one mutant allele did not show any noticeable characteristics in their corresponding phenotype. In contrast, the Y54H mutation was observed in neither any of the 348 patients with inflammatory bowel disease nor in any control subject. Conclusions. Our data indicate that both keratin 8 mutations, G62C and Y54H, do not play a relevant pathogenic role in inflammatory bowel disease.


Background. Keratin 8 is a major component of intermediate filaments in single-layered epithelia of the gastrointestinal tract. Keratin 8 deficient mice display signs of colitis and diarrhoea characteristic for inflammatory bowel disease. Very recently, two keratin 8 mutations, Y54H and G62C, were identified. Aims. We investigated if these keratin 8 missense mutations were associated with inflammatory bowel disease. Patients. In total, 217 German patients with Crohn's disease, 131 German patients with ulcerative colitis, and 560 German control subjects were enrolled in this study. Methods. Samples were analysed by PCR amplification and subsequent melting curve analysis using fluorescence resonance energy transfer probes. Results. The G62C mutation was detected in five (2.3%) patients presenting with Crohn's disease and in three (2.3%) with ulcerative colitis. In comparison, 9 (1.6%) out of 560 controls were heterozygous for this mutation. No patient or control was homozygous for this mutation. Patients carrying one mutant allele did not show any noticeable characteristics in their corresponding phenotype. In contrast, the Y54H mutation was observed in neither any of the 348 patients with inflammatory bowel disease nor in any control subject. Conclusions. Our data indicate that both keratin 8 mutations, G62C and Y54H, do not play a relevant pathogenic role in inflammatory bowel disease.
Background. Keratin 8 (K8) and 18 (K18) are the major components of the intermediate filament cytoskeleton of pancreatic acinar cells and play a relevant role in pancreatic exocrine homeostasis. Transgenic mice for K8 have shown to display progressive exocrine pancreas alterations, including dysplasia, loss of acinar architecture, redifferentiation of acinar to ductal cells, inflammation, fibrosis, and substitution of exocrine tissue by adipose tissue. Aim. To investigate whether mutations in the keratin 8 gene are associated with chronic pancreatitis.

Methods. Mutations in the keratin 8 gene were determined by polymerase chain reaction/restriction fragment length polymorphism in 67 chronic pancreatitis patients and 100 normal controls. Sequence analysis was performed when necessary.

Results. Glycine-to-cysteine mutations at position 61 (G61C) of the keratin 8 gene were found in six patients (8.9 vs. 0%, pcConclusion. G61C mutation of the keratin 8 gene, together with other environmental factors and/or genetic factors, could predispose to chronic pancreatitis, by interfering with the normal organization of keratin filaments.


http://www.sciencedirect.com/science/article/B7582-4CSG44H-1/2/3db057e19b282c4b50fe2d2d29b0f45d

Background. Several studies have demonstrated that bone marrow contains a subpopulation of stem cells capable of participating in the hepatic regenerative process, even if some reports indicate quite a low level of liver repopulation by human stem cells in the normal and transiently injured liver. Aims. In order to overcome the low engraftment levels seen in previous models, we tried the direct intraperitoneal administration of human cord blood stem cells, using a model of hepatic damage induced by allyl alcohol in NOD/SCID mice.

Methods. We designed a protocol based on stem cell infusion following liver damage in the absence of irradiation. Flow cytometry, histology, immunohistochemistry and RT-PCR for human hepatic markers were performed to monitor human cell engraftment.

Results. Human stem cells were able to transdifferentiate into hepatocytes, to improve liver regeneration after damage and to reduce the mortality rate both in both protocols, even if with qualitative and quantitative differences in the transdifferentiation process.

Conclusions. We demonstrated for the first time that the intraperitoneal administration of stem cells can guarantee a rapid liver engraftment. Moreover, the new protocol based on stem cell infusion following liver damage in the absence of irradiation may represent a step forward for the clinical application of stem cell transplantation.


Background. Exfoliated colonic epithelial cells in faeces provide a source of human DNA which may be analysed for the presence of tumour-induced modification. Aim. In the present study we investigated K-ras and p53 mutations in faeces of patients with colorectal carcinoma, to verify whether analysis of these mutations might identify a high percentage of patients with colorectal cancer. Patients and methods. Faeces, tumour and normal mucosa samples were taken from 26 patients. Polymerase chain reaction amplification and restriction enzyme analysis were performed to detect K-ras mutations; p53 gene mutations were identified by using polymerase chain reaction amplification and single strand conformation polymorphism. Results. We were able to amplify the K-ras gene and exons 5-9 of the p53 gene in 100% of the faecal samples studied. K-ras and p53
gene mutations were detected in faeces in 26.9% and 50% of the cases, respectively. The two
mutations were present together in 5 out of 26 patients. There was full agreement between the K-
ras and p53 pattern observed in faecal DNA and that in tumour tissue DNA.Conclusions.
Application of K-ras and p53 mutation gene analysis in the faeces may have clinical applications
in the future. Since this genetic analysis is able to detect only 57.7% of patients with colorectal
cancer, the study of other genes involved in colorectal carcinogenesis is necessary.

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P/2/752fac721a1a838172a932c03523cc35

Background. In vitro studies showed that Helicobacter pylori strains carrying the cag
pathogenicity island are able to induce epithelial secretion of Interleukin-8.Aims. To evaluate the
assessment of cag pathogenicity island and the expression of Interleukin-8 in the gastric mucosa of
Helicobacter pylori-infected patients and correlate these data with the activity of gastritis and
Helicobacter pylori density.Methods. cag status was determined by polymerase chain reaction
directly on gastric biopsies from 13 Helicobacter pylori+ patients with non-ulcer dyspepsia and 13
Helicobacter pylori+ with duodenal ulcer. Interleukin-8 gene transcription and protein expression
were analysed by in situ hybridization and immunofluorescence, respectively. Gastritis activity
and Helicobacter pylori density were also investigated.Results. cag was present in 20/26 of
Helicobacter pylori+ patients: in 7/13 non-ulcer dyspepsia (53.8%) and in 13/13 duodenal ulcer
patients (100%), (pcag+ than in cag- patients (pHelicobacter pylori density was enhanced in cag+
(pConclusions. The present study demonstrates that in Helicobacter pylori-infected human gastric
mucosa, cag+ infection is associated with enhanced Interleukin-8 expression, higher levels of
active gastritis and bacterial density, and presence of duodenal ulcer.

active coeliac disease is reversed after a gluten-free diet." Digestive and Liver Disease 36(5):
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Coeliac disease is an autoimmune enteropathy characterized by an enhanced permeability of the
intestinal epithelial barrier. In epithelial cells paracellular permeability is regulated by intercellular
tight junction. The cytoplasmic protein ZO-1 interacts directly with F-actin and plays a pivotal role
in the structural and functional organization of tight junction.Aim. The aim of this study was to
investigate the expression and localization of ZO-1 in the intestinal mucosa of coeliac
patients.Patients and methods. Twenty patients with active coeliac disease, seven of whom
underwent a repeat biopsy following a gluten-free diet and 27 control subjects, were studied. In all
subjects, three biopsies were obtained from distal duodenum during upper gastrointestinal
endoscopy. ZO-1 protein localization and levels were detected by immunofluorescence followed
by confocal microscopy analysis and immunoblotting. ZO-1 mRNA expression was assessed by
RT-PCR. F-actin distribution was also investigated.Results. In patients with active coeliac
disease, both ZO-1 protein levels and mRNA were clearly reduced. Cytoskeletal organization was
disrupted with F-actin staining concentrated at the subcortical and basal surface regions.
Abnormalities in ZO-1 expression and actin organization were reversed after a gluten-free
diet.Conclusions. In active coeliac disease, ZO-1 protein expression is downregulated at the
transcriptional level in association with F-actin redistribution. These changes are completely
reversed after a gluten-free diet and could contribute to the increased intestinal paracellular permeability observed in this disorder.


http://www.sciencedirect.com/science/article/B7582-4B1SKT2-10/2/ef05617cb932667ad27399b63524a440

Background. The mammalian augmenter of liver regeneration gene encodes a protein involved in the unique process of liver regeneration. The augmenter of liver regeneration respective protein stimulates hepatocyte proliferation in hepatectomized rats and inhibits cytotoxic activity of liver-derived Natural Killer cells from intact rats. Augmenter of liver regeneration protein shares homology with a Saccaromyces Cerevisiae protein essential for the viability, oxidative phosphorylation and cell-division cycle. Aims. To demonstrate if augmenter of liver regeneration protein, like the homologous in the yeast, plays a role in the regulation of biogenesis of mitochondria. Methods. Augmenter of liver regeneration protein was injected in intact rats and, in the hepatic tissue, the expression of two genes located in two different regions of the mitochondrial genome, mitochondrial ATPase 6/8, and ND1 subunit, and of a nuclear gene, mitochondrial Transcription Factor A, were considered. In addition, cytochrome content and oxidative phosphorylation capacity of liver-derived mitochondria were evaluated. Results. The augmenter of liver regeneration protein administration induces an increase in the mitochondrial gene expression and enhances cytochrome content and oxidative phosphorylation capacity of liver-derived mitochondria. Conclusions. The present data demonstrate a comparable role in the regulation of mitochondria biogenesis in the eukaryotic cell like the yeast protein. This phenomenon could be part of the complex mechanism through which augmenter of liver regeneration regulates hepatocyte proliferation.


http://www.sciencedirect.com/science/article/B7582-4B2H630-1P/2/4a473cd7baa8504d93f7c4bc70f8b955

Background. We have shown that the administration of exogenous Augmenter of Liver Regeneration protein in intact rats i) regulates mitochondrial gene expression by inducing the transcription and translation of the nuclear-encoded mitochondrial transcription factor A, and ii) inhibits the lytic activity of liver-resident Natural Killer cells. Aims. The present investigation was carried out to study the effect, in intact rats, of exogenous administration of Augmenter of Liver Regeneration protein on Interferon-[gamma], a cytokine produced by activated Natural Killer cells and known to control the expression of mitochondrial transcription factor A, a nuclear gene responsible for mitochondrial metabolism. Methods. Interferon-[gamma] was measured as messenger RNA in liver-derived mononuclear leukocytes and as protein in liver-derived Natural Killer cells after a single injection of Augmenter of Liver Regeneration protein. Results. The data obtained demonstrate that: i) in intact rats, Augmenter of Liver Regeneration protein administration induces a reduction of Interferon-[gamma] in the liver-resident Natural Killer cells and ii) the administration of Interferon-[gamma] in 70% hepatectomized rats is followed by a significant reduction both of the mitochondrial transcription factor A expression and of liver regeneration. Conclusions. These data demonstrate the pivotal role of Augmenter of Liver Regeneration as Growth Factor and as immunoregulator by controlling, through Interferon-
[gamma] levels, the mitochondrial transcription factor A expression and the lytic activity of liver-resident Natural Killer cells.


http://www.sciencedirect.com/science/article/B7582-4B1W7C/2/8591fd99e97d5d8eb29e96bf3d6fd737

Background. Host response plays a major role in pathogenesis of Helicobacter pylori-induced gastroduodenal diseases including adenocarcinoma of distal stomach. Epidermal growth factor-related growth factors are important modulators of gastric homeostasis in normal and damaged gastrointestinal mucosa. Aim. To evaluate expression of heparin binding epidermal growth factor and amphiregulin in antral mucosa of Helicobacter pylori-infected and non-infected dyspeptic patients and to correlate levels of heparin binding-epidermal growth factor and amphiregulin mRNA with mitogenic activity of gastric epithelial cells. Methods. A total of 10 Helicobacter pylori-infected and 15 Helicobacter pylori non-infected (10 with and 5 without gastritis) dyspeptic patients were studied. Diagnosis of Helicobacter pylori infection was based on rapid urease test and histology. Heparin binding-epidermal growth factor and amphiregulin mRNA expression in antral mucosa were assessed by reverse transcriptase-polymerase chain reaction. Protein expression and localization of both peptides were determined by immunohistochemistry. Mitogenic activity of antral gastric mucosa was assessed by determination of proliferating cell nuclear antigen labelling index by immunohistochemistry. Results. Heparin binding-epidermal growth factor and amphiregulin mRNA expression increased in Helicobacter pylori-infected vs Helicobacter pylori non-infected patients. Heparin binding-epidermal growth factor and amphiregulin immunostaining was more intense and deeper in gastric gland compartment in infected mucosa than in noninfected mucosa. Increase in heparin binding-epidermal growth factor and amphiregulin mPNA expression significantly correlated with increase in proliferating cell nuclear antigen labelling index. Conclusions. Helicobacter pylori gastritis is associated with up-regulation of heparin binding-epidermal growth factor and amphiregulin which correlates with increased mitogenic activity of gastric mucosa. Increased heparin binding-epidermal growth factor and amphiregulin expression is postulated to contribute to reparative response of gastric mucosa to Helicobacter pylori infection.

DNA Repair (15)


DNA from therapy-related acute leukemia/myelodysplastic syndrome cases (tAL/MDS) from the GIMEMA [Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto] Archive was examined for the microsatellite instability (MSI+) phenotype that is diagnostic for defective DNA mismatch repair. More than 60% (16/25) of tAL/MDS cases were MSI+ in contrast to de novo cases.
hMLH1 gene silencing was rare and evidence of promoter methylation was found in less than one-third of the MSI+ cases. Among the GIMEMA patients who had been treated for breast cancer there was an apparent trend towards early onset primary breast disease. This suggests that there might be common predisposing factors for breast cancer and tAL/MDS. There were also three examples of mutations in the MRE11 gene among the 25 tAL/MDS cases suggesting that defective recombinational DNA repair may promote the development of secondary malignancy. MSI+ tAL/MDS was significantly associated with previous chemotherapy and the frequency of MSI+ among radiotherapy patients was considerably lower. In view of the established relationship between drug resistance and mismatch repair defects, we suggest that selection for therapeutic drug resistance may contribute to the incidence of MSI+ tAL/MDS.


http://www.sciencedirect.com/science/article/B6T2B-47GGK7W-1Y/2/11e50c161110b6b69633a06e19ba9be2

The patterns of expression of 3 human DNA-repair genes (ERCC1, ERCC2, ERCC6) were assessed in 52 bone-marrow specimens obtained from cancer patients prepared for autologous bone-marrow transplantation. Marrow was collected prior to the initiation of treatment in patients with sarcoma or testicular cancer; marrow was collected after initial cytoreductive therapy for patients with non-Hodgkin's lymphoma, Hodgkin's disease, and other tumors. Slot-blot analysis of marrow RNA showed a bimodal pattern of ERCC1, ERCC2 and ERCC6 gene expression with relative expression values ranging more than 200-fold. This pattern was seen in all patient groups and appeared to be independent of whether or not patients had received prior chemotherapy. In all patient groups, when expression was low for ERCC1, expression was also low for ERCC2 and ERCC6, suggesting that expression of these genes may be coordinated within an individual although they are located on two different chromosomes. Southern blot analyses of Pst I digest of DNA from 6 bone-marrow samples indicate no differences in ERCC1 gene copy number between high expressors and low expressors. There is absence of restriction fragment length polymorphism for ERCC1 suggesting that the different levels of expression in high and low expressors were not due to major deletions or rearrangements of the ERCC1 gene. We conclude that expression of these ERCC genes may vary widely between individuals, and that within an individual, their expression may be linked and coordinated by a common regulatory mechanism.


http://www.sciencedirect.com/science/article/B6T2B-3RD0T5D-4/2/34cc67fe387930f444ad065ba938d1c9

A new approach to monitoring UV damage and repair in the human genome has been developed. The proposed approach is based on a combination of features unique to interspersed repetitive Alu elements, and the ability of certain DNA lesions to block Taq polymerase-mediated DNA synthesis: namely, the extraordinary abundance of Alu repeats throughout the human genome in conjunction with distinct sequence motifs comprising long runs of T residues which are likely targets for formation of UV lesions. Hence, Taq polymerase-mediated extension synthesis with Alu specific primers was employed to visualize formation of discrete predicted adducts within the element. Several variations of the Alu-primer driven amplification protocol were developed to monitor the following aspects of damage: (i) induction of UV-photoproducts at predicted sites within the Alu sequence, (ii) modification of extension synthesis profiles, (iii) UV dose dependent, quantitative inhibition of Alu-primer driven amplification. The assays reveal sites of predicted Taq
polymerase blockage within the Alu sequence, a global decrease in the mean length of extension products, and a measurable reduction in the quantity of extension products that is inversely proportional to UV dose. Thus, the exceptional abundance of Alu repeats and their primary sequence features, in combination with the ability of UV lesions to block elongation by Taq polymerase, provide a novel and sensitive system for detecting UV damage in the human genome. The system detects UV damage at levels that are compatible with cellular DNA repair, and provides a unique amplification-based protocol for probing the overall integrity of human DNA.


http://www.sciencedirect.com/science/article/B6T2B-3WF7M8X-4/2/397304a310fed992018ff4d4a22ce43

The Saccharomyces cerevisiae RAD52 gene was introduced into the human fibrosarcoma-derived cell line HT1080. Transfected cell lines that expressed the yeast transgene catalyzed inter-plasmid homologous DNA recombination at frequencies approx. 12-fold higher than did control cells. Additional experiments revealed that yeast RAD52 gene expression increased the level of resistance to the DNA damaging agents diepoxybutane, and methyl methanesulfonate, but did not alter sensitivity to ultraviolet radiation. These results indicate that the S. cerevisiae Rad52 protein can function in a human somatic cell background and provide support for the idea that a homologous recombination-based DNA repair process functions in mammalian somatic cells.


http://www.sciencedirect.com/science/article/B6X17-464P4Y9-1/2/8452f38860ec3e02d002e68f77e0ecd6

The gene p53 is a critical tumor suppressor that can respond to multiple signals of cellular gatekeepers for growth and division. The mdm2 gene is one of the downstream target genes for transcriptional activation by the product of p53 tumor suppressor gene. Transactivation of mdm2 gene is represented by the presence of a functional P53 protein. To understand the biological function of mutant p53 in tumorigenesis, we constructed a number of p53 mutants by site-directed mutagenesis (H179Y, L194R, S240R, R249S, A276D, E286Q), followed by characterization of each P53 mutant's ability to transactivate mdm2, bax and p21waf. The transactivation properties of p53 mutants were compared by co-transfection with pGL-3-mdm2, pGL-3-bax and pGL-3-p21waf into the P53 null cell line H1299 derived from a non-small cell lung carcinoma. Among them mt p53 S240R and E286Q were shown to have enhanced transactivating activity of pGL3-mdm2, at about 43.2 and 28.2% of the wt p53 vector, respectively, while the remaining four had nearly the same level of activity as the negative control did. Furthermore, data indicated that mt p53 S240R had as high an ability to suppress the growth of the p53 null cell line H1299 as wild type p53. Therefore, mutant p53 alone is an insufficient indicator of poor prognosis. Instead, functional p53 may affect lung cancer prognosis.

In previously reported studies, we transfected repair-proficient murine fibroblasts with the denV gene of bacteriophage T4 and showed that expression of encoded endonuclease V markedly enhanced cyclobutane pyrimidine dimer (CPD) repair and reduced the frequency of ultraviolet radiation (UV)-induced mutations. In the present studies, we compared the spectra of UV-induced mutations at the hprt locus in denV-transfected and control cells. A significant difference in mutation types was observed. While multiple base deletions and single base insertions were found in denV-transfected but not control cells, multiple tandem and non-tandem point mutations identified in control cells were absent in denV-transfected cells. When we compared colony survival following UV exposure in the two cell lines, it appeared that endonuclease V expression did not enhance UV resistance, instead denV-transfected cells had increased susceptibility to low fluences of UV. The effects of endonuclease V expression on UV resistance and on UV mutational spectrum are likely to be due both to the removal of CPDs and to the novel enzymatic activity of endonuclease V.


Apurinic/apyrimidinic endonuclease (here designated APE/REF) carries out repair incision at abasic or single-strand break damages in mammals. This multifunctional protein also has putative role(s) as a cysteine 'reducing factor' (REF) in cell-stress transcriptional responses. To assess the significance of APE/REF for embryonic teratogenesis we constructed a more precisely targeted Ape/Ref-deficient genotype in mice. Ape/Ref gene replacement in ES cells eliminated the potential of APE/REF protein synthesis while retaining the Ape/Ref bi-directional promoter that avoided potential inactivation of an upstream gene. Chimeric animals crossed into Tac:N:NIH-BC produced germline transmission. Homozygous null Ape/Ref-embryos exhibited successful implantation and nearly normal developmental progression until embryonic day 7.5 followed by morphogenetic failure and adsorption of embryos by day 9.5. We characterized the cellular events proceeding to embryonic lethality and examined ionizing radiation sensitivity of pre-implantation Ape/Ref-null embryos. After intermating of heterozygotes, Mendelian numbers of putative Ape/Ref-null progeny embryos at day 6.5 displayed a several-fold elevation of pycnotic, fragmenting cell nuclei within the embryo proper--the epiblast. Increased cell-nucleus degeneration occurred within epiblast cells while mitosis continued and before obvious morphogenetic disruption. Mitogenic response to epiblast cell death, if any, was ineffective for replacement of lost cells. Extra-embryonic yolk sac, a trophectoderm derived lineage retained normal appearance to day 9. Explanted homozygous Ape/Ref-null blastocysts displayed increased sensitivity to [gamma]-irradiation, most likely a manifestation of APE/REF incision defect. Our study establishes that this new Ape/Ref deficiency genotype is definitely capable of post-implantation developmental progression to the onset of gastrulation. Function(s) of APE/REF in base damage incision and also conceivably in mitogenic responses towards epiblast cell death are critical for transit through the gastrulation stage of embryonic growth and development.

A search for genetic alterations within the XPG gene has been conducted on skin and blood cells cultured from a newly characterized xeroderma pigmentosum (XP) patient (XP20BE). This patient is the ninth known case that falls into the extremely rare XP complementation group G. Four genetic markers within the XPG gene (including two polymorphisms) demonstrated the Mendelian distribution of this gene from the parents to the patient and to an unaffected sibling. The patient (XP20BE) inherited a G to T transversion from his father in exon 1 of the XPG gene that resulted in the conversion of a glutamic acid at codon 11 to a termination codon. The patient also inherited an XP-G allele from his mother that produces an unstable or poorly expressed message. The cause of the latter defect is still uncertain. In addition to these alterations, XP20BE cDNA contained an mRNA species with a large splicing defect that encompassed a deletion from exon 1 to exon 14. This splicing defect, however, appears to be a naturally occurring low-frequency event that results from abnormal splicing that occurs between certain conserved non-consensus splicing signals within the human XPG gene.


We have cloned a 13 kb genomic DNA fragment from the Chinese hamster ovary cell line, CHO-KI, and determined the nucleotide sequence of a 4 kb stretch of DNA which encompasses the complete sequence (2.277 kb) of the hamster apurinic/apyrimidinic endonuclease (chAPE1) gene. The intron/exon boundaries, identified by RT-PCR, follow GT/AG rule. The structure of the chAPE1 gene is similar to other mammalian apurinic/apyrimidinic (AP) endonuclease (hAPE1, BAP1, RAPEN and mAPE1) genes in that it has five exons and four introns with the first exon unexpressed. This structure, however, differs from one of the two structures that have been proposed for mAPE1 gene. Three transcription start sites (TSS) for the chAPE1 gene were identified by primer extension analysis at +1, +14 and +18 positions. The sequence also includes 1.72 kb of the upstream region of the chAPE1 gene. In this region, a CCAAT box but no TATA box that could initiate the transcription at the initiation sites was identified. The upstream region also includes the binding sites for a variety of other transcription factors. A polyadenylation site, 13 nucleotides downstream to the polyadenylation signal, was identified by 3'-RACE analysis. The observed 1.28 kb transcript of the chAPE1 gene is smaller than the 1.5 kb transcript of the human AP endonuclease gene. The translation of chAPE1 gene starts within the second exon with ATG and terminates in the fifth exon with UGA codons, 318 and 2121 nucleotides downstream to the first TSS, respectively. The encoded peptide of 317 amino acid residues is similar in size and is highly homologous in its amino acid sequence to mouse, rat, human, and bovine AP endonucleases.

The role of poly(ADP-ribose) polymerase (PADPRP) in nuclear DNA repair and other nuclear processes has been intensely studied and debated for decades. Recent studies have begun to shed new light on these arguments with firm experimental data for its role, primarily, as a remodeler of chromatin structure. Those studies imply that PADPRP plays an indirect role in DNA repair, serving to expose DNA to repair enzymes through chromatin remodeling. Only DNA that is tightly packaged would require PADPRP activity for its repair; while DNA in an open conformation would be accessible to DNA repair enzymes and not require PADPRP activity. The purpose of the current studies was to address the above hypothesis directly. Using quantitative Southern blot analysis, we studied repair in transcribed and nontranscribed nuclear DNA sequences in ADPRT 351 cells 95% deficient in PADPRP activity. Cells were exposed to methylnitrosourea (MNU) for 1 h and allowed to repair for 8 or 24 h. Densitometric scans of autoradiographs revealed that, when compared to their parental V79 cell line, ADPRT 351 cells 95% deficient in PADPRP activity were equally as efficient in repair of N-methylpurines in the transcribed sequence containing the dihydrofolate reductase gene. However, the ADPRT 351 cells were deficient in the ability to repair these lesions in the nontranscribed sequence containing the IgE gene compared to repair of the same sequence in the parental V79 cells. Nucleoid sedimentation assays demonstrated that the ADPRT 351 cells are deficient in repair across the entire genome when compared to the parental V79 cells. These studies indicate that PADPRP activity is not required for repair of N-methylpurines in transcribed nuclear DNA sequences but is necessary for the repair of these lesions in nontranscribed nuclear DNA sequences as well as across the entire genome since the DNA in a given cell is predominantly nontranscribed.


We have developed a rapid method to synthesize radioactively labeled single-stranded DNA probes suitable for strand-specific analysis of single copy genes on Southern blot. Linear PCR with 10 [mu]Ci [alpha]32P-aATP (3000 Ci/mmole) as the only dATP source enabled us to generate strand-specific DNA probes with high specific activity. The probes synthesized by this method have higher specific activities and the same strand specificity compared to the end-labeled single-stranded DNA probes obtained from single-stranded M13mp18/19 vectors. Application of the method for strand-specific analysis of ultraviolet-induced DNA lesions in defined DNA sequences significantly improved the hybridization signal.


Base excision repair (BER) is a tightly coordinated mechanism for repair of DNA base damage (via alkylation and oxidation) and base loss. From E. coli to yeast to human cells, subtle alterations in expression of BER proteins lead to mutagenic or genome instability phenotypes. DNA polymerase [beta] ([beta]-pol), the major BER polymerase, has been found to be over-
expressed in human tumor tissues and more recently it has been shown that over-expression of [beta]-pol results in a mutator and genome instability phenotype. These previous reports imply that [beta]-pol over-expression is deleterious and suggests that such an imbalance may cause an overall functional deficiency in the BER pathway. In the present study, we have developed a bicistronic tetracycline-responsive transgenic system to over-express [beta]-pol in mice. We find that over-expression of [beta]-pol in the lens epithelium results in the early onset of severe cortical cataract, with cataractogenesis beginning within 4 days after birth. In utero and post-natal suppression of transgenic Flag-[beta]-pol expression by doxycycline administration completely prevents cataract formation through adulthood, yet cataract is subsequently observed following removal of doxycycline and re-expression of the transgene. Cataract development accompanies increased expression of cyclooxygenase-2 in the lenticular fibers of the lens, implicating oxidative stress in the development of this cataractous phenotype. Although the mechanism for the transgene mediated cataractogenesis is not clear at this time, it is nevertheless intriguing that increased expression of [beta]-pol leads to such a phenotype. These results suggest that either a [beta]-pol expression imbalance negatively affects overall fidelity and/or BER capacity or that [beta]-pol has a role in lens epithelial cell differentiation.


http://www.sciencedirect.com/science/article/B6T2B-3WF7M8X-3/2/c2a0dd04ca5f235480e1c448c5f59fb4

XP12BE is a commonly studied XP-A cell line that exhibits slightly increased resistance to UV compared with the majority of XP-A cell lines. The elevated UV survival is common to a subset of XP-A cell lines and correlates with delayed onset of the neurological disease in patients. We identified the XPA mutations in XP12BE by single strand conformation polymorphism (SSCP) analyses and nucleotide sequencing. XP12BE is a compound heterozygote and both mutations affect mRNA splicing. One mutation is a G to C transversion within the splice donor site of intron 4 that is common to several cell lines from XP-A patients with delayed onset of neurological disease. The other mutation is a G to T transversion at the same position as a G to C transversion in the splice acceptor site of intron 3 that is common in Japanese XP-A patients. We also demonstrated the persistence of the XP12BE mutations in cell line Z-O-A2 which has been shown to express XPA protein. These results suggest that the intron 4 splice donor mutation likely produces some, at least partially functional, XPA protein that accounts for the increased UV survival of XP-A cell lines derived from patients with delayed onset of neurological disease.


http://www.sciencedirect.com/science/article/B6T2B-3VXYRFN-4/2/74d7dec1b7157c4f199ca06b0074c24c

The REV3 gene of Saccharomyces cerevisiae encodes the catalytic subunit of DNA polymerase [zeta] which is involved in translesion synthesis. The mouse homolog of this gene, Rev3l, was cloned and sequenced. The gene encodes a putative protein of 3122 amino acids. The sequence conservation to its yeast counterpart is restricted to several regions. In the carboxy-terminal part of the protein all six domains are present that are characteristic for [alpha]-type DNA polymerases. In the amino-terminal part of the protein two regions can be identified with considerable similarity to the NT boxes of mouse polymerase [delta]. In addition, a region of 60 residues unique for the REV3 homologs can be found in the middle part of the protein. The
mouse REV3L protein shows strong sequence conservation with the recently cloned human REV3L protein (86% identity overall). Northern blot analysis of various tissues of the mouse revealed that transcription of the Rev3l gene was highest in brain, ovaries and testis. The human REV3L gene was localised to the long arm of chromosome 6, region 21-22. The mouse equivalent maps to chromosome 10, distal to the c-myb gene, close to the Macs gene.


http://www.sciencedirect.com/science/article/B6T2B-3WD5C3J-1/2/703efb76af1bb8b03f37c1b178dfca33

We report a sensitive, SINE (Short Interspersed DNA Element)-mediated, PCR-based, DNA damage detection assay. Here, the SINE assay is used for detection of UVB-induced DNA damage and repair in cultured mouse cells and in vivo, in mouse skin. The unique feature of the SINE assay is its ability to support simultaneous amplification of multiple, random segments of genomic DNA. This can be accomplished due to the remarkable abundance, dispersion and conservation of SINEs in mammalian genomes. The most abundant SINEs in the mouse genome are the B1 elements, at a copy number of 50,000-80,000. Due to their strong sequence conservation, primers complementary to the B1 consensus sequence anneal to the majority of their targets in the genome. Consequently, long segments of genomic DNA located between pairs of B1 elements are efficiently amplified by PCR. Thus, in conjunction with the fact that many types of DNA adducts form blocks for thermostable polymerase, the B1 element anchored PCR makes a sensitive and versatile tool for assessing the overall integrity of the transcribed regions in mouse genome. We measured UVB-dose (0.1-3 kJ m-2) dependent formation of photoproducts in DNA from cultured cells, and after 20 h observed a substantial removal of damage at doses lower or equal to 0.6 kJ m-2. The sensitivity of detection of UVB-photoproducts formation and repair was compared to that of the conventional, single locus-targeting QPCR. Using the SINE assay we also have shown the distribution of UVB and UVC induced DNA adducts at a single nucleotide resolution within the B1 elements in mouse DNA. Lastly, we demonstrated that the sensitivity of the SINE assay is adequate for measurement of UVB-dose (1-6 kJ m-2) dependent formation and subsequent removal of photoproducts in vivo, in mouse skin.

Drug Metab. Dispos.  (9)


http://dmd.aspetjournals.org/cgi/content/abstract/32/12/1341

Cytochrome P450 2B6 (CYP2B6) metabolizes a number of therapeutic drugs and its metabolic activity varies markedly in human liver. Although genetic polymorphisms of CYP2B6 have been reported in noncoding and coding regions, little information is available regarding single nucleotide polymorphisms (SNPs) and their haplotypes in noncoding regions in Asians. Fourteen previously reported SNPs were determined by polymerase chain reaction-restriction fragment
length polymorphism or SNaPshot analysis in a Korean population and their haplotypes were inferred from genotype data using an expectation-maximization algorithm. The most common haplotypes were haplotype I, the reference sequence (frequency 0.35), haplotype II (0.19), haplotype III (0.19), and haplotype V (0.12), which together accounted for 85% of all haplotypes. The frequency of haplotype III, which contains -2320C, -1778G, -1186G, -750C, and 15582T, was found to be 2.4-fold higher than that of the *1J allele in Caucasians, and the frequency of haplotype V, which contains -8207C, -1456C, -750C, 516T, and 785G, was 55% of that of the *6B allele in Caucasians. Moreover, haplotype V, the *6B allele, appeared to be completely linked to -8207 within a putative nuclear receptor binding motif, suggesting that lower expressions of the *6B allele may be associated with the presence of noncoding SNPs such as -8207G>C linked to nonsynonymous SNPs. In conclusion, we found 11 previously described polymorphisms and identified four major haplotypes of CYP2B6 in Koreans. The frequencies of the *1J or *6B alleles, which may reduce CYP2B6 enzyme expression, were found to be significantly different between Koreans and Caucasians.


http://dmd.aspetjournals.org/cgi/content/abstract/33/3/365

In contrast to the beneficial effects of tert-butylhydroquinone (tBHQ) as a food antioxidant, a number of studies have shown that chronic exposure to tBHQ may induce carcinogenicity. Therefore, we examined the ability of tBHQ to induce the cytochrome P450 1a1 (Cyp1a1), an enzyme known to play an important role in the chemical activation of xenobiotics to carcinogenic derivatives. A significant concentration-dependent increase in Cyp1a1 mRNA, protein, and activity occurred after treatment of murine hepatoma Hepa 1c1c7 cells with tBHQ. The increase in mRNA was apparent 3 h after treatment. The RNA polymerase inhibitor, actinomycin D, completely blocked the Cyp1a1 induction by tBHQ, indicating a requirement of de novo RNA synthesis through transcriptional activation. The protein synthesis inhibitor cycloheximide superinduced the tBHQ-mediated induction of Cyp1a1 mRNA and completely prevented the increase in Cyp1a1 activity, indicating that the induction of enzyme activity by tBHQ is dependent on de novo protein synthesis. In addition, the aryl hydrocarbon receptor (AHR) antagonist, resveratrol, inhibited the increase in Cyp1a1 activity by tBHQ. Gel electrophoretic mobility shift assays showed that tBHQ causes activation or transformation of the AHR in nuclear extracts, indicating that AHR-dependent mechanisms contributed to the Cyp1a1 induction. Similar to murine Hepa 1c1c7 cells, tBHQ caused a concentration-dependent increase in CYP1A1 at the mRNA and activity levels in human HepG2 cells. This is the first demonstration that the phenolic antioxidant, tBHQ, can directly induce Cyp1a1 gene expression in an AHR-dependent manner and may represent a novel mechanism by which tBHQ promotes carcinogenicity.


http://dmd.aspetjournals.org/cgi/content/abstract/31/5/548

Human cytochrome P450 3A4 (CYP3A4) is the most abundant hepatic and intestinal phase I drug-metabolizing enzyme, and participates in the oxidative metabolism of approximately 50% of drugs on the market. In the present study, a transgenic-CYP3A4 (Tg-CYP3A4) mouse model that expresses CYP3A4 in the intestine and is phenotypically normal was generated, which was genotyped by both polymerase chain reaction and Southern blotting. Intestinal microsomes prepared from Tg-CYP3A4 mice metabolized midazolam (MDZ) to 1'-hydroxymidazolam about 2
times, and to 4-hydroxymidazolam around 3 times faster than that from wild-type (WT) mice. These increased MDZ hydroxylation activities were completely inhibited by an anti-CYP3A4 monoclonal antibody. The time course of plasma MDZ and its metabolite concentrations was measured after intravenous (0.25 mg/kg) and oral (2.5 mg/kg) administration of MDZ, and pharmacokinetic parameters were estimated by fitting to a noncompartmental model. Pretreatment with ketoconazole increased orally dosed MDZ maximum plasma concentration (Cmax), time of the maximum concentration, area under the plasma concentration-time curve from zero to infinity (AUC0-\([\infty]\)), and elimination half-life (t1/2) to 3.2-, 1.7-, 7.7-, 2-fold, and decreased MDZ apparent oral clearance about 8-fold in Tg-CYP3A4 mice. The ratios of MDZ Cmax, AUC0-\([\infty]\), t1/2 and bioavailability between Tg-CYP3A4 and WT mice after the oral dose of MDZ were 0.3, 0.6, 0.5, and 0.5, respectively. These results suggest that this Tg-CYP3A4 mouse would be an appropriate in vivo animal model for the evaluation of human intestine CYP3A4 metabolism of drug candidates and potential food-drug and drug-drug interactions in preclinical drug development.


http://dmd.aspetjournals.org/cgi/content/abstract/31/5/677

In this case report, we present genetic differences in two morphine-related gene sequences, UDP-glucuronosyltransferase 2B7 (UGT2B7) and \{micro\} opioid receptors (MOR1), in two cancer patients whose clinical responses to morphine were very different [i.e., sensitive (patient 1) and low responder (patient 2)]. In addition, allelic variants in the UGT2B7 gene were analyzed in 46 Japanese individuals. Amplified DNA fragments for the two genes of interest were screened using single strand conformation polymorphism and then sequenced. In the UGT2B7 gene, 12 single nucleotide polymorphisms (SNPs) were newly identified with an allelic frequency ranging from 0.022 to 0.978. Six SNPs in the promoter region (A-1302G, T-1295C, T-1111C, G-899A, A-327G, and T-125C) and two coding SNPs (UGT2B7*2 in exon 2 and C1059G in exon 4) appeared to be consistently linked. Remarkable differences in the nucleotide sequence of UGT2B7 were observed between the two patients; in contrast to patient 1 who had "reference" alleles at almost SNP positions, but a rare ATTGAT\(^2\)(AT)C haplotype as homozygosity, patient 2 was a homozygous carrier for the predominant GCCAGC\(^1\)(TC)G sequence. Serum morphine and two glucuronide concentrations in patient 2 suggest that the predominant GCCAGC\(^1\)G sequence was not associated with a "poor metabolizer" phenotype. In the MOR1 gene, patient 1 had no SNPs, whereas patient 2 was a heterozygous carrier for both the G-1784A and A118G alleles. The present study describes substantial differences in genotype patterns of two genes of interest between the two patients. The results necessitate larger trials to confirm these observations in larger case control studies.


http://dmd.aspetjournals.org/cgi/content/abstract/33/1/94

The aim of the present study was to assess the contribution of polymorphisms in the breast cancer resistance protein/ATP-binding cassette transporter G2 (BCRP/ABCG2) gene to the placental expression from a new perspective, allelic imbalance. Polymorphisms were screened by polymerase chain reaction (PCR)-single-strand conformation polymorphism analysis followed by sequencing with DNA extracted from 100 placenta. To examine whether polymorphisms of the
BCRP gene correlate with the placental BCRP expression, we determined mRNA and protein levels by quantitative real-time PCR and Western blotting, respectively. In placentas, G34A (Val12Met) and C421A (Gln141Lys) were frequently observed (18-36%), but C376T, which creates a stop codon (Gln126 stop codon), was found with an allelic frequency of 1%. The mean of the BCRP protein level was significantly lower (p < 0.05) in homozygotes for the A421 allele than in those for the C421 allele, and heterozygotes had an intermediate value. To evaluate whether the C421A polymorphism acts as a cis-element in BCRP transcription, allelic imbalance was determined using informative lymphoblasts and 56 samples of placental cDNA. In most of the placental samples we tested, the difference in expression levels between the two alleles was small, and only two samples indicated a monoallelic expression (i.e., preferential expression of one allele). These results suggest that 1) the predominant allelic expression pattern of BCRP in placental samples is biallelic, and 2) the mutation C421A is not a genetic variant acting in cis, but is considered to influence the translation efficiency.


http://dmd.aspetjournals.org/cgi/content/abstract/dmd.104.002741v1

Constitutive active (or androstane) receptor (CAR, NR113), a member of the nuclear receptor family, is a major regulator for induction of cytochrome P450 2B (CYP2B) genes by phenobarbital. Phenobarbital-like inducer, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), is a potent mouse CAR (mCAR) ligand that has been used to study CAR target genes in mice but does not activate human CAR (hCAR) or rat CAR (rCAR). Although 6-(4-chlorophenyl) imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzyl)oxime (CITCO) was reported to be an hCAR agonistic ligand, activation of hCAR by CITCO in cell-based reporter assay was weak. Therefore, we performed a screening of 50 drugs and chemicals using cell-based reporter assays to identify activators of hCAR. Among them, HMG-CoA reductase inhibitors (cerivastatin, simvastatin, fluvastatin and atorvastatin) enhanced the hCAR-mediated transcriptional activation of PBREM reporter gene by up to 3 fold. Similar activation by HMG-CoA reductase inhibitors was also observed with mouse and rat CARs. On the other hand, pravastatin did not activate hCAR at the concentrations tested (up to 30 (micro)M). The extent of activation by the HMG-CoA reductase inhibitors was stronger than that by CITCO. Cerivastatin, simvastatin, fluvastatin and atorvastatin induced CYP2B6 mRNA in stable hCAR-expressed FLC7 cells but not in original FLC7 cells. Therefore, we concluded that CAR mediates the effects of HMG-CoA reductase inhibitors on the induction of CYP2B genes, although HMG-CoA reductase inhibitors also activate pregnane X receptor. HMG-CoA reductase inhibitors such as cerivastatin would be useful to study for elucidating molecular and cellular mechanisms of hCAR.


http://dmd.aspetjournals.org/cgi/content/abstract/33/2/254

Human carbonyl reductase (CBR) activity accounts for a significant fraction of the metabolism of endogenous and xenobiotic carbonyl compounds. It is possible that genetic polymorphisms in CBR1 and CBR3 are key for the wide interindividual variability in the disposition of CBR drug substrates. We pinpointed a single nucleotide polymorphism in CBR3 (CBR3 V244M) that encodes for a V244 to M244 change. Blacks showed a higher frequency of the M244 allele (q = 0.51, n = 49) than did whites (q = 0.31, n = 70; p = 0.003). In addition, DNA variation panels from
10 ethnic groups presented a wide range of CBR3 V244M genotype distributions. Kinetic experiments with the recombinant CBR3 protein variants and menadione revealed that CBR3 M244 has significantly higher Vmax than does CBR3 V244 (Vmax CBR3 M244 = 40.6 \(+/-\) 1.3 \(\mu\)mol/min \(\cdot\) mg versus Vmax CBR3 V244 = 19.6 \(+/-\) 2.0 \(\mu\)mol/min \(\cdot\) mg, \(p = 0.002\)). In contrast, both isoforms presented similar Km values (Km CBR3 M244 = 22.9 \(+/-\) 2.9 \(\mu\)M versus Km CBR3 V244 = 24.6 \(+/-\) 3.2 \(\mu\)M, \(p = 0.43\)). Assays with NADP(H) demonstrated a higher VmaxNADP(H) (1.6-fold) and increased catalytic efficiency (VmaxNADP(H)/KmNADP(H)) for CBR3 M244 compared with CBR3 V244 (\(p = 0.013\)). Comparative three-dimensional analyses based on the structure of the homologous porcine carbonyl reductase suggested that the V244M substitution is positioned in a region critical for interactions with the NADP(H) cofactor. These studies demonstrate that the common CBR3 V244M polymorphism encodes for CBR3 isoforms with distinctive enzymatic properties.


The expression levels of mRNAs for MDR1 (P-glycoprotein), multidrug resistance-associated proteins (MRP1, MRP2), and cytochrome P450 3A (CYP3A) in Caco-2 cells were quantitatively compared with those in human duodenal enterocytes, normal colorectal tissues, and colorectal adenocarcinomas. Caco-2 cells (passages 36-88) were kindly supplied by several laboratories in Japan. Human duodenal enterocytes were obtained from five healthy male volunteers. Normal colorectal tissues and colorectal adenocarcinomas were simultaneously obtained from seven patients with primary colorectal adenocarcinoma. MDR1, MRP1, MRP2, and CYP3A mRNA levels were determined by real-time quantitative polymerase chain reactions (PCR). Relative concentrations of mRNAs for target proteins (MDR1, MRP1, MRP2, and CYP3A) and glyceraldehyde-3-phosphate dehydrogenase in Caco-2 cells were 1.00 \(+/-\) 0.15, 1.02 \(+/-\) 0.06, 0.94 \(+/-\) 0.10, and 0.68 \(+/-\) 0.60, respectively, and those in human enterocytes were about 12-, 3-, 7-, and 8000-fold higher than in the Caco-2 cells, respectively. In contrast, MDR1, MRP1, and CYP3A mRNA levels in Caco-2 cells were comparable to those in normal colorectal tissue and colorectal adenocarcinoma.


Etoposide is a DNA topoisomerase II inhibitor widely used in the treatment of a variety of malignancies that is also associated with therapy-related leukemia. The cytochrome P450 (P450)-derived catechol and quinone metabolites of etoposide may be important in the damage to the MLL (mixed lineage leukemia) gene and other genes resulting in leukemia-associated chromosomal translocations. Kinetic analysis of catechol formation by recombinant P450s was determined using liquid chromatography/selected reaction monitoring/mass spectrometry. CYP3A4 was found to play a major role in etoposide metabolism (Km = 77.7 \(+/-\) 27.8 \(\mu\)M; Vmax = 314 \(+/-\) 84 pmol of catechol/min/nmol of P450). However, CYP3A5 (Km = 13.9 \(+/-\) 3.1 \(\mu\)M; Vmax = 19.4 \(+/-\) 0.4 pmol of catechol/min/nmol of P450) may be involved in etoposide metabolism at therapeutic concentrations of free drug. Other P450s do not appear to be involved in etoposide catechol formation. Real-time polymerase chain reaction and Western blot analysis revealed significantly increased CYP3A4 mRNA and protein levels in hepatocytes treated with 10 \(\mu\)M rifampicin compared with untreated cells, but only modest effects of rifampicin on
CYP3A5 induction. Etoposide (40, 5, 1, and 0.25 \( \mu \text{M} \)) caused a slight increase in CYP3A4 mRNA in three of five batches of hepatocytes but did not result in proportionately increased CYP3A4 protein levels. At high concentrations, etoposide induced only a modest increase in CYP3A5 mRNA and protein levels in four of five batches of hepatocytes. Alternatively, coadministration of other drugs with etoposide may account for the increase in etoposide catechol formation during therapy with etoposide.

**EMBO J.** (9)


http://embojournal.npgjournals.com/cgi/content/abstract/22/23/6299

The human AP-endonuclease (APE1/Ref-1), a multifunctional protein central to repairing abasic sites and single-strand breaks in DNA, also plays a role in transcriptional regulation. Besides activating some transcription factors, APE1 is directly involved in Ca\(^{2+}\)-dependent downregulation of parathyroid hormone (PTH) expression by binding to negative calcium response elements (nCaREs) present in the PTH promoter. Here we show that APE1 is acetylated both in vivo and in vitro by the transcriptional co-activator p300 which is activated by Ca\(^{2+}\). Acetylation at Lys6 or Lys7 enhances binding of APE1 to nCaRE. APE1 stably interacts with class I histone deacetylases (HDACs) in vivo. An increase in extracellular calcium enhances the level of acetylated APE1 which acts as a repressor for the PTH promoter. Moreover, chromatin immunoprecipitation (ChIP) assay revealed that acetylation of APE1 enhanced binding of the APE1-HDACs complex to the PTH promoter. These results indicate that acetylation of APE1 plays an important role in this key repair protein's action in transcriptional regulation.


http://embojournal.npgjournals.com/cgi/content/abstract/21/21/5899

L1 elements are ubiquitous human transposons that replicate via an RNA intermediate. We have reconstituted the initial stages of L1 element transposition in vitro. The reaction requires only the L1 ORF2 protein, L1 3' RNA, a target DNA and appropriate buffer components. We detect branched molecules consisting of junctions between transposon 3' end cDNA and the target DNA, resulting from priming at a nick in the target DNA. 5' junctions of transposon cDNA and target DNA are also observed. The nicking and reverse transcription steps in the reaction can be uncoupled, as priming at pre-existing nicks and even double-strand breaks can occur. We find evidence for specific positioning of the L1 RNA with the ORF2 protein, probably mediated in part by the polyadenosine portion of L1 RNA. Polyguanosine, similar to a conserved region of the L1 3' UTR, potently inhibits L1 endonuclease (L1 EN) activity. L1 EN activity is also repressed in the context of the full-length ORF2 protein, but it and a second cryptic nuclease activity are released by ORF2p proteolysis. Additionally, heterologous RNA species such as Alu element RNA and L1 transcripts with 3' extensions are substrates for the reaction.
We report that the cyclophilin USA-CyP is part of distinct complexes with two spliceosomal proteins and is involved in both steps of pre-mRNA splicing. The splicing factors hPrp18 and hPrp4 have a short region of homology that defines a high affinity binding site for USA-CyP in each protein. USA-CyP forms separate, stable complexes with hPrp18 and hPrp4 in which the active site of the cyclophilin is exposed. The cyclophilin inhibitor cyclosporin A slows pre-mRNA splicing in vitro, and we show that its inhibition of the second step of splicing is caused by blocking the action of USA-CyP within its complex with hPrp18. Cyclosporin A also slows splicing in vivo, and we show that this slowing results specifically from inhibition of USA-CyP. Our results lead to a model in which USA-CyP is carried into the spliceosome in complexes with hPrp4 and hPrp18, and USA-CyP acts during splicing within these complexes. These results provide an example of the function of a cyclophilin in a complex process and provide insight into the mechanisms of action of cyclophilins.

The detection of thousands of volatile odorants is mediated by several hundreds of different G protein-coupled olfactory receptors (ORs). The main strategy in encoding odorant identities is a combinatorial receptor code scheme in that different odorants are recognized by different sets of ORs. Despite increasing information on agonist-OR combinations, little is known about the antagonism of ORs in the mammalian olfactory system. Here we show that odorants inhibit odorant responses of OR(s), evidence of antagonism between odorants at the receptor level. The antagonism was demonstrated in a heterologous OR-expression system and in single olfactory neurons that expressed a given OR, and was also visualized at the level of the olfactory epithelium. Dual functions of odorants as an agonist and an antagonist to ORs indicate a new aspect in the receptor code determination for odorant mixtures that often give rise to novel perceptual qualities that are not present in each component. The current study also provides insight into strategies to modulate perceived odorant quality.

Since the discovery of RNA recombination in polioviruses, there has been a general belief that this mechanism operates only in positive-sense RNA viruses. Recently, studying wild-type Tula hantavirus, we observed a mosaic-like structure of the S RNA segment that was consistent with generation by recombination between viruses from two genetic lineages. Here we show transfection-mediated rescue of Tula virus carrying recombinant S RNA segment. Independent attempts yielded S RNA molecules of similar structure; the majority of them carried a break point located close to one of the break points suggested for natural recombinants. Recombinant virus purified from the original variant was able to grow to the same titers in cell culture and showed the same characteristic immunofluorescence pattern when stained for the nucleocapsid protein. While competent, the recombinant virus appeared to be slightly less competitive than the wild type.
type. Sequence analysis of the S cDNA clones obtained from the purified recombinant virus confirmed that all S RNA molecules were of recombinant origin. This provides the first example of a negative-sense RNA virus constructed using homologous recombination.


http://embojournal.npgjournals.com/cgi/content/abstract/21/19/5017

We discovered that the hepatitis C virus (HCV) envelope glycoprotein E2 binds to human hepatoma cell lines independently of the previously proposed HCV receptor CD81. Comparative binding studies using recombinant E2 from the most prevalent 1a and 1b genotypes revealed that E2 recognition by hepatoma cells is independent from the viral isolate, while E2-CD81 interaction is isolate specific. Binding of soluble E2 to human hepatoma cells was impaired by deletion of the hypervariable region 1 (HVR1), but the wild-type phenotype was recovered by introducing a compensatory mutation reported previously to rescue infectivity of an HVR1-deleted HCV infectious clone. We have identified the receptor responsible for E2 binding to human hepatic cells as the human scavenger receptor class B type I (SR-BI). E2-SR-BI interaction is very selective since neither mouse SR-BI nor the closely related human scavenger receptor CD36, were able to bind E2. Finally, E2 recognition by SR-BI was competed out in an isolate-specific manner both on the hepatoma cell line and on the human SR-BI-transfected cell line by an anti-HVR1 monoclonal antibody.


http://embojournal.npgjournals.com/cgi/content/abstract/21/13/3434

We recently described an erythroid \{epsilon\}-globin gene repressor activity, which we named DRED (direct repeat erythroid-definitive). We show that DRED binds with high affinity to DR1 sites in the human embryonic (\{epsilon\}-) and fetal (\{gamma\}-) globin gene promoters, but the adult (\{beta\}-)globin promoter has no DR1 element. DRED is a 540 kDa complex; sequence determination showed that it contains the nuclear orphan receptors TR2 and TR4. TR2 and TR4 form a heterodimer that binds to the \{epsilon\}on and \{gamma\} promoter DR1 sites. One mutation in a DR1 site causes elevated \{gamma\}-globin transcription in human HPFH (hereditary persistence of fetal hemoglobin) syndrome, and we show that this mutation reduces TR2/TR4 binding in vitro. The two receptor mRNAs are expressed at all stages of murine and human erythropoiesis; their forced transgenic expression reduces endogenous embryonic (\{epsilon\})-globin transcription. These data suggest that TR2/TR4 forms the core of a larger DRED complex that represses embryonic and fetal globin transcription in definitive erythroid cells, and therefore that inhibition of its activity might be an attractive intervention point for treating sickle cell anemia.


http://embojournal.npgjournals.com/cgi/content/abstract/21/22/6025

Here we take advantage of the well-characterized and simple nervous system of Caenorhabditis elegans to further our understanding of the functions of RNA editing. We describe the two
C.elegans ADAR genes, adr-1 and adr-2, and characterize strains containing homozygous deletions in each, or both, of these genes. We find that adr-1 is expressed in most, if not all, cells of the C.elegans nervous system and also in the developing vulva. Using chemotaxis assays, we show that both ADARs are important for normal behavior. Biochemical, molecular and phenotypic analyses indicate that ADR-1 and ADR-2 have distinct roles in C.elegans, but sometimes act together.


http://embojournal.npgjournals.com/cgi/content/abstract/21/11/2703

Wnt signals regulate differentiation of neural crest cells through the (beta)-catenin associated with a nuclear mediator of the lymphoid-enhancing factor 1 (LEF-1)/T-cell factors (TCFs) family. Here we show the interaction between the basic helix-loop-helix and leucine-zipper region of microphthalmia-associated transcription factor (MITF) and LEF-1. MITF is essential for melanocyte differentiation and its heterozygous mutations cause auditory-pigmentary syndromes. Functional cooperation of MITF with LEF-1 results in synergistic transactivation of the dopachrome tautomerase (DCT) gene promoter, an early melanoblast marker. This activation depends on the separate cis-acting elements, which are also responsible for the induction of the DCT promoter by lithium chloride that mimics Wnt signaling. (beta)-catenin is required for efficient transactivation, but dispensable for the interaction between MITF and LEF-1. The interaction with MITF is unique to LEF-1 and not detectable with TCF-1. LEF-1 also cooperates with the MITF-related proteins, such as TFE3, to transactivate the DCT promoter. This study therefore suggests that the MITF/TFE3 family is a new class of nuclear modulators for LEF-1, which may ensure efficient propagation of Wnt signals in many types of cells.

EMBO Rep. (1)


http://emboreports.npgjournals.com/cgi/content/abstract/5/10/989

BRCA2 is a breast cancer susceptibility gene implicated in the repair of double-strand breaks by homologous recombination with RAD51. BRCA2 associates with a 70-amino-acid protein, DSS1, but the functional significance of this interaction has remained unclear. Recently, deficiency of a DSS1 orthologue in the fungus Ustilago maydis has been shown to cause a defect in recombinational DNA repair. Here we have investigated the consequences of DSS1 depletion in mammalian cells. We show that like BRCA2, DSS1 is required for DNA damage-induced RAD51 focus formation and for the maintenance of genomic stability, indicating a function conserved from lower eukaryotes to humans. However, DSS1 seems to be not required for BRCA2 or RAD51 stability or for BRCA2 and RAD51 to interact, raising the possibility that DSS1 may be required for the BRCA2-RAD51 complex to become associated with sites of DNA damage.

We have been investigating gene-expression profiles in estrogen receptor (ER)-negative breast cancers to identify molecules involved in breast carcinogenesis and to select genes or gene products that might be useful as diagnostic markers or targets for new molecular therapies. Here we report evidence that the gene encoding retinoic acid-induced protein 3 (RAI3) is a potential molecular target for treatment of breast cancers. Using quantitative reverse transcription-PCR (RT-PCR), we documented increased expression of RAI3 in 19 of 25 primary breast cancers and in 6 of 11 breast-cancer cell lines examined, by comparison with normal mammary-gland tissue. Treatment of human embryonic kidney (HEK293) cells with siRNA against RAI3 suppressed expression of RAI3 and also suppressed cell growth. Transfection of siRNA into breast-cancer cell lines MCF7 and T47D also suppressed RAI3 mRNA and growth of the cancer cells. Because our data imply that up-regulation of RAI3 function is a frequent feature of breast carcinogenesis, we suggest that selective suppression of signal from RAI3 might hold promise for development of a new strategy for treating breast cancers.


The presence of erm genes was investigated among macrolide-resistant Gram-positive bacteria isolated from soil samples collected from four Danish farms that had been treated with animal waste. Soil samples were collected before, a few days after spread and 1 months and 5 months later. In 33% (9/27) of these isolates, an erm gene was detected using PCR. Eight isolates were positive for erm(B) and one isolate was positive for erm(C). No isolates contained erm(A), erm(D) or erm(F). The positive isolates were identified to genus level. Two erm(B) positive isolates were identified as Enterococcus spp., and the erm(C)-positive isolate as a Streptococcus spp., probably indicating survival from animal waste. The remaining six erm(B) positive isolates all belonged to the Bacillus cereus group. The erm(B) gene has not previously been identified in B. cereus but is frequently found among enterococci. This result could indicate horizontal transfer from bacteria of animal origin to indigenous soil bacteria.
Febrile convulsions (FCs) represent the majority of childhood seizures, and patients have a genetic predisposition to their development. The genetic susceptibility to FCs seems to involve multiple genes in most instances. Recent studies provided evidence that mutations in SCN1A represent the most frequent cause of generalized epilepsy with febrile seizures plus an autosomal-dominant epilepsy syndrome. SCN1A mutations alter channel inactivation, resulting in persistent inward sodium current. It is not known if polymorphisms in those genes involved in familial epilepsies also contribute to the pathogenesis of FCs. By performing an association study, we used single nucleotide polymorphisms to investigate the distribution of genotypes of SCN1A in patients with FCs. A total of 104 Taiwanese children with FCs and 83 normal control subjects were included in the study. Polymerase chain reaction was used to identify the A/G polymorphism of the SCN1A gene. The results showed that genotypes and allelic frequencies for the SCN1A gene polymorphisms in both groups were not significantly different. These data suggest that the SCN1A gene might not be one of the susceptibility factors for FCs. Pure FCs and febrile convulsions associated with idiopathic generalized epilepsy may not share a common genetic etiology.

Various studies have shown that brain-derived neurotrophic factor (BDNF) increased neuronal excitability. We tested that BDNF might be involved in the etiology of febrile seizures (FSs). A total of 186 Taiwanese children were divided into two groups: (1) FSs (n = 104); (2) normal control subjects (n = 83). A single base pair polymorphism SNP6265 (Val66Met) at position 196 was analyzed. Our findings suggest that the BDNF polymorphisms were not candidate genetic markers.

Disruption of the function of the mouse jerky gene by transgene insertion causes generalized recurrent seizures reminiscent of human idiopathic generalized epilepsy (IGE). A human homologue, JRK/JH8, has been cloned, which maps to 8q24, a chromosomal region associated with several forms of IGE. JRK/JH8 is, therefore, a candidate locus for at least some forms of IGE. We report corrected cDNA sequences and extended open reading frames for the mouse.
jerky and human JRK/JH8 genes, which add 48 amino acids to the N-terminus of the Jerky protein and which extends the region of homology with the N-terminal DNA-binding domain of the centromere-binding protein, CENP-B. Systematic sequencing of the coding region of the extended JRK/JH8 gene identified single nucleotide polymorphisms that define three haplotypes, which were used for association studies in patients with idiopathic generalized epilepsy. We report one subject with childhood absence epilepsy (CAE) that evolved to juvenile myoclonic epilepsy (JME) that has a unique de novo mutation that results in a non-conservative amino acid change at a potential protein glycosylation site. Familial analysis supports a causal role for this mutation in the disease.

Eukaryot. Cell (2)


http://ec.asm.org/cgi/content/abstract/1/1/137

We describe a novel gene family that forms clusters in subtelomeric regions of Trypanosoma brucei chromosomes and partially accounts for the observed clustering of retrotransposons. The ingi and ribosomal inserted mobile element (RIME) non-LTR retrotransposons share 250 bp at both extremities and are the most abundant putatively mobile elements, with about 500 copies per haploid genome. From cDNA clones and subsequently in the T. brucei genomic DNA databases, we identified 52 homologous gene and pseudogene sequences, 16 of which contain a RIME and/or ingi retrotransposon inserted at exactly the same relative position. Here these genes are called the RHS family, for retrotransposon hot spot. Comparison of the protein sequences encoded by RHS genes (21 copies) and pseudogenes (24 copies) revealed a conserved central region containing an ATP/GTP-binding motif and the RIME/ingi insertion site. The RHS proteins share between 13 and 96% identity, and six subfamilies, RHS1 to RHS6, can be defined on the basis of their divergent C-terminal domains. Immunofluorescence and Western blot analyses using RHS subfamily-specific immune sera show that RHS proteins are constitutively expressed and occur mainly in the nucleus. Analysis of Genome Survey Sequence databases indicated that the Trypanosoma brucei diploid genome contains about 280 RHS (pseudo)genes. Among the 52 identified RHS (pseudo)genes, 48 copies are in three RHS clusters located in subtelomeric regions of chromosomes Ia and II and adjacent to the active bloodstream form expression site in T. brucei strain TREU927/4 GUTat10.1. RHS genes comprise the remaining sequence of the size-polymorphic "repetitive region" described for T. brucei chromosome I, and a homologous gene family is present in the Trypanosoma cruzi genome.


http://ec.asm.org/cgi/content/abstract/1/6/895

The dimorphic fungi Blastomyces dermatitidis and Histoplasma capsulatum cause systemic mycoses in humans and other animals. Forward genetic approaches to generating and screening
mutants for biologically important phenotypes have been underutilized for these pathogens. The plant-transforming bacterium Agrobacterium tumefaciens was tested to determine whether it could transform these fungi and if the fate of transforming DNA was suited for use as an insertional mutagen. Yeast cells from both fungi and germinating conidia from B. dermatitidis were transformed via A. tumefaciens by using hygromycin resistance for selection. Transformation frequencies up to 1 per 100 yeast cells were obtained at high effector-to-target ratios of 3,000:1. B. dermatitidis and H. capsulatum ura5 lines were complemented with transfer DNA vectors expressing URA5 at efficiencies 5 to 10 times greater than those obtained using hygromycin selection. Southern blot analyses indicated that in 80% of transformants the transferred DNA was integrated into chromosomal DNA at single, unique sites in the genome. Progeny of B. dermatitidis transformants unexpectedly showed that a single round of colony growth under hygromycin selection or visible selection of transformants by lacZ expression generated homokaryotic progeny from multinucleate yeast. Theoretical analysis of random organelle sorting suggests that the majority of B. dermatitidis cells would be homokaryons after the ca. 20 generations necessary for colony formation. Taken together, the results demonstrate that A. tumefaciens efficiently transfers DNA into B. dermatitidis and H. capsulatum and has the properties necessary for use as an insertional mutagen in these fungi.

Eur. Heart J. (2)


http://eurheartj.oupjournals.org/cgi/content/abstract/26/6/584

Aims To assess, in families with premature coronary artery disease (CAD), the possible association, with linkage, of the X-linked AT2 receptor (-1332 G/A) gene polymorphism and premature CAD. Methods and results We investigated 509 families with a history of premature CAD that consisted of one sibling affected with premature CAD and two unaffected siblings. Genotyping of subjects was performed using a restriction enzyme digestion of an initial 310 bp polymerase chain reaction fragment that included the AT2 (-1332 G/A) locus. The mean age of the 611 individuals affected by premature CAD at the time of event was 49.5(±)8.1 years. Conditional logistic regression analysis confirmed a significant predictive value of premature CAD for the covariates of hypertension, diabetes, dyslipidaemia, history of smoking, and male gender. The genetic data were analysed for these families using the X-linked sibling transmission/deletion test (XS-TDT) statistics program. In hemizygous men we observed evidence for association in the presence of linkage, for the AT2 (-1332 G/A) locus and premature CAD (P-exact value=0.024) and also a trend towards association, in the presence of linkage, for this polymorphism and hypertension (P-exact value=0.08). Conclusions We have observed evidence of association between the presence of linkage for the X-linked AT2 (-1332 G/A) polymorphism and premature CAD in hemizygous males.


http://eurheartj.oupjournals.org/cgi/content/abstract/25/5/377
Aims We tested the hypothesis that cardiac angiotensin II (Ang II) receptor gene transcription may predict the development of transplant coronary artery disease (TCAD) following heart transplantation. Methods and results We examined the gene transcripts of Ang II type 1 (AT1R) and type 2 receptors (AT2R) in endomyocardial biopsy specimens from 50 heart transplant recipients. The progression of TCAD was measured as change in maximal intimal thickness (CMIT) and change in plaque volume (CPV) by intravascular ultrasound (IVUS) examinations from baseline to one year after transplantation. The development of transplant vasculopathy was defined as a CMIT of \( \geq 0.3 \) mm over one year. The level of AT1R mRNA was associated with that of AT2R in transplanted hearts (regression coefficient=1.77, 95% CI 0.85-2.89; [IMG]f1.gif" BORDER="0">). AT1R and AT2R gene transcripts were univariate predictors of CMIT (AT1R: regression coefficient 0.10, 95% CI 0.06-0.14; [IMG]f1.gif" BORDER="0">; AT2R: regression coefficient 0.28, 95% CI 0.17-0.40; [IMG]f1.gif" BORDER="0">) or CPV (AT1R: regression coefficient 0.41, 95% CI 0.17-0.65; [IMG]f1.gif" BORDER="0">; AT2R: regression coefficient 1.25, 95% CI 0.49-2.01; [IMG]f2.gif" BORDER="0">). By one year, 21 (46%) transplant recipients showed evidence of transplant vasculopathy and the rest did not. The vasculopathic group demonstrated a higher level of expression of cardiac AT1R than the non-vasculopathic group (3.7(+/-)2.9 vs 1.6(+/-)1.7 folds; [IMG]f3.gif" BORDER="0">). The level of AT1R mRNA in transplanted heart was identified as a discriminator that predicted the development of transplant vasculopathy with a sensitivity of 75% and specificity of 83%. Conclusions Cardiac Ang II receptor gene transcripts are associated with the progression of TCAD following heart transplantation. Only AT1R gene transcripts predicted the development of transplant vasculopathy in this preliminary study. These findings potentially support a role of Ang II receptors in the progression of TCAD following cardiac transplantation.


http://content.febsjournal.org/cgi/content/abstract/269/4/1293

The \( \alpha \)s1-casein (\( \alpha \)s1-Cas) locus in the goat is characterized by a polymorphism, the main feature of which is to be qualitative as well as quantitative. A systematic analysis performed in an autochthon southern Italy breed identified a new rare allele (M), which was characterized at both the protein and genomic level. The M protein displays the slowest electrophoretic mobility of the \( \alpha \)s1-Cas variants described so far. MS and automated Edman degradation experiments showed that this behavior was due to the loss of two phosphate residues in the multiple phosphorylation site (64SP-SP-SP-SP-SP-E-70E) consecutively to a Ser[&gt;Leu substitution at position 66 of the peptide chain (64S-SP-L-SP-SP-E-70E). This was confirmed by sequencing a genomic DNA fragment encompassing exon 9 where the 8th codon (TCG) was shown to be mutated to TTG. Sequencing of amplified genomic DNA segments spanning the 5' and 3' flanking regions of each exon allowed us to identify 23 single nucleotide polymorphisms and two insertion/deletion events in the coding as well as the noncoding regions. A comparison of specific haplotypes defined for each of the(\( \alpha \))s1-CasF, A and M alleles indicates that the M allele probably arises from interallelic recombination between alleles A and B 2, followed by a C[-&gt;G]T transition at nucleotide 23 of the ninth exon. The region encompassing the recombination break point was putatively located between nucleotides 86 upstream and nucleotide 40 downstream of exon 8. Interallelic recombination therefore appears to be a possible means of generating allelic diversity at the(\( \alpha \))s1-Cas locus, at least in the goat. The previously
proposed molecular phylogeny must now be revised, possibly starting from two ancestral allelic lineages.


http://content.febsjournal.org/cgi/content/abstract/269/18/4566

Thyrotropin-releasing hormone receptor (TRHR) has already been cloned in mammals where thyrotropin-releasing hormone (TRH) is known to act as a powerful stimulator of thyroid-stimulating hormone (TSH) secretion. The TRH receptor of amphibians has not yet been characterized, although TRH is specifically important in the adaptation of skin color to environmental changes via the secretion of (alpha)-melanocyte-stimulating hormone (alpha)-MSH. Using a degenerate PCR strategy, we report on the isolation of three distinct cDNA species encoding TRHR from the brain of Xenopus laevis. We have designated these as xTRHR1, xTRHR2 and xTRHR3. Analysis of the predicted amino acid sequences revealed that the three Xenopus TRHRs are only 54-62% identical and contain all the highly conserved residues constituting the TRH binding pocket. Amino acid sequences and phylogenetic analysis revealed that xTRHR1 is a member of TRHR subfamily 1 and xTRHR2 belongs to subfamily 2, while xTRHR3 is a new TRHR subtype awaiting discovery in other animal species. The three Xenopus TRHRs have distinct patterns of expression. xTRHR3 was abundant in the brain and much scarcer in the peripheral tissues, whereas xTRHR1 was found mainly in the stomach and xTRHR2 in the heart. The Xenopus TRHR subtype 1 was found specifically in the intestine, lung and urinary bladder. These observations suggest that the three xTRHRs each have specific functions that remain to be elucidated. Expression in Xenopus oocytes and HEK-293 cells indicates that the three Xenopus TRHRs are fully functional and are coupled to the inositol phosphate/calcium pathway. Interestingly, activation of xTRHR3 required larger concentrations of TRH compared with the other two receptors, suggesting marked differences in receptor binding, coupling or regulation.


http://content.febsjournal.org/cgi/content/abstract/270/7/1493

Several clinical trials have revealed that individuals who were given (beta)-carotene and vitamin A did not have a reduced risk of cancer compared to those given placebo; rather, vitamin A could actually have caused an adverse effect in the lungs of smokers [Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Valanis, B., Williams, J.H., Barnhart, S. & Hammar, S. N. Engl. J. Med. (1996) 334, 1150-1155; Hennekens, C.H., Burin, J.E., Manson, J.E., Stampfer, M., Rosner, B., Cook, N.R., Belanger, C., LaMotte, F., Gaziano, J.M., Ridker, P.M., Willet, W. & Peto, R. (1996) N. Engl. J. Med. 334, 1145-1149]. Using differential display techniques, an initial survey using rats showed that liver RNA expression of c-H-Ras was decreased and p53 increased in rats with chronic vitamin A deficiency. These findings prompted us to evaluate the expression of c-Jun, p53 and p21 WAF1/CIF1 (by RT-PCR) in liver and lung of rats. This study showed that c-Jun levels were lower and that p53 and p21 WAF1/CIF1 levels were higher in chronic vitamin A deficiency. Vitamin A supplementation increased expression of c-Jun, while decreasing the expression of p53 and p21 WAF1/CIF1. Western-blot analysis demonstrated that c-Jun and p53 showed a similar pattern to that found in the RT-PCR analyses. Binding of retinoic acid receptors (RAR) to the c-Jun promoter was
decreased in chronic vitamin A deficiency when compared to control hepatocytes, but contrasting results were found with acute vitamin A supplemented cells. DNA fragmentation and cytochrome c release from mitochondria were analyzed and no changes were found. In lung, an increase in the expression of c-Jun produced a significant increase in cyclin D1 expression. These results may explain, at least in part, the conflicting results found in patients supplemented with vitamin A and illustrate that the changes are not restricted to lung. Furthermore, these results suggest that pharmacological vitamin A supplementation may increase the risk of adverse effects including the risk of oncogenesis.


http://content.febsjournal.org/cgi/content/abstract/271/13/2755

Phospholipase D (PLD) plays a major role in the activation of the neutrophil respiratory burst. However, the repertoire of PLD isoforms present in these primary cells, the precise mechanism of activation, and the impact of cell priming on PLD activity and localization remain poorly defined. RT-PCR analysis showed that both PLD1 and PLD2 isoforms are expressed in human neutrophils, with PLD1 expressed at a higher level. Endogenous PLD1 was detected by immunoprecipitation and Western blotting, and was predominantly membrane-associated under control and primed/stimulated conditions. Immunofluorescence showed that PLD had a punctate distribution throughout the cell, which was not altered after stimulation by soluble agonists. In contrast, PLD localized to the phagolysosome membrane after ingestion of nonopsonized zymosan particles. We also demonstrate that tumour necrosis factor α (TNFα) greatly potentiates agonist-stimulated PLD activation, myeloperoxidase release, and superoxide anion generation, and that PLD activation occurs via a phosphatidylinositol 3-kinase-sensitive and brefeldin-sensitive ADP-ribosylation factor GTPase-regulated mechanism. Moreover, propranolol, which causes an increase in PLD-derived phosphatidic acid accumulation, caused a selective increase in agonist-stimulated myeloperoxidase release. Our results indicate that priming is a critical regulator of PLD activation, that the PLD-generated lipid products exert divergent effects on neutrophil functional responses, that PLD1 is the major PLD isoform present in human neutrophils, and that PLD1 actively translocates to the phagosomal wall after particle ingestion.


http://content.febsjournal.org/cgi/content/abstract/271/1/108

Volkensin, a type 2 ribosome-inactivating protein from the roots of Adenia volkensii Harms (kilyambiti plant) was characterized both at the protein and nucleotide level by direct amino acid sequencing and cloning of the gene encoding the protein. Gene sequence analysis revealed that volkensin is encoded by a 1569-bp ORF (523 amino acid residues) without introns, with an internal linker sequence of 45 bp. Differences in residues present at several sequence positions (reproduced after repeated protein sequence analyses), with respect to the gene sequence, suggest several isoforms for the volkensin A-chain. Based on the crystallographic coordinates of ricin, which shares a high sequence identity with volkensin, a molecular model of volkensin was obtained. The 3D model suggests that the amino acid residues of the active site of the ricin A-chain are conserved at identical spatial positions, including Ser203, a novel amino acid residue found to be conserved in all known ribosome-inactivating proteins. The sugar binding site 1 of the ricin B-chain is also conserved in the volkensin B-chain, whilst in binding site 2, His246 replaces Tyr248. Native volkensin contains two free cysteinyI residues out of 14 derived from the gene
sequence, thus suggesting a further disulphide bridge in the B chain, in addition to the inter-and intrachain disulphide bond pattern common to other type 2 ribosome-inactivating proteins.


http://content.febsjournal.org/cgi/content/abstract/269/24/6162

The marine snail Conus is the sole invertebrate wherein both the vitamin K-dependent carboxylase and its product, \(\gamma\)-carboxyglutamic acid, have been identified. To examine its biosynthesis of \(\gamma\)-carboxyglutamic acid, we studied the carboxylase from Conus venom ducts. The carboxylase cDNA from Conus textile has an ORF that encodes a 811-amino-acid protein which exhibits sequence similarity to the vertebrate carboxylases, with 41% identity and \(\text{approx}\) 60% sequence similarity to the bovine carboxylase. Expression of this cDNA in COS cells or insect cells yielded vitamin K-dependent carboxylase activity and vitamin K-dependent epoxidase activity. The recombinant carboxylase has a molecular mass of \(\text{approx}\) 130 kDa. The recombinant Conus carboxylase carboxylated Phe-Leu-Glu-Glu-Leu and the 28-residue peptides based on residues -18 to +10 of human prothrombin and proFactor IX with \(K_m\) values of 420 \(\mu\)m, 1.7 \(\mu\)m and 6 \(\mu\)m, respectively; the \(K_m\) for vitamin K is 52 \(\mu\)m. The \(K_m\) values for peptides based on the sequence of the conotoxin (epsilon)-TxiX and two precursor analogs containing 12 or 29 amino acids of the propeptide region are 565 \(\mu\)m, 75 \(\mu\)m and 74 \(\mu\)m, respectively. The recombinant Conus carboxylase, in the absence of endogenous substrates, is stimulated up to fivefold by vertebrate propeptides but not by Conus propeptides. These results suggest two propeptide-binding sites in the carboxylase, one that binds the Conus and vertebrate propeptides and is required for substrate binding, and the other that binds only the vertebrate propeptide and is required for enzyme stimulation. The marked functional and structural similarities between the Conus carboxylase and vertebrate vitamin K-dependent \(\gamma\)-carboxylases argue for conservation of a vitamin K-dependent carboxylase across animal species and the importance of \(\gamma\)-carboxyglutamic acid synthesis in diverse biological systems.


http://content.febsjournal.org/cgi/content/abstract/270/1/163

The ST2 gene, which is specifically induced by growth stimulation in fibroblasts, encodes interleukin-1 receptor-related proteins and is widely expressed in hematopoietic, helper T, and various cancer cells. However, the physiological as well as pathological functions of the ST2 gene products are not yet fully understood. In this study, we analyzed the expression of the ST2 gene in human glioma cell lines and human brain tumor samples with real-time polymerase chain reaction method, the results of which revealed that the expression level of the ST2 gene in glioma cell lines and glioblastoma samples is significantly lower than that in a fibroblastic cell line, TM12, and benign brain tumors, suggesting the reverse relationship between malignancy and ST2 expression. As we could not detect the soluble ST2 protein in the culture fluid of the T98G glioblastoma cell line by ELISA, we established stable transformants of T98G that continuously produce and secrete the ST2 protein, in order to study the effect of the ST2 protein on malignancy. Although we could not detect a remarkable difference in proliferation between transformants and control cells in conventional tissue culture dishes, the efficiency of colony formation in soft agar was significantly decreased in the case of cells that continuously produce the ST2 protein. Furthermore, inhibition of colony formation in soft agar was observed in wild-type
T98G cells when purified soluble ST2 protein was added to the culture, in a dose-dependent manner. Taken together, the results suggest that the expression of ST2 suppressed the anchorage-independent growth and malignancy.


http://content.febsjournal.org/cgi/content/abstract/269/21/5137

The structurally homologous mononuclear iron and manganese superoxide dismutases (FeSOD and MnSOD, respectively) contain a highly conserved glutamine residue in the active site which projects toward the active-site metal centre and participates in an extensive hydrogen bonding network. The position of this residue is different for each SOD isoenzyme (Q69 in FeSOD and Q146 in MnSOD of Escherichia coli). Although site-directed mutant enzymes lacking this glutamine residue (FeSOD[Q69G] and MnSOD[Q146A]) demonstrated a higher degree of selectivity for their respective metal, they showed little or no activity compared with wild types. FeSOD double mutants (FeSOD[Q69G/A141Q]), which mimic the glutamine position in MnSOD, elicited 25% the activity of wild-type FeSOD while the activity of the corresponding MnSOD double mutant (MnSOD[G77Q/Q146A]) increased to 150% (relative to wild-type MnSOD). Both double mutants showed reduced selectivity toward their metal. Differences exhibited in the thermostability of SOD activity was most obvious in the mutants that contained two glutamine residues (FeSOD[A141Q] and MnSOD[G77Q]), where the MnSOD mutant was thermostable and the FeSOD mutant was thermolabile. Significantly, the MnSOD double mutant exhibited a thermal-inactivation profile similar to that of wild-type FeSOD while that of the FeSOD double mutant was similar to wild-type MnSOD. We conclude therefore that the position of this glutamine residue contributes to metal selectivity and is responsible for some of the different physicochemical properties of these SODs, and in particular their characteristic thermostability.


http://content.febsjournal.org/cgi/content/abstract/271/13/2584

A novel hypoxically regulated intercellular junction protein (claudin-like protein of 24 kDa, CLP24) has been identified that shows homology to the myelin protein 22/epithelial membrane protein 1/claudin family of cell junction proteins, which are involved in the modulation of paracellular permeability. The CLP24 protein contains four predicted transmembrane domains and a C-terminal protein-protein interaction domain. These domains are characteristic of the four transmembrane spanning (tetraspan) family of proteins, which includes myelin protein 22, and are involved in cell adhesion at tight, gap and adherens junctions. Expression profiling analyses show that CLP24 is highly expressed in lung, heart, kidney and placental tissues. Cellular studies confirm that the CLP24 protein localizes to cell-cell junctions and co-localizes with the {beta}-catenin adherens junction-associated protein but not with tight junctions. Over-expression of CLP24 results in decreased adhesion between cells, and functional paracellular flux studies confirm that over-expression of the CLP24 protein modulates the junctional barrier function. These data therefore suggest that CLP24 is a novel, hypoxically regulated tetraspan adherens junction protein that modulates cell adhesion, paracellular permeability and angiogenesis.

http://content.febsjournal.org/cgi/content/abstract/270/2/230

Human \{beta\}2-glycoprotein I (\{beta\}2GPI), also known as apolipoprotein H, has been implicated in haemostasis and the production of anti-phospholipid antibodies. There is a wide range of interindividual variation in \{beta\}2GPI plasma levels that is thought to be under genetic control, but its molecular basis remains unknown. To understand the genetic basis of \{beta\}2GPI variation, we analyzed the 5' flanking region of the \{beta\}2GPI gene for mutation detection by DHPLC and identified a point mutation at the transcriptional initiation site (-1C\[\rightarrow\]A) with a carrier frequency of 12.1%. The mutation was associated with significantly lower \{beta\}2GPI plasma levels (P < 0.0001) and low occurrence of anti-phospholipid antibodies in lupus patients (4.8% antibody-positive group vs. 16.6% in the antibody-negative group; P = 0.019). Northern blot analysis confirmed that the -1C\[\rightarrow\]A mutation was associated with lower mRNA levels and it reduced the reporter (luciferase) gene expression by twofold. Electrophoretic gel mobility shift assay (EMSA) revealed that the -1C\[\rightarrow\]A mutation disrupts the binding for crude hepatic nuclear extracts and purified TFIID. These results suggest that the substitution of C with A at the \{beta\}2GPI transcriptional initiation site is a causative mutation that affects its gene expression at the transcriptional level and ultimately \{beta\}2GPI plasma levels and the occurrence of anti-phospholipid antibodies.


http://content.febsjournal.org/cgi/content/abstract/270/2/213

We report the cloning, expression, pharmacological characterization and tissue distribution of a melanocortin (MC) receptor gene in a shark, the spiny dogfish (Squalus acanthias) (Sac). Phylogenetic analysis showed that this receptor is an ortholog of the MC4 subtype, sharing 71% overall amino acid identity with the human (Hsa) MC4 receptor. When expressed and characterized by radioligand binding assay for the natural MSH (melanocyte-stimulating hormone) peptides \{alpha\}-, \{beta\}-, and \{gamma\}-MSH, the SacMC4 receptor showed pharmacological properties very similar to the HsaMC4 receptor. Stimulation of SacMC4 receptor transfected cells with \{alpha\}-MSH caused a dose-dependent increase in intracellular cAMP levels. The SacMC4 receptor has Ala in position 59 where all other cloned MC receptors have Glu. We confirmed that this was not due to individual polymorphism and subsequently mutated the residue back' to Glu but the mutation did not affect the pharmacological properties of the receptor. SacMC4 receptor mRNA was detected by RT-PCR in the optic tectum, hypothalamus, brain stem, telencephalon and olfactory bulb but not in cerebellum or in peripheral tissues. This study describes the first characterization of an MC receptor in a cartilaginous fish, the most distant MC receptor gene cloned to date. Conservation of gene structure, pharmacological properties and tissue distribution suggests that this receptor may have similar roles in sharks as in mammals and that these were established more than 450 million years ago.

Bothrops snake venoms are known to induce local tissue damage such as hemorrhage and myonecrosis. The opossum Didelphis marsupialis is resistant to these snake venoms and has natural venom inhibitors in its plasma. The aim of this work was to clone and study the chemical, physicochemical and biological properties of DM64, an antmyotoxic protein from opossum serum. DM64 is an acidic protein showing 15% glycosylation and with a molecular mass of 63 659 Da when analysed by MALDI-TOF MS. It was cloned and the amino acid sequence was found to be homologous to DM43, a metalloproteinase inhibitor from D. marsupialis serum, and to human (alpha)1 B-glycoprotein, indicating the presence of five immunoglobulin-like domains. DM64 neutralized both the in vivo myotoxicity and the in vitro cytotoxicity of myotoxins I (mt-I/Asp49) and II (mt-II/Lys49) from Bothrops asper venom. The inhibitor formed noncovalent complexes with both toxins, but did not inhibit the PLA2 activity of mt-I. Accordingly, DM64 did not neutralize the anticoagulant effect of mt-I nor its intracerebroventricular lethality, effects that depend on its enzymatic activity, and which demonstrate the dissociation between the catalytic and toxic activities of this Asp49 myotoxic PLA2. Furthermore, despite its similarity with metalloproteinase inhibitors, DM64 presented no antihemorrhagic activity against Bothrops jararaca or Bothrops asper crude venoms, and did not inhibit the fibrinogenolytic activity of jararhagin or bothrolysin. This is the first report of a myotoxin inhibitor with an immunoglobulin-like structure isolated and characterized from animal blood.


Retinyl esters are a major endogenous storage source of vitamin A in vertebrates and their hydrolysis to retinol is a key step in the regulation of the supply of retinoids to all tissues. Some members of nonspecific carboxylesterase family (EC 3.1.1.1) have been shown to hydrolyze retinyl esters. However, the number of different isoenzymes that are expressed in the liver and their retinyl palmitate hydrolase activity is not known. Six different carboxylesterases were identified and purified from rat liver microsomal extracts. Each isoenzyme was identified by mass spectrometry of its tryptic peptides. In addition to previously characterized rat liver carboxylesterases ES10, ES4, ES3, the protein products for two cloned genes, AB010635 and D50580 (GenBank accession numbers), were also identified. The sixth isoenzyme was a novel carboxylesterase and its complete cDNA was cloned and sequenced (AY034877). Three isoenzymes, ES10, ES4 and ES3, account for more than 95% of rat liver microsomal carboxylesterase activity. They obey Michaelis-Menten kinetics for hydrolysis of retinyl palmitate with Km values of about 1 {micro}m and specific activities between 3 and 8 nmol[middle dot]min-1[middle dot]mg-1 protein. D50580 and AY034877 also hydrolyzed retinyl palmitate. Gene-specific oligonucleotide probing of multiple-tissue Northern blot indicates differential expression in various tissues. Multiple genes are highly expressed in liver and small intestine, important tissues for retinoid metabolism. The level of expression of any one of the six different carboxylesterase isoenzymes will regulate the metabolism of retinyl palmitate in specific rat cells and tissues.

A novel cosmid (pABC6.5) whose DNA insert from Streptomyces capreolus, the A201A antibiotic producer, overlaps the inserts of the previously reported pCAR11 and pCAR13 cosmids, has been isolated. These two latter cosmids were known to contain the aminonucleoside antibiotic A201A resistance determinants ard2 and ard1, respectively. Together, these three cosmids have permitted the identification of a DNA stretch of 19 kb between ard1 and ard2, which should comprise a large region of a putative A201A biosynthetic (ata) gene cluster. The sequence of the 7 kb upstream of ard1 towards ard2 reveals seven consecutive open reading frames: ataP3, ataP5, ataP4, ataP10, ataP7, ata12 and ataPKS1. Except for the last two, their deduced products present high similarities to an identical number of counterparts from the pur cluster of Streptomyces alboniger that were either known or proposed to be implicated in the biosynthesis of the N6,N6-dimethyl-3'-amino-3'-deoxyadenosine moiety of puromycin. Because A201A contains this chemical moiety, these ataP genes are most likely implicated in its biosynthesis. Accordingly, the ataP4, ataP5 and ataP10 genes complemented specific puromycin nonproducing {Delta}pur4, {Delta}pur5 and {Delta}pur10 mutants of S. alboniger, respectively. Amino acid sequence comparisons suggest that ata12 and ataPKS1 could be implicated in the biosynthesis of the d-rhamnose and {alpha}-p-coumaric acid moieties of A201A. Further sequencing of 2 kb of DNA downstream of ard1 has disclosed a region which might contain one end of the ata cluster.


http://content.febsjournal.org/cgi/content/abstract/269/21/5119

Campylobacter jejuni infections are one of the leading causes of human gastroenteritis and are suspected of being a precursor to Guillain-Barre and Miller-Fisher syndromes. Recently, the complete genome sequence of C. jejuni NCTC 11168 was described. In this study, the molecular structure of the lipooligosaccharide and capsular polysaccharide of C. jejuni NCTC 11168 was investigated. The lipooligosaccharide was shown to exhibit carbohydrate structures analogous to the GM1a and GM2 carbohydrate epitopes of human gangliosides (shown below): ![GM1a and GM2 structures](medium/ejb3201.fu1.gif) The high Mr capsule polysaccharide was composed of (beta)-d-Ribp, (beta)-d-GalfNAc, (alpha)-d-GlcpA6(NGro), a uronic acid amidated with 2-amino-2-deoxyglycerol at C-6, and 6-O-methyl-d-glycero-(alpha)-l-gluco-heptopyranose as a side-branch (shown below): ![High Mr capsule polysaccharide structure](medium/ejb3201.fu2.gif) The structural information presented here will aid in the identification and characterization of specific enzymes that are involved in the biosynthesis of these structures and may lead to the discovery of potential therapeutic targets. In addition, the correlation of carbohydrate structure with gene complement will aid in the elucidation of the role of these surface carbohydrates in C. jejuni pathogenesis.

Eur. J. Endocrinol. (1)


http://www.eje-online.org/cgi/content/abstract/152/4/545
Background: Cyclooxygenase-2 (COX-2) seems to play a role in the development and carcinogenesis of papillary thyroid carcinoma. Its incidence of expression and potential application as a tumor marker remain to be elucidated. Materials and methods: Immunohistochemical staining for COX-2 expression was performed for 30 papillary thyroid carcinoma (PTC) and 40 benign thyroid specimens. COX-2 mRNA expression was analyzed using a reverse transcriptase-polymerase chain reaction (RT-PCR) for paired fresh frozen tissues removed from surgically resected PTC specimens. Results: COX-2 expression was detected by immunohistochemistry in 27 of 30 (90%) PTC but was absent in 40 benign thyroid specimens, including 27 nodular hyperplasia, 7 follicular adenoma and 6 lymphocytic thyroiditis. Two of the three COX-2 negative carcinomas were follicular variant of PTC. RT-PCR analysis confirmed COX-2 mRNA over-expression in 14 of 20 (70%) paired specimens of PTC. Real-time quantitative RT-PCR showed that the level of COX-2 mRNA expression was significantly higher in PTC than in both the adjacent non-cancerous tissues and the benign thyroid specimens. Conclusion: COX-2 is frequently expressed in PTC but not in benign thyroid specimens. COX-2 expression may serve as a useful molecular marker for PTC in cases of diagnostic difficulty.

Eur. Respir. J. (2)


http://erj.ersjournals.com/cgi/content/abstract/24/1/30

Mutations in the surfactant protein C gene (SFTPC) were recently reported in patients with interstitial lung disease. In a 13-month-old infant with severe respiratory insufficiency, a lung biopsy elicited combined histological patterns of nonspecific interstitial pneumonia and pulmonary alveolar proteinosis. Immunohistochemical and biochemical analyses showed an intra-alveolar accumulation of surfactant protein (SP)-A, precursors of SP-B, mature SP-B, aberrantly processed proSP-C, as well as mono- and dimeric SP-C. Sequencing of genomic DNA detected a de novo heterozygous missense mutation of the SFTPC gene (g.1286T>C) resulting in a substitution of threonine for isoleucine (I73T) in the C-terminal propeptide. At the ultrastructural level, abnormal transport vesicles were detected in type-II pneumocytes. Fusion proteins, consisting of enhanced green fluorescent protein and wild-type or mutant proSP-C, were used to evaluate protein trafficking in vitro. In contrast to wild-type proSP-C, mutant proSP-C was routed to early endosomes when transfected into A549 epithelial cells. In contrast to previously reported mutations, the I73T represents a new class of surfactant protein C gene mutations, which is marked by a distinct trafficking, processing, palmitoylation, and secretion of the mutant and wild-type surfactant protein C. This report heralds the emerging diversity of phenotypes associated with the expression of mutant surfactant C proteins.


http://erj.ersjournals.com/cgi/content/abstract/22/2/317

Links between immune responses to respiratory syncytial virus (RSV), age and atopic sensitisation are poorly understood. This study investigated the induction of target organ type-1,
type-2 and pro-inflammatory cytokine responses to RSV and/or phytohaemagglutinin (PHA) in tonsillar mononuclear cells from children, in relation to age and atopic status. In comparison with the control medium, RSV induced production of the type-1 cytokines interferon (IFN)-\(\gamma\) and interleukin (IL)-18, the pro-inflammatory cytokines IL-6, -8 and RANTES (regulated on activation, normal T-cell expressed and secreted), but not any of the type-2 cytokines IL-4, -5, -10 and -13. Induction of IL-6, -8 and RANTES, but not IFN-\(\gamma\) or IL-18, were shown to be dependent on virus replication. PHA induced all except IL-12, -13, and -15. Induction of IFN-\(\gamma\) (gamma), IL-6, -8, and RANTES was significantly increased in atopic children. Induction of both IFN-\(\gamma\) (gamma) and IL-4 increased in parallel in relation to age, with no change in the IFN-\(\gamma\) (gamma):IL-4 ratio. These data are compatible with the hypothesis that immature type-1 immunity during early childhood plays a role in both respiratory syncytial virus bronchiolitis and in its relationship with atopy.

European Journal of Cancer  (36)


http://www.sciencedirect.com/science/article/B6T68-3Y2G9FW-6/2/efefdc7af6f05693ae6e2d3e14b1e833

Although human papillomaviruses (HPVs) have been found in many, but not all, tumours of the oral cavity, nose, pharynx and larynx, the true role of HPV in malignant tumours of the head and neck is still unclear. The presence of HPV DNA was investigated in 45 fresh squamous cell carcinoma (SCC) specimens and in 29 normal mucosa specimens collected from 45 primary laryngeal SCC patients. HPV DNA was detected using the polymerase chain reaction (PCR) with consensus primers that detect HPV types 6, 11, 16 and 18. 9 of the 45 patients (20%) were HPV positive; the presence of HPV was also detected in the corresponding normal laryngeal mucosa of four of the 29 specimens (14%). No statistically significant differences were found between the presence of HPV DNA in normal specimens and in neoplastic mucosa specimens. No correlation was found between HPV DNA positive tumours and size, T classification, lymph node involvement and histological grading. This study adds further evidence suggesting a possible role of HPV DNA infection in laryngeal carcinogenesis.


http://www.sciencedirect.com/science/article/B6T68-3YDFY6MP2/daec4ff752923e76e911725e82773739

The development of therapy-induced drug resistance is still one of the most important therapeutic limitations. Nevertheless, an integrating view of the molecular mechanisms underlying resistance development in general is missing. In order to shed some light on the network of this resistance development, we established drug-resistant (doxorubicin (DX), methotrexate (MTX), cisplatin (cisPt), vincristine (Vin)) derivatives of six tumour cell lines (Jurkat, U937, HL60, DoHH-2, K562...
and ARH77) of haematopoetic origin. Differential gene expression of drug-sensitive parental cell lines and the drug-resistant derivatives thereof was analysed by suppressive subtractive hybridisation. After dot blot screening for differential expression and sequencing of the cloned PCR fragments, differential expression was confirmed by Northern blot analysis. In an attempt to discriminate for differentially expressed genes only related to one or the other of the investigated drugs, the cDNAs of various resistant sublines (doxorubicin-, methotrexate-, cisplatin-resistant Jurkat cells) were pooled and compared with the sensitive parental cell line. In addition, cDNAs of the resistant derivatives of the different haematopoetic tumour cell lines were pooled and compared with the pooled cDNAs of the corresponding sensitive haematopoetic cell lines to eliminate cell line to cell line variations that were not related to drug resistance. As a result of this screening, the following genes showed a higher (at least 2-fold) or exclusive expression in the drug-resistant variants: serglycin, sorcin, BMPG (bone marrow proteoglycan gene) and PTI-1 (prostate-tumour-inducing gene 1). In addition, elevated expression of hsp90, previously found by our group to be upregulated in the drug-resistant colon carcinoma cell line LoVo H67P was found to be overexpressed in drug-resistant HL60 cells.


http://www.sciencedirect.com/science/article/B6T68-4CP0YYH-4/2/6702d79174c49189297f53c4f9a4e15a

The purpose of this study was to demonstrate the effects of lycopene, the major tomato carotenoid, on the expression of the BRCA1 and BRCA2 genes in three breast tumour cell lines, MCF-7, HBL-100, MDA-MB-231 and the fibrocystic breast cell line MCF-10a. Flow cytometry analysis showed a G1/S phase cell cycle-arrest after treatment of the cells with 10 [mu]M lycopene for 48 h. mRNA expression was studied by quantitative reverse transcription-polymerase chain reaction using the Taqman(R) method. We observed an increase of BRCA1 and BRCA2 mRNA in the oestrogen receptor (ER)-positive cell lines (MCF-7 and HBL-100), and a decrease (MDA-MB-231) or no change (MCF-10a) in the ER-negative cell lines. BRCA1 and BRCA2 proteins were quantified by perfusion affinity chromatography. No variation in their expression was observed. These preliminary results on the effects of lycopene on the expression of BRCA1 and BRCA2 oncosuppressor genes in breast cancer may reflect cross-talk between the oestrogen and retinoic acid receptor (RAR) pathways.


http://www.sciencedirect.com/science/article/B6T68-3W48PK9-X/2/d8829082eb72e6a78f14d3760c782cbb

The aim of this study was to assess the expression of cytokine transcripts, reflecting the type of ongoing immune responses at the site of human papillomavirus (HPV) infection, in relation to the development of cervical neoplasia. To this end reverse transcription-polymerase chain reaction (RT-PCR) was performed for interferon (IFN)[gamma], interleukin (IL)-2, IL-4, IL-5, IL-10, IL-12 (p35 and p40), and transforming growth factor (TGF[beta]1) in snap-frozen cervical biopsies, which were tested for the presence of high risk HPV DNA and histologically classified from normal to invasive carcinoma (n=40). IFN[gamma], IL-10 and IL-12 (p35 and p40) transcripts were found to be expressed at significantly lower frequencies in invasive carcinoma as compared with premalignant biopsies (P=0.006, P=0.007 and P=0.002, respectively). IFN[gamma] and IL-10 mRNA were associated with the presence of the IL-12 p35 and p40 transcripts (P=0.008 and P<0.00001, respectively). These results are consistent with a locally reduced cellular (type 1)
immunity correlating with HPV-induced invasive cervical carcinoma.


http://www.sciencedirect.com/science/article/B6T68-4BNVS1W-2/2/bdcc1e5a4767cc72a706f712eba9a2e0

Previous studies have shown that activating mutations of c-KIT/PDGFRA, potential therapeutic targets for imatinib mesylate, are implicated in the pathophysiology of gastrointestinal stromal tumours (GISTs). In this study, GISTs from 37 patients enrolled in an European Organisation for Research and Treatment of Cancer (EORTC) phase I/II clinical study of imatinib were examined for mutations of c-KIT/PDGFRA in order to explore whether the mutational status of the tumour predicts the clinical response to therapy. Mutations were screened by denaturing high-pressure liquid chromatography (DHPLC) and characterised by bi-directional DNA sequencing. Activating mutations of c-KIT or PDGFRA were found in 29 (78%) and 2 (6%) GISTs, respectively. Most c-KIT mutations involved exon 11 (n=24; 83%), all but one being an in-frame deletion; no isolated point mutations were found. The other c-KIT mutations included exon 9 AY 502-503 duplication (n=4; 14%) and exon 13 Lys->Glu642 missense mutation (n=1; 3%). Two tumours with no detectable c-KIT mutations demonstrated PDGFRA Asp->Glu842 amino acid substitutions. Patients with GISTs harbouring exon 11 mutations were more likely to achieve a partial response (PR) on imatinib therapy (83%) than all of the others (23%). The overall survival and progression-free survival rates for the entire group at 106 weeks were 78.3% and 46.9%, respectively. Based on a Kaplan-Meier analysis, patients with GISTs harbouring c-KIT mutations had longer median survival times and were less likely to progress than the other patients. These findings indicate that the mutational status of the c-KIT/PDGFRA oncoproteins could be useful to predict the clinical response of patients imatinib therapy.


http://www.sciencedirect.com/science/article/B6T68-3YMFV22-9M/2/19c7efad67ca32dd52325cf1ffbcfbf3

Polymerase chain reaction (PCR) products representative of the DNA sequence coding for the variable heavy (vH) and the variable light (VL) chains of an antiMUC1 mucin monoclonal antibody, C595, have been produced. These products were cloned, sequenced, and the primary amino acid sequences of the VH and VL regions deduced. The hypervariable complementarity determining regions (CDRs) and framework regions in the heavy and light chains were located, and homologies with canonical forms for the CDR loops L1, L2, L3, H1 and H2 were identified by database searching. The structure for the H3 loop was calculated directly. Computational molecular modelling was accomplished using the fully automated AbM package (Oxford Molecular, Oxford, U.K.). Energy minimisation was performed using the program InsightII (Biosym, San Diego, California, U.S.A.). The investigation provides a basis for the molecular analysis of the antigen binding site of the C595 antibody with the aim to identify key residues and interactions involved in the immune recognition of the C595 antibody defined epitope, which is expressed in the majority of breast and ovarian carcinomas.

This report describes an unusual clinical presentation of Li-Fraumeni syndrome. Family history revealed a mild aggregation of adult cancers in one generation, and an unusual clustering of brain tumours of early childhood in the following generation. In order to evaluate the genetic basis for cancer predisposition in this family, molecular genetic analysis for the occurrence of germline TP53 tumour suppressor gene mutations was performed on 12 siblings of two generations. Indirect mutation analysis was performed by the single-strand conformation polymorphism (SSCP) technique. Alterations were characterised by automated direct fluorescence sequencing analysis. Tumour material was also examined for p53 protein accumulation by immunohistochemistry. Initially, a TP53 gene germline missense mutation was detected in an 11-year-old kindred with acute myeloid leukaemia (AML) following intensive treatment of a brain tumour. In peripheral blood and bone marrow samples of this proband, a reduction to hemizygosity occurred. During AML treatment, detection of LOH of 17p was used as a marker for clonality and treatment control. The mutation was found to be inherited from the proband's mother, who was diagnosed with breast cancer at the age of 48 years. Further, three siblings were carriers, and two are apparently healthy at the age of 21 and 23 years. Knowledge of germline mutations may allow accurate DNA-based carrier diagnosis which is of important clinical significance for treatment strategy and control. Furthermore, the occurrence of unaffected carriers in this family raises questions about appropriate methods of cancer surveillance and counselling for these people.


The aim of this study was to describe and characterise a founder mutation of the BRCA1 gene in western Sweden. Of 62 families screened for BRCA mutations, 24 had BRCA1 mutations and two had BRCA2 mutations. Tumours that occurred in family members were histologically reviewed and mutational status was analysed using archival paraffin-embedded tissues. The same BRCA1 mutation, 3171ins5, was found in 16 families who were clustered along the western coast of Sweden. Mutation analysis revealed a maternal linkage in 13 families and a paternal linkage in 3. There was complete agreement between mutation analysis results obtained from blood and archival tissues. The penetrance of breast or ovarian cancer by age 70 years was estimated to be between 59 and 93%. There were no differences in survivals between breast or ovarian cancer patients with the mutation and age-matched controls. Thus, a predominant BRCA1 gene founder mutation associated with a high risk of breast and ovarian cancer has been identified and found to occur in a restricted geographical area, thereby allowing timely and cost-effective mutation screening using blood samples or archival histological material.

Individuals with an inherited predisposition to cancer development are at an increased risk of developing multiple tumours. Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common hereditary cancer syndromes and is estimated to account for approximately 2% of colorectal cancers. However, HNPCC individuals are at an increased risk of developing other tumour types such as cancers of the endometrium, urothelium and small intestine. We have utilised a population-based regional cancer registry to identify all patients with double primary colorectal cancers and at least one additional malignancy and characterised the tumour spectrum in this patient group. We subsequently selected those 47 individuals who had developed at least four malignancies, including two colorectal cancers, for studies of the tumour characteristics associated with HNPCC. In total, these individuals developed 209 tumours, 156 of which were successfully retrieved. Microsatellite instability (MSI), a phenomenon caused by defective mismatch-repair (MMR), was identified in 63/154 (41%) evaluable tumours with a MSI-high pattern in 59 and a MSI-low pattern in four tumours. All tumours were immunohistochemically stained for the MMR proteins MLH1 and MSH2, with loss of expression in 55/63 (87%) MSI tumours and in 2/89 (2%) microsatellite stable (MSS) tumours. This loss affected MLH1 in 24 tumours and MSH2 in 33 tumours. A concordant loss of expression for the same MMR protein in several tumours from the same individual, a pattern that strongly suggests an underlying germline MMR gene mutation, was found in 17/45 (38%) patients and affected MLH1 in 8 patients and MSH2 in 9 patients. We conclude that the development of multiple primary tumours, including synchronous or metachronous colorectal cancers, is associated with an increased frequency of MSI and loss of immunohistochemical expression of MLH1 and MSH2.


In tumour cells, replicative immortality is attained through stabilisation of telomeres by telomerase. Recent evidence suggests that telomerase plays an anti-apoptotic role. Since apoptosis is the primary mode of cell death induced by several drugs, telomerase could be involved in determining the chemosensitivity profile of tumour cells. We investigated whether inhibition of telomerase activity through a hammerhead ribozyme targeting the RNA template of telomerase influences the susceptibility of human melanoma cells to a variety of anticancer agents (platinum compounds, taxanes, topoisomerase I inhibitors). The ribozyme sequence was inserted into an expression vector and the JR8 human melanoma cell line was transfected with it. The cell clones obtained showed a reduced telomerase activity. Growth inhibition curves generated after exposure of ribozyme-transfectant clones to individual drugs were superimposable to those obtained from parental cells. Moreover, telomerase inhibition did not promote apoptosis as a cellular response to drug treatment. Overall, our results indicate that downregulation of telomerase activity does not increase the sensitivity of melanoma cells to anticancer drugs.

The aim of this study was to investigate the expression of p53 and bcl2 proteins in a series of 107 non-small cell lung cancers (NSCLC), and to relate such protein expression to neovascularisation and the expression of vascular endothelial growth factor (VEGF). Moreover, we analysed the prognostic impact of these biological parameters on overall survival, both in univariate and multivariate analyses. An inverse association was found between bcl2 expression and microvessel count (MVC; P=0.0004) and bcl2 and VEGF (P=0.007). In contrast, a significant association was found between p53 expression and MVC (P=0.03) and p53 and VEGF expression (P=0.04). In univariate analysis, nodal status (PPP=0.002), p53 (P=0.03) and VEGF expression (P<0.000001) significantly affected overall survival, but in multivariate analysis only MVC and VEGF expression retained their prognostic influence. Our results suggest that bcl2 and p53 possibly control the development of tumour angiogenesis in NSCLC, with putative mediation by VEGF. Moreover, the important influence of angiogenesis in the progression of NSCLC is further highlighted.


Non-small cell lung cancer is associated with approximately 85% mortality due to its high metastatic potential. Therapeutic efforts have failed to produce a significant improvement in prognosis. In this situation, a better understanding of the key factors of metastasis may be useful for designing new molecular targets of therapy. In order to identify these factors, we compared the expression profiles of two subpopulations of an adenocarcinoma cell line with a high metastatic potential, PC9/f9 and PC9/f14, with the parent cell line, PC9, using a cDNA array. The expression of 15 genes was found to be significantly enhanced or reduced in the highly metastatic subpopulations. The expression of matrix metalloproteinase-2 (MMP-2), plasminogen activator inhibitor-1 (PAI-1) and interleukin-1 (IL-1[alpha]) were upregulated in the highly metastatic subpopulations, while the expression of carcinoembryonic antigen (CEA), caspase-5, Fas ligand, Prk/FNK, cyclin E, cyclin B1, Ki-67, proliferating cell nuclear antigen (PCNA), Smad4, macrophage proinflammatory human chemokine-3[alpha] (MIP-3[alpha])/LARC, Met and CD44 were downregulated. Data from the literature suggest that the altered expression of MMP-2, PAI-1, IL-1[alpha], CEA, caspase-5, Fas ligand, Prk/FNK and Smad4 promotes the highly metastatic phenotype. The differential expression of these genes was confirmed by Northern blot analysis, standard reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR. This analysis in subpopulations of a lung cancer cell line indicated that the highly metastatic potential of lung cancer may be induced not by an alteration in the expression of a single gene, but by the accumulation of alterations in the expression of several genes involved in extracellular matrix (ECM) adhesion disruption, ECM degradation, escape from apoptosis, and resistance to transforming growth factor-[ss]1 (TGF-[ss]1). Strategies for inhibiting metastasis of pulmonary adenocarcinoma should be designed accordingly.

cell-mediated immune responses and are considered to be beneficial for antitumour immunity. Type 2 cytokines, such as IL-4, IL-5, and IL-10, inhibit Type 1 responses and promote humoral responses. We have previously reported an association between low intratumoral IFN[gamma] mRNA levels and poor clinical outcome in patients with invasive cervical carcinoma. In this study, by using quantitative polymerase chain reaction (PCR), we identified a group of cervical carcinoma patients with undetectable intratumoral T cell-derived cytokine mRNAs, as IFN[gamma], IL-4 and IL-17 expression could not be detected in 5, 25 and 8 of the 52 biopsies analysed, respectively. Global downregulation of Type 1 and Type 2 cytokines was observed in a subgroup of patients who more frequently presented advanced stage tumours. Biopsies of patients with no IFN[gamma] gene expression did not appear to be less infiltrated by T cells than control biopsies with measurable IFN[gamma] gene expression. These results clearly demonstrate that, in some clinical situations, the decrease in intratumoral Type 1 cytokines is not associated with a Type 2 polarisation, but rather reflects global deactivation of T cells at the tumour site. These data provide support for immunotherapy protocols designed to reverse the anergic state of T cells in cancer.


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A significant proportion of cervical carcinomas show loss of major histocompatibility complex human leucocyte antigen (HLA) class I expression while upregulating HLA class II expression. These changes may have direct consequences for immune surveillance of the human papilloma virus (HPV) infection which is strongly associated with cervical malignancy. A relationship between changes in HLA expression and HPV infection may be evident in the evolution of premalignant disease. This immunohistological study of 104 colposcopic biopsies establishes that HLA class II expression occurs in a significant proportion of squamous epithelia showing histological evidence of wart virus infection and cervical intraepithelial neoplasia (CIN) I to III. In comparison, alteration of HLA class I expression in cervical premalignant lesions is rare. There is no correlation between the detection of high risk HPV DNA (types 16, 18, 31 and 33) by polymerase chain reaction (PCR) and the MHC class II phenotype of the lesion. This suggests that altered HLA class II expression is neither a consequence nor a prerequisite for HPV infection.


http://www.sciencedirect.com/science/article/B6T68-3W316N0-14/2/2f6cc6592daf9663218e43b7ccf40e2

O6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein, which removes alkyl groups from the O6 atom of guanine residues. Tumour cells which lack MGMT are sensitive to cytostatic drugs such as dacarbazine (DTIC), whose active species bind to this site. To explore whether analyses of MGMT expression can be used as a predictive test for clinical sensitivity to DTIC in melanomas, we developed a method to assay MGMT mRNA levels in cells obtained by fine needle aspiration biopsies of metastases. cDNA was synthesised from mRNA prepared from biopsy material. Polymerase chain reaction was performed using primers complementary to MGMT cDNA and to [beta]-actin, which served as an internal control. Analyses of 44 biopsies from 35 patients showed a considerable variation in MGMT mRNA, with 15 samples (34%)
lacking detectable mRNA. In 6 out of 8 patients in whom more than one tumour was analysed, separate metastases had different levels of MGMT mRNA. There was no correlation between MGMT activity studied by a biochemical assay and MGMT mRNA levels when these were compared in 10 surgical biopsies.


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Heparanase (hep) degrades heparan sulphate proteoglycans (HSPGs), which are the main components of the extracellular matrix. This process has been considered as the first step of tumour invasion or metastasis. However, HSPGs play an important role in signal transduction. Thus, the degradation of HSPGs by hep may suppress tumour cell growth. In the present study, we investigated the clinicopathological importance of enhanced hep mRNA expression in 48 hepatocellular carcinomas (HCCs) and in 48 non-cancerous liver samples obtained from the same patients by quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR). Spontaneous apoptosis in the hepatocytes was evaluated by immunohistochemistry. The relative hep mRNA expression levels were described as hep/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) ratios. The hep mRNA levels of HCCs were significantly lower than those of non-cancerous livers (Phep mRNA levels decreased with increasing liver fibrosis. A significant positive correlation between hep gene expression and spontaneous apoptosis was detected. Hep expression in the tumours did not correlate with tumour differentiation or with tumour stage. However, low hep gene expression was associated with a poor disease-free survival of the patients. Thus, hep gene expression may play an important role in programmed cell death and this gene expression may be lost during the malignant transformation of hepatocytes.


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Several tumour-forming cell lines are known to overproduce the lysosomal cysteine peptidase cathepsin L. We have used an antisense approach to investigate whether inhibition of cathepsin L overexpression in two malignant cell lines (myeloma SP cells and L cells) reduces their tumorigenic potential. Two different cDNA fragments of murine cathepsin L were inserted in the antisense direction into the pcDNA3 vector, and SP and L cells were stably transfected with these plasmid constructs. Several of the selected clones expressing the antisense transcript showed specific reduction of the mRNA level and the intracellular activity of cathepsin L, and a greatly diminished amount of secreted procathepsin L. When tested in Balb/c nu/nu mice, the cell lines with low cathepsin L activity exhibited a significantly decreased potential for tumour growth when compared with control cells expressing wild-type levels of cathepsin L activity. This observation suggests that cathepsin L is a critical factor in tumour growth.

Using four cell lines including drug-sensitive K562/Parent cells, P-glycoprotein (Pgp)-mediated multidrug resistant (MDR) K562/VCR, K562/ADR and revertant K562/ADR-R cells, two fluorescent agents, Fluo-3 and rhodamine-123 (Rh-123), were compared as indicators in a functional assay of MDR. Cells were incubated with 4 [μM] Fluo-3 or 1 [μM] Rh-123 for 45 min and then the intracellular accumulation of the agent was measured using a flow cytometer. Verapamil (20 [μM]) or cepharanthine (biscoclaurine alkaloid, 10 [μM]) was added just before the fluorescent agents. Efflux patterns were also studied 60 min after incubation with or without verapamil and cepharanthine. Increased intracellular accumulation and a delayed efflux pattern of Fluo-3 by verapamil and cepharanthine were demonstrated in multidrug resistant K562/VCR and K562/ADR cells, indicating that Fluo-3 is another good indicator of MDR. However, a similar, but lower, increase in uptake and a delayed efflux pattern of Fluo-3 by verapamil and cepharanthine were also demonstrated even in Pgp-non-overexpressed K562/Parent cells. In contrast, accumulation of Rh-123 was not affected by verapamil and cepharanthine. To further study the Pgp dependency of Fluo-3, another cell line, K562/NC16 expressing minimum MDR1 mRNA, was cloned. Increased uptake and a delayed efflux pattern of Fluo-3, but not Rh-123, with verapamil or cepharanthine were again demonstrated in K562/NC16 cells, indicating that intracellular accumulation of Fluo-3 may be nonspecifically influenced by verapamil and cepharanthine at very low levels of Pgp-related MDR, while the influx and efflux patterns of Rh-123 may be specifically affected by Pgp overexpression.


At least 10% of all ovarian cancers are estimated to have a hereditary background. Hereditary breast-ovarian cancer (HBOC) due to mutations in the BRCA genes is a major cause of hereditary ovarian cancer, although its frequency and relationship to age and family history in unselected series of ovarian cancers is not completely known. We report here the results of a full mutational screening analysis for germ line BRCA1 and BRCA2 mutations in 161 patients with invasive epithelial ovarian carcinomas. Age at diagnosis ranged from 22 to 82 years (mean 59 years). Deleterious (frame-shift, nonsense and missense) mutations were detected in 13/161 (8%) of the patients and affected BRCA1 in 12 cases and BRCA2 in one case. Four additional missense variants (one in BRCA1 and three in BRCA2) with a possible association with an increased risk ovarian cancer were revealed, resulting in a total frequency of BRCA gene alterations of 17/161 (11%). The 13 patients with deleterious mutations had a mean age of 57 years (range 41-76 years) and only three of these patients were below 50 years of age. A family history of at least one breast cancer and/or ovarian cancer was reported in all but 1 of the patients with BRCA mutations compared with only 24% of patients without mutations. Our findings in this prospective study confirm approximately 1 in 10 patients with ovarian cancer carry a germline BRCA gene mutation associated with HBOC, and also indicate that a large number of these patients are over 50 years of age at diagnosis.

Overexpression of the epidermal growth factor receptor (EGFR) often correlates with an aggressive tumour phenotype and poor prognosis. To examine the relevance of EGFR in colorectal cancer, we determined the expression of EGFR protein in 249 colorectal adenocarcinomas and 42 lymph node metastases using immunohistochemistry. Moreover, we investigated a (CA)n dinucleotide repeat polymorphism of the EGFR gene in a subset of 114 tumours. High levels of EGFR protein were observed in 123/249 (49.4%) samples. EGFR expression in colorectal carcinomas correlated with differentiation grade (P=0.014). However, there were no associations with Dukes' stage, site, patient age or gender. EGFR protein expression did not influence survival in this colorectal cancer patient cohort (P>0.05). Expression was not identical in paired colorectal tumours and lymph node metastases, with only 17/42 (40.5%) samples showing equivalent EGFR levels (P>0.05). The distribution of the (CA)n dinucleotide repeat alleles in colorectal adenocarcinomas was not associated with EGFR protein expression (P>0.05). These results indicate that while EGFR overexpression is a common event in colorectal carcinogenesis, it does not influence patient prognosis.


http://www.sciencedirect.com/science/article/B6T68-3YWWYBW-G/2/524952fdd08e2f9d94e93f30b40b13f

A number of genes, including IGF2 and H19, are normally imprinted with preferential expression of the paternal or maternal allele, respectively. Loss of imprinting (LOI) of IGF2 and H19 is found in a number of tumours, suggesting that LOI of IGF2 and/or H19 may play an important role in tumorigenesis. The IGF2 gene codes for a fetal growth factor and the H19 gene is likely to act as an RNA with an antitumour effect. We investigated the imprinting status of IGF2 and H19 in human meningiomas. The normally imprinted IGF2 gene lacks imprint in the leptomeninges and choroid plexus of the brain. To examine the imprinting status of IGF2 and H19 in human meningiomas we used the ApaI polymorphism in exon 9 for the IGF2 gene and the AluI polymorphism in exon 5 for the H19 gene. In total, 24 meningiomas of WHO grade I, II and III were analysed. 15 meningiomas (63%) were informative for the ApaI polymorphism in the IGF2 gene. Monoallelic expression (MAE) for IGF2 was found in 11 out of 15 tumours (73%) which is in contrast to the lack of imprinting status of IGF2 in leptomeninges. Ten cases (42%) were heterozygous for the H19 gene and biallelic expression was found in 3 out of 10 meningiomas (30%). These results indicate that modulation of the imprinting status of IGF2 and H19 may play an important role for the development of meningiomas.


http://www.sciencedirect.com/science/article/B6T68-3WNMG0K-C/2/45cd43efa09304da88e3e4b7d1877ca

We analysed microsatellite instability (MSI) in a consecutive series of 165 rectal carcinomas. Data on a personal and/or family history of cancer were collected from all patients and revealed metachronous cancer in 9 patients, 2 of whom had developed colorectal cancer, and a suspected familial aggregation of colorectal cancer in three families. Only three of the 165 (2%) rectal cancers showed MSI. The patients whose tumours displayed MSI had clinical histories suggesting hereditary cancer--a family history of colorectal cancer and/or synchronous colorectal
cancers. Denaturing gradient gel (DGGE) analysis was used to screen the MSI+ patients for mutations in the hMLH1 and hMSH2 genes and revealed two new germline mutations; a 1 bp deletion in exon 10 of hMSH2 creating a premature stop-codon and a splice donor site mutation in intron 16 of hMLH1. Considering colorectal carcinomas as a group, MSI has been reported to occur in approximately 10-20% of the tumours and thus can not, per se be used for clinical detection of hereditary tumours. This study shows, however, that MSI is rare in rectal carcinomas and when present strongly suggests a hereditary predisposition for colorectal cancer development.


http://www.sciencedirect.com/science/article/B6T68-4CDHRH3-B9/2/a13207966e8cce006c993efef81b0e88c

Mutations in ras genes have been found in the DNA of numerous cancer types including melanomas, but the expression of these mutations in melanomas has not yet been addressed. We have used the polymerase chain reaction (PCR) and allele-specific restriction analysis (ASRA) to determine the frequency of expressed N-ras mutations on 25 short-term melanoma tissue culture samples. N-ras cDNA generated using reverse transcriptase from whole cells was used as the PCR template. 14 secondary melanoma cultures that varied in differentiation patterns were analysed. Only 2 were found to express N-ras mutations; in both, the mutation was localised to one of the first two positions of the 61st codon of N-ras. These tumour lines, KMI-M8412a and KMI-M8412b, were established from separate tumour deposits in the same patient. Codons 12 and 13 were found to be free of mutations in all of the lines studied. 8 primary melanomas and 3 unclassified skin lesions were also analysed and found free of N-ras mutations. These results suggest that N-ras may not play such an important role in melanoma tumorigenesis as is speculated by others.


http://www.sciencedirect.com/science/article/B6T68-4D5P20G-1/2/100d2633f3f13f371dcbd4df4fad196

The mitochondria plays a role in apoptosis. Its genome is also more susceptible to mutations because of high levels of reactive oxygen species and limited repair mechanisms. The D-loop of mitochondrial DNA (mtDNA) contains essential transcription and replication elements, and mutations in this region might alter the rate of DNA replication. We examined genetic alterations in the D-loop region of mtDNA in uterine serous carcinoma (USC) samples and their paired normal adjacent endometrium. DNA was extracted after laser-capture microdissection of paraffin-embedded tissues from eight patients with USC. The entire D-loop genome was amplified using nine pairs of overlapping primers. Denatured polymerase chain reaction (PCR) products were subjected to single-strand conformation polymorphism (SSCP) analysis. Somatic mtDNA alterations were detected in five tumours (63%). Our study indicates that mtDNA D-loop sequence alterations occur at a high frequency in USC suggesting that mtDNA mutations may play a role in the development of USC.

http://www.sciencedirect.com/science/article/B6T68-45MDR6F-2/2/a6c88edeb107341bd06bee26579a36d19

The correlation between inactivation of the TP53 gene through mutation or the presence of high-risk human papillomavirus (HPV) DNA and intrinsic paclitaxel sensitivity was studied in 27 gynaecological cancer cell lines. IC50 values, as a measure of drug sensitivity, were determined using a 96-well clonogenic assay. TP53 mutations were investigated with polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and direct DNA sequencing. HPV status was studied with PCR using HPV consensus primers. TP53 mutations were found in 7/11 vulvar SCC cell lines. Only 2/9 endometrial and 1/7 ovarian cancer cell lines carried TP53 mutations. One vulvar and one endometrial cancer cell line were HPV-positive; both carrying HPV type-16 DNA. Thus, TP53 was functionally normal in 3/11 vulvar, 6/9 endometrial and 6/7 ovarian cancer cell lines. The IC50 values for paclitaxel were 0.60-2.9, 0.49-2.3 and 0.40-3.4 nM in the vulvar, endometrial and ovarian cancer cell lines, respectively. No correlation could be demonstrated between inactivation of the TP53 gene and paclitaxel sensitivity in vitro; the cell lines were evaluated as one group or according to their anatomical origin or histology. Previous reports have given inconclusive results, partly due to the cell types used, i.e. normal, cancerous or transformed cells. Our results support the view that paclitaxel sensitivity of tumour-derived cancer cell lines is not related to the TP53 status.


http://www.sciencedirect.com/science/article/B6T68-40B2BW5-D/2/27a0ccfd4f88c380e41ce6cdff9ded

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been widely used as a control RNA in Northern blotting and in reverse transcriptase-polymerase chain reaction (RT-PCR) analyses. We investigated the expression of GAPDH in a large series of primary breast cancers and in MCF7 human mammary epithelial breast cancer cells treated with oestradiol. The expression of GAPDH was quantified by a real-time one-step RT-PCR assay, based upon the 5' nuclease activity of Taq polymerase using an Abi Prism 7700 Sequence Detector System (Perkin Elmer, France). Using the Spearman test, GAPDH expression was found to correlate inversely with the age of the patients at diagnosis (P=0.003; r=-0.147), oestradiol receptors (ER) (Pr=-0.327) and progesterone receptors (PgR) (Pr=-0.206). A positive correlation was observed between GAPDH expression and the histo-prognostic grading (HPG) (Pr=0.344). Moreover, the overall survival (OS) and the relapse-free survival (RFS) were significantly reduced in patients whose tumours showed an enhanced level of GAPDH expression (OS, P=0.046; RFS, P=0.021). Multivariate analyses demonstrated that GAPDH was not an independent prognostic factor. Finally, in MCF7 cells treated with oestradiol, a statistically significant dose-dependent increase in GAPDH expression was observed. These results show that GAPDH expression is associated with breast cancer cell proliferation and with the aggressiveness of tumours. The present study demonstrates that, in cancer, the use of GAPDH gene expression should not be used as a control RNA.

In this study, we investigated whether (a) carcinoembryonic antigen (CEA), cytokeratin-20 (CK-20) and guanylyl cyclase C (GCC) are clinically useful markers for the molecular detection of submicroscopic metastases in colorectal cancer (CRC) and (b) whether overexpression of CEA, CK-20 and GCC can be reliably detected in formalin-fixed, paraffin-embedded tissues as well as frozen lymph nodes. We studied 175 frozen lymph nodes and 158 formalin-fixed, paraffin-embedded lymph nodes from 28 cases of CRC. CEA or CK-20 or GCC-specific polymerase chain reaction (PCR) was carried out on mRNA transcripts extracted from the nodal tissues. Ten out of 11 Dukes' B CRC cases had detectable CEA and CK-20 while 6 out of 11 Dukes' B CRC cases had detectable GCC. In general, the difference of re-staged cases when comparing frozen and paraffin-embedded samples was marked; the only statistically significant correlation between frozen and paraffin tissue was for the CEA marker. Our results indicated a high incidence (>50%) of detecting micrometastases in histologically-negative lymph nodes at the molecular level.


Hyalinizing trabecular tumour (HTT) of the thyroid is a neoplasm of follicular derivation that shares several morphological similarities with papillary thyroid carcinoma (PTC). In this study, we investigated the prevalence of B-raf point mutations, RET/PTC rearrangements and N-ras point mutations in a large HTT series (28 samples). Twenty benign thyroid lesions and 10 PTC served as control cases. A high (47%) prevalence of RET/PTC rearrangements was found in HTT. By contrast, neither B-raf nor N-ras mutations were found in HTT. These findings suggest that, although RET/PTC, N-ras, and B-raf proteins may act along the same signalling cascade, the biological and morphological outcome of their oncogenic activation is not completely overlapping. Thus, in clinical practice, the detection of B-raf mutations in a thyroid follicular tumour may prove to be a valuable tool, supplementing histological examination, and allowing a differential diagnosis between PTC and HTT.


The aim of this study was to determine whether nucleic acids are detectable in cell-free bronchial lavage supernatants, and whether it is possible to find alterations in this DNA and RNA of genes known to be present in lung tumour cells. DNA was isolated from cell-free lavage supernatants from 30 and RNA from 25 lung cancer patients. The DNA was examined for microsatellite alterations (MA) and the RNA analysed for the expression of seven tumour-associated genes. Intact DNA and mRNA could be isolated from all cell-free bronchial lavage supernatants. MA were found in lavage supernatants of 12/30 patients and in lavage cells of 6/30 patients. Altogether alterations were found in 14/30 patients. Analyses of tumour-associated gene expression showed positive results, with at least one marker in the lavage supernatants of all 25 patients. Thus, we could demonstrate, for the first time, that it is possible to isolate intact DNA...
and RNA from cell-free bronchial lavage supernatants. Their quantity and quality is sufficient for further amplification by polymerase chain reaction (PCR)/reverse transcriptase (RT)-PCR. Altogether, tumour-associated changes were detected in DNA samples from 47% of the patients and in RNA samples from all of the patients analysed.


http://www.sciencedirect.com/science/article/B6T68-3YS90M5-B/2/3588ee870912e234dd799de0831021cd

The aim of the study was to clarify the role of telomerase component genes in hepatocarcinogenesis and to examine both the relationship between the expression of telomerase component genes and histological differentiation in hepatocellular carcinoma (HCC) and the relationship between expression levels of telomerase component genes and telomerase activity in HCCs. Telomerase is a ribonucleoprotein enzyme composed of a template RNA and several proteins. Recently, three such telomerase component genes have been identified: human telomerase reverse transcriptase (hTERT); human telomerase RNA component (hTERC); and telomerase-associated protein 1 (TEP1). The expression of these components was evaluated in 34 HCCs and 24 non-cancerous liver tissues by reverse transcriptase-polymerase chain reaction (RT-PCR). Expression of hTERT mRNA was detected in most HCCs, but not in the non-cancerous tissues (PPP<0.01). These results suggest that telomerase reactivation during hepatocarcinogenesis might be regulated by only hTERT and an increase in telomerase activity level in tumour progression might be regulated by both hTERT and hTERC.


http://www.sciencedirect.com/science/article/B6T68-41FTRGV-9/2/7f11dc9e08d5858bb75e8b17e5b1d09

Breast cancer in young women is uncommon and often presents with unfavourable biopathological features. Although early age at onset could suggest a genetic susceptibility to cancer, the appropriateness of BRCA1 testing for women with early-onset breast cancer and modest family history (FH) is controversial. 40 Women diagnosed with breast cancer at the age of 35 years or less, unselected for FH, were screened for germ line BRCA1 mutations by automated sequencing of exons 2, 5, 6, 11, 13 and 20. Overall, deleterious mutations were evidenced in 6 (15%) patients. With regard to FH, mutations were detected in 14%, 11% and 29% of women with none, weak and strong FH, respectively. Large tumour size, grade 3, lack of oestrogen receptors and high proliferation rate were significantly more common in mutation carriers (MC). Our data support both the appropriateness of testing young breast cancer patients and the frequency of unfavourable features in BRCA1-related breast cancer. It is hypothesised that BRCA1 mutations partially justify the high rate of aggressive breast cancer in young patients and that combining age and breast cancer phenotype could help to identify probable MC.

The relative contribution of promoter hypermethylation and aberrant splicing to the inactivation of the fragile histidine triad (FHIT) gene is unclear. Using genetic and epigenetic analyses, the current investigation examines the loss of protein and mRNA expression, and 5’CpG hypermethylation and allelic imbalance of the FHIT gene in a series of 129 non-small cell lung cancer (NSCLC) samples, in parallel with clinicopathological analyses. We found that 50% of NSCLC patients had aberrant protein expression, which was more frequent in squamous cell carcinomas (SQ) (69%) than in adenocarcinomas (AD) (28%) (P < 0.05). FHIT was identified in 31% of patients. Abnormally-sized FHIT transcripts were also observed in 24% of patients and were attributed to various exonic deletions, mainly in the region of exons 4-8. Allelic imbalance of the FHIT locus and its correlation with the status of Fhit expression, 5’CpG hypermethylation, and aberrant splicing, indicated that biallelic inactivation of Fhit expression could be induced by 5’CpG hypermethylation of one allele and alternative splicing in the other allele. Moreover, an 83% concordance in the methylation status of FHIT was demonstrated between 12 samples of bronchial precancerous lesions taken before surgery and their matched resected tumours. Our data suggest that FHIT 5’CpG hypermethylation and splicing alterations are both predominant mechanisms involved in the aberrant expression of the FHIT gene, and that FHIT 5’CpG methylation may be potentially used as a supplemental detection marker for NSCLC.


Dihydropyrimidine dehydrogenase (DPD) is responsible for the breakdown of the widely used antineoplastic agent 5-fluorouracil (5-FU), thereby limiting the efficacy of the therapy. It has been suggested that patients suffering from 5-FU toxicides due to a low activity of DPD are genotypically heterozygous for a mutant allele of the gene encoding DPD. In this study we investigated the cDNA and a genomic region of the DPD gene of a cancer patient experiencing severe toxicity following 5-FU treatment for the presence of mutations. Although normal activity of DPD was observed in fibroblasts, the DPD activity in leucocytes of the cancer patient proved to be in the heterozygous range. Analysis of the DPD cDNA showed heterozygosity for a 165 bp deletion that results from exon skipping. Sequence analysis of the genomic region encompassing the skipped exon showed that the tumour patient was heterozygous for a G ->A point mutation in the invariant GT splice donor sequence in the intron downstream of the skipped exon. So far, the G->A point mutation has also been found in 8 out of 11 patients suffering from a complete deficiency of DPD. Considering the frequent use of 5-FU in the treatment of cancer patients, the severe 5-FU-related toxicities in patients with a low activity of DPD and the high frequency of the G->A mutation in DPD deficient patients, analysis of the DPD activity and screening for the G->A mutation should be routinely carried out prior to the start of the treatment with 5-FU.

The patched/hedgehog/smoothened signalling pathway has been implicated in the development of sporadic tumours associated with the naevoid basal cell carcinoma (Gorlin) syndrome (NBCCS). Mutations in sporadic basal cell carcinomas (BCCs) of the skin and medulloblastomas have been found in genes encoding all three proteins of the pathway. A substantial proportion of breast carcinomas has recently been suggested to contain missense mutations in the human patched (PTCH) and sonic hedgehog (SHH) homologues. However, an independent study showed that the implicated mutation in SHH (H133Y) was absent in a large number of BCCs, medulloblastomas, breast, ovary and colorectal tumours. We searched for the H133Y SHH mutation in 84 primary breast carcinomas, but did not detect this change in any sample. In addition, a subset of 45 primary breast tumours was analysed for mutations in the PTCH coding region and 48 samples in previously implicated exons of human smoothened, but no mutations were found. Although our results do not exclude the presence of clonal alterations of these genes in a small proportion of breast carcinomas, these data do not support the existence of frequent mutations in genes encoding major protein partners of this signalling pathway. The absence of nucleotide changes in PTCH may point to another linked gene in the chromosome region 9q22-q23, previously suggested to contain a breast cancer susceptibility gene.


http://www.sciencedirect.com/science/article/B6T68-49979VC-D/2/241269c2a1cfec0e7a8cece3e1e1d9fe

Epigenetic silencing of the p16 and p15 genes by promoter methylation are commonly observed in human epithelial malignancies, including head and neck squamous cell carcinomas (HNSCC). In this study, a methylation-specific polymerase chain reaction (MSP) was used to evaluate the methylation status of the p16 and p15 genes in 73 HNSCC surgical specimens. p16 and p15 gene methylation was also examined in 29 paired metastatic lymph nodes and 29 paired histologically, normal resection margin mucosae. The quantity of cell-free methylated p16 and p15 DNA in the plasma samples of 20 HNSCC patients and 24 healthy controls was also examined using a fluorescence-based real-time PCR assay. The frequencies of p16 and p15 methylation in the primary tumour were 49% and 60%, respectively. Concordant methylation of p16 and p15 in tumour samples and metastatic lymph nodes was found in 59 and 38% of cases, respectively. A significantly higher prevalence of p15 methylation was found in histologically-normal surgical margin epithelia of HNSCC patients with chronic smoking and drinking habits compared with non-smokers and non-drinkers. In addition, methylated p16 and p15 DNA levels were significantly higher in the plasma of HNSCC patients (mean 56 copies/ml plasma and 65 copies/ml plasma, respectively) compared with normal controls (mean 6 copies/ml plasma and 16 copies/ml plasma, respectively). In conclusion, promoter methylation of the p16 and p15 genes is involved in the pathogenesis of HNSCC and may be related to chronic smoking and drinking. The differential levels of methylated p16 and p15 DNA in plasma might be potential useful markers in screening high-risk populations for early HNSCC and monitoring their treatment response.


http://www.sciencedirect.com/science/article/B6T68-3THY0JD-T/2/fbdfo980ed6a5fec5dof2e0afc69bd89

TSG101 is a recently identified putative tumour suppressor gene which has been implicated in human breast cancer. To address whether germline disruption of TSG101 predisposes
individuals to this disease, we analysed genomic DNA and mRNA isolated from peripheral blood from 20 familial breast cancer cases. No evidence of large intragenic insertions/deletions or point mutations in TSG101 was found by Southern blot analysis and sequence analysis of the entire coding region. However, in 11 of 20 samples, ‘aberrant’ transcripts were detected. Sequence analysis suggested that these variants were generated by the use of different cryptic splicing sites. Such alternative/aberrant splicing events were not restricted to cancer patients, but were also detected in peripheral blood of non-cancer patients and in normal tissues.

**European Journal of Cancer. Part B: Oral Oncology** (1)


http://www.sciencedirect.com/science/article/B7GGS-4C06G66-8/2/d2df3e2c17e4c16f69e9547926eaf564

The polymerase chain reaction was used to examine paraffin-embedded tissues of 37 nasopharyngeal carcinomas (NPC) for Epstein-Barr virus (EBV) genomic sequences. EBV DNA was found in 2/14 keratinising squamous cell (WHO 1) carcinomas and in all of 23 non-keratinising and undifferentiated (WHO 2 and 3) NPC. The study confirms the infrequent association of keratinising NPC and EBV, in contrast with the 100% association of the less differentiated NPCs and the virus. The results may indicate a different carcinogenesis for the WHO 1 NPC subtype.

**European Journal of Cell Biology** (1)


http://www.sciencedirect.com/science/article/B7GJ2-4F3NXW0-1/2/90366c2978d7507dcd46a932bb7452f8

Biomechanical forces are major epigenetic factors that determine the form and differentiation of skeletal tissues, and may be transduced through cell adhesion to the intracellular biochemical signaling pathway. To test the hypothesis that stepwise stretching is translated to molecular signals during early chondrogenesis, we developed a culture system to study the proliferation and differentiation of chondrocytes. Rat embryonic day-12 limb buds were microdissected and dissociated into cells, which were then micromass cultured on a silicone membrane and maintained for up to 7 days. Stepwise-increased stretching was applied to the silicone membrane, which exerted shearing stress on the cultures on day 4 after the initiation of chondrogenesis. Under stretched conditions, type II collagen expression was significantly
inhibited by 44% on day 1 and by 67% on day 2, and this difference in type II collagen reached 80% after 3 days of culture. Accumulation of type II collagen protein and the size of the chondrogenic nodules had decreased by 50% on day 3. On the other hand, expression of the non-chondrogenic marker fibronectin was significantly up-regulated by 1.8-fold on day 3, while the up-regulation of type I collagen was minimal, even by day 3. The down-regulation in the expression of chondrogenic markers was completely recovered when cell-extracellular matrix attachment was inhibited by Gly-Arg-Gly-Asp-Ser-Pro-Lys peptide or by the application of blocking antibodies for [alpha]2, [alpha]5 or [beta]1 integrins. We conclude that shearing stress generated by stepwise stretching inhibits chondrogenesis through integrins, and propose that signal transduction from biomechanical stimuli may be mediated by cell-extracellular matrix adhesion.

European Journal of Heart Failure (1)


http://www.sciencedirect.com/science/article/B6VS9-46RVHN-1/2/e971c74160e904a9f2d11e2e2dbb5759

Background: Angiotensin II (Ang II) is a potent vasoconstrictor and a deleterious factor in cardiovascular pathophysiology. Ang II receptor blockers (ARBs) have recently been introduced into clinical practice for treatment of hypertension and congestive heart failure. Aims: This study was undertaken to evaluate the inhibitory effects of ARBs on vasoconstriction in humans.

Methods: Vasomotor tone was analyzed in endothelium denuded, human coronary artery (HCA) segments. Ang II type 1 (AT1) and type 2 (AT2) receptor mRNA expression was examined by reverse transcriptase-polymerase chain reaction (RT-PCR). Results: Ang II was a potent vasoconstrictor (pEC50=7.7). At 1 nM of the AT1 receptor antagonists, candesartan and valsartan, the maximum contraction was depressed to 57 and 50% of Ang II, respectively, indicating insurmountability. Although generally considered surmountable, the presence of 100 nM losartan elicited a depression of the Ang II response to 32%. Its active metabolite, EXP 3174 (1 nM), abolished the Ang II contraction. The AT1 receptor antagonists had the following order of blocking effect; EXP 3174>candesartan=valsartan>losartan. The AT2 receptor antagonist, PD 123319 (100 nM) significantly attenuated the Ang II contraction (Emax=62% of Ang II). RT-PCR of HCA smooth muscle cells demonstrated expression of both AT1 and AT2 receptor mRNA. Conclusions: Ang II contraction in HCA is mediated mainly by AT1 but also involves AT2 receptors. The active metabolite of losartan, EXP 3174, is the most efficacious AT1 receptor antagonist in HCA.

European Journal of Obstetrics & Gynecology and Reproductive Biology (5)

Objective: To investigate the presence of mutations in the open reading frame (ORF), as well as on the 5' and 3', flanking regions of the SRY gene in patients with mixed gonadal dysgenesis (MGD) or with Turner syndrome (TS) and Y mosaicism. Study design: We studied 13 patients with MGD and three patients with TS and Y mosaicism. DNA was isolated from blood leukocytes for subsequent polymerase chain reaction (PCR) and direct sequencing were performed in the ORF, as well as from the 5' and 3' flanking regions of the SRY gene. Results: No mutations were present in any of the patients studied. Conclusion: The absence of mutations in these regions indicated that mutations were an unlikely cause of MGD or TS with Y mosaicism and suggested that there are other genes playing an important role in sex development.


Objective: To investigate the correlation of the androgen receptor gene microsatellite polymorphism (CAG trinucleotide repeat polymorphism on exon 1) with bone mineral density and their relationship to osteoporosis in postmenopausal women. Study design: A number of 168 of 477 postmenopausal women were randomly recruited. The androgen receptor gene microsatellite polymorphism was determined using polymerase chain reaction-based microsatellite analysis. Bone mineral density of the lumbar spine and proximal femur was measured using dual-energy X-ray absorptiometry. Results: The AR genotype was classified from "9" to "32" according to the number of CAG trinucleotide repeats they contained to represent "signposts". After adjustment for potential confounding factors such as age, height, weight, years since menopause, and daily calcium intake, subjects with genotype 20+ (n=64) had lower bone mineral density values and a significantly greater risk for osteoporosis (OR 4.2, 95% CI 1.0-17.2) when compared with subjects with genotype 20- (n=104) at the femoral neck. Conclusion: The present study suggests that the androgen receptor gene microsatellite polymorphism may be a candidate genetic marker for risk of osteoporosis in postmenopausal women.


Objective: To investigate the maturation of the paracrine system's endothelial nitric oxide synthase (eNOS), inducible NOS (iNOS), endothelin-1 (ET-1) and adrenomedullin (AM) in human placenta during the 2nd and 3rd trimester of pregnancy. Study design: Placental tissue from 14 healthy women with normal pregnancy and from 13 patients giving birth to premature infants following premature labor was obtained. Messenger RNA expression was determined using quantitative TaqMan real-time PCR. Results: Placental eNOS/GAPDH and ET-1/GAPDH mRNA expression significantly increased as a function of gestational age (r=0.63, Pr=0.53, P=0.007,
respectively). There was no change in gene expression of neither iNOS nor AM mRNA/GAPDH during gestation (r=0.02, P=0.75 and r=0.001, P=0.99, respectively). Conclusion: There is a maturation of eNOS and ET-1 in human placenta with gestation reflecting developmental changes of important paracrine endothelial and trophoblastic regulators. AM and iNOS show no maturation during pregnancy.


http://www.sciencedirect.com/science/article/B6T69-44X87SS-F/2/66961222457fa61e6d2e2b62684b0560

Objective: The human placenta expresses a variety of vasoactive substances and neuropeptides, which play an important role in the regulation of placental blood flow in both the maternal and foetal compartment and are therefore of critical importance for foetal growth and development. Our study was planned to examine placental mRNA amounts of vasodilatory adrenomedullin (AM), calcitonin gene-related peptide (CGRP) and their receptors (AM-R and CGRP-R) in preeclampsia and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets). These are severe maternal conditions leading to an altered uteroplacental and fetoplacental perfusion and a higher risk for foetal growth retardation, premature delivery, infant mortality, and even maternal death. Study Design: We included 17 patients with preeclampsia, four women with HELLP syndrome and 34 controls. After delivery, the mRNA levels of AM, AM-R, CGRP, CGRP-R, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and [beta]-actin were measured in placental villi and chorionic plates using quantitative real-time PCR. Results: AM/[beta]-actin and AM/GAPDH mRNA ratios were significantly lower in placental villi in preeclampsia than in controls (PPConclusion: Our data show a reduction of AM and CGRP mRNAs in contrast to unchanged mRNA levels of their receptors in placenta specimens of women with preeclampsia or HELLP syndrome.


http://www.sciencedirect.com/science/article/B6T69-44TD2KX-5/2/a5bdac7a07253f58e2b6f05a123b0f14

Objective: To investigate the content of tumor necrosis factor [alpha], interleukin-1[beta], interleukin-6 and interleukin-8 messenger ribonucleic acid (mRNA) in the lower uterine segment during term parturition. Study design: mRNA extracts from the lower uterine segment obtained from 53 women undergoing non-elective caesarean section at term were analyzed by semi-quantitative reverse transcriptase polymerase chain reaction. The patients were grouped according to cervical dilatation (less than 2 cm, n=18; 2 to less than 4 cm, n=13; 4-6 cm, n=7; more than 6 cm, n=15) at the time of caesarean section. Results: Interleukin-1[beta] and interleukin-8 mRNA-contents at more than 6 cm cervical dilatation were significantly higher than at less than 2 cm. The levels of interleukin-6 were already significantly increased in the 4-6 cm group, while the tumor necrosis factor [alpha] ribonucleic acid content did not change during parturition. Conclusion: The process of cervical dilatation during parturition at term is associated with an increased expression of interleukin-1[beta], interleukin-6 and interleukin-8 mRNA in the lower uterine segment. These findings support the theory that the activation of the inflammatory network plays an important role in the progress of cervical dilatation.
http://www.sciencedirect.com/science/article/B6WF2-4DJBP8R-2/2/eaa4a5ce8e6bbcfe42eca8f6b7befa6b

Summary
NADH-ubiquinone oxidoreductase or complex I deficiency is a frequently diagnosed enzyme defect of the oxidative phosphorylation (OXPHOS) system in humans. However, in many patients, with complex I deficiency and clinical symptoms suggestive of mitochondrial disease, often no genetic defect can be found after investigation of the most common mitochondrial DNA (mtDNA) mutations. In this study, 20 patients were selected with a biochemically documented complex I defect and no common mtDNA mutation. We used the Denaturing Gradient Gel Electrophoresis (DGGE) method with primers encompassing all mitochondrial encoded fragments, to search in a systematic manner for mutations in the mitochondrial genome of complex I. In our group of patients, we were able to detect a total of 96 nucleotide changes. We were not able to find any disease causing mutation in the mitochondrial encoded subunits of complex I. These results suggested that the complex I deficiency in this group of patients is most probably caused by a defect in one of the nuclear encoded structural genes of complex I, or in one of the genes involved in proper assembly of the enzyme.

http://www.sciencedirect.com/science/article/B6T25-47K3J7V-1/2/ebf21b1c703489ad77ef3c8c42b14a36

PCR amplification has emerged as a very important tool in biological research. The utility of the PCR is, however, hampered by the fact that it is a slow technique. Faster heating cycles are therefore needed, both to enhance the activity of the enzyme, and to enable shortening of the reaction times. In this paper, polymerase chain reactions with focused microwave irradiation as the source of heat were demonstrated for the first time. Thus, it was established that continuous microwave heating does not terminate the enzymatic function of the polymerase. The results indicate the possibility to shorten the total reaction time. In addition, the technique may give the possibility to perform PCR reactions in millilitre scale.

The effects of 12 Ca2+ antagonists on MDR1 were examined by two independent models: the inhibitory effect on MDR1-mediated transport of [3H]digoxin using MDR1-overexpressing LLC-GA5-COL150 cell monolayers and the reversal effect on cytotoxicity of vinblastine or paclitaxel using MDR1-overexpressing Hvr100-6 cells. The inhibitory effects on [3H]digoxin transport were assessed as the 50% inhibitory concentration during 4 h exposure, and the values were the lowest for nicardipine (4.54 [μM]), manidipine (4.65 [μM]) and benidipine (4.96 [μM]), followed by bepridil (10.6 [μM]), barnidipine (12.6 [μM]), efonidipine (13.0 [μM]), verapamil (13.2 [μM]) and nilvadipine (18.0 [μM]). The reversal effect on cytotoxicity was assessed by the 50% growth inhibitory concentration after 3 days exposure, and the resistance to vinblastine or paclitaxel in Hvr100-6 cells was reversed by manidipine, verapamil, benidipine, barnidipine, and nicardipine, in that order. Bepridil, barnidipine, efonidipine, verapamil and nilvadipine showed similar inhibitory effects on [3H]digoxin transport, but barnidipine and verapamil showed a stronger effect in reversal of cytotoxicity. Real-time quantitative RT-PCR assay indicated a decrease in MDR1 mRNA expression by barnidipine and verapamil. It is concluded that Ca2+ antagonists cannot only be direct inhibitors of MDR1 but that some may at the same time act as inhibitors of expression of MDR1 via down-regulation of MDR1 mRNA.


The effect of electric field gradients are examined on the speed, selectivity, read length, and accuracy for DNA sequencing using capillary array electrophoresis. Modified electric field gradients was realized to read over 800 bases within 140 min. The method developed is effectively applicable to single nucleotide polymorphism analysis for genomic drug discovery and pharmacogenomics.

European Journal of Pharmacology (30)


Modifications of rat prostatic [alpha]1-adrenoceptors were investigated in testosterone-induced prostatic hypertrophy. prazosin bound to a single class of binding sites with a dissociation constant of 57.9+/-5.02 pM. The greater part of the binding capacity (24.6+/-1.02 fmol/mg protein) was made up of chloroethylclonidine-resistant binding sites that showed high-affinity for
oxymetazoline and 5-methyl-urapidil, and was identified as \([\text{alpha}]1\text{A}-\text{adrenoceptors}\). The remaining chloroethylclonidine-sensitive binding sites that showed low-affinity for oxymetazoline and 5-methyl-urapidil were preferentially identified as \([\text{alpha}]1\text{B}-\text{adrenoceptors}\). mRNA for the three \([\text{alpha}]1\)-adrenoceptors \([\text{alpha}]1\text{a}, [\text{alpha}]1\text{b} \text{ and } [\text{alpha}]1\text{d}\) was detected. Testosterone administration produced a 23% decrease of \([\text{alpha}]1\)-adrenoceptor density, likely by an increase of prostatic glandular epithelium and a decrease in the relative proportion of smooth muscle, thus of \([\text{alpha}]1\)-adrenoceptor density. The steady state level of mRNAs for \([\text{alpha}]1\)-adrenoceptors was not modified by testosterone treatment. These results indicate that prostate \([\text{alpha}]1\)-adrenoceptors are not affected in the prostatic hypertrophy induced by testosterone.


http://www.sciencedirect.com/science/article/B6T1J-3W2Y75F-1J/2/8590278bef2b76e9135e13f4e547e727

To elucidate which neuropeptide Y receptor subtype is responsible for the neuropeptide Y-induced potentiation of the noradrenaline-evoked contraction in human omental arteries we used antisense oligodeoxynucleotide (Antisense), the new selective neuropeptide Y Y1 receptor antagonist, BIBP3226 \(\{(R)-\text{N2-(diphenylacetyl)N-((4-hydroxyphenyl) methyl)-arginine-amide}\}\) and the reverse transcriptase-polymerase chain reaction (RT-PCR). Neuropeptide Y significantly potentiated the noradrenaline-induced contraction in non-incubated vessels \(\text{(pEC50 6.4 +/- 0.2 vs. 5.9 +/- 0.2)}\) and in vessels incubated with 1 \(\mu\text{M}\) Sense oligodeoxynucleotide (Sense) \(\text{(pEC50 6.0 +/- 0.1 vs. 5.6 +/- 0.2)}\). In vessels incubated with 1 \(\mu\text{M}\) Antisense the potentiating effect of neuropeptide Y was completely abolished. BIBP3226 \(1 \mu\text{M}\) inhibited the neuropeptide Y-induced potentiation in human omental arteries \(\text{(pEC50 5.8 +/- 0.3 vs. 6.4 +/- 0.2)}\). Finally, messenger RNA for the neuropeptide Y Y1 receptor was detected using RT-PCR. On the basis of our results we conclude that the neuropeptide Y-induced potentiation of the noradrenaline-induced contraction is mediated by the neuropeptide Y Y1 receptor.


http://www.sciencedirect.com/science/article/B6T1J-44VGBP-2/2/73024e45e161a11c45e9c1721813a05d

Using a combination of reverse transcription polymerase chain reaction (RT-PCR) and inverse-PCR techniques, we amplified, cloned and sequenced a full-length porcine 5-hydroxytryptamine 1F (5-ht1F) receptor complementary DNA (cDNA) derived from porcine trigeminal ganglion. Sequence analysis revealed 1101 base pairs (bp) encoding an open reading frame of 366 amino acids showing a high similarity (>90%) with the 5-ht1F receptor sequences from other species, including human. The recombinant porcine 5-ht1F receptor was expressed in African green monkey kidney cell lines (COS-7 cells) and its ligand binding profile was determined using \(\text{[3H]5-HT}\). The affinities of several agonists \(\text{(LY334370 (5-(4-fluorobenzoyl)amino-3-(1-methylpiperidin-4-yl)-1H-indole fumarate)=CP122638 (N-methyl-3 [pyrrolidin 2(R)-yl methyl]-1H-indol-5-ylmethyl sulphonamide)=naratriptan=eletriptan=sumatriptan=frovatriptan=avitriptan=dihydroergotamine>zolmitriptan=carboxamidotryptamine=rizatriptan=alniditan=donitriptan=L694247 (2-[5-[3-(4-methylsulphonylamino)benzyl]-1,2,4-oxadiazol-5-yl]-1H-indole-3-yl ethylamine) and putative antagonists (methiothepin>GR127935 (N-[4-methoxy-3-(4-methyl-1-piperaziny1) phenyl]-2'-methyl 4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride)=ritanserin>SB224289 (2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'-(5-methyl-1,2,4-}
oxadiazol-3-y1) biphenyl-4-carbonyl] furo [2,3-f] indole-3-spiro-4’-piperidine hydrochloride)>BRL155572 ([1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R)
hydroxypropanyl)piperazine] hydrochloride)>ketanserin=pindolol) correlated highly with those
described for the recombinant human 5-ht1F receptor (Spearman correlation coefficient;
rs=0.942). Nevertheless, as compared to the human homologue, some triptans (i.e. sumatriptan,
zolmitriptan and rizatriptan) displayed a 10- to 15-fold lower affinity for the porcine 5-ht1F
receptor. Using RT-PCR technique, the expression of porcine 5-ht1F receptor mRNA was
observed in cerebral cortex, trigeminal ganglion and several blood vessels, but not in skeletal
muscles. In conclusion, we have cloned and established the amino acid sequence and ligand
binding profile of the porcine 5-ht1F receptor as well as the distribution of its mRNA. This
information may be helpful in exploring the role of 5-ht1F receptor in physiological processes and
diseases, such as migraine.

the toxicity of a cyclooxygenase inhibitor in Gram-negative sepsis." European Journal of

http://www.sciencedirect.com/science/article/B6T1J-3W0NCT2-9/2/81d585cd506a8dc96060f1bb62fd59e4

To investigate the effect of cyclooxygenase inhibition in experimental Gram-negative sepsis,
indomethacin was administered to mice at different times (1 or 5 days, or 1 h) before sublethal
infection with an intravenous inoculum of Pseudomonas aeruginosa. Early indomethacin
exposure did not alter the outcome of infection, yet treatment at the time of bacterial challenge
resulted in a high mortality rate. Polymerase chain reaction-assisted mRNA amplification in the
spleens of infected mice revealed that tumor necrosis factor [alpha] (TNF-[alpha]) messenger was
selectively expressed by the drug-treated and infected mice during the 24 h preceding death.
Higher TNF-[alpha] levels were found in sera from these mice, whose macrophages produced
increased levels of nitric oxide in vitro. Both pentoxifylline, an inhibitor of TNF-[alpha] synthesis,
and an inhibitor of nitric oxide production improved survival in the indomethacin-treated and
infected mice, although no such effect followed the administration of TNF-neutralizing antibodies.
These data support the notion that cyclooxygenase inhibitors may exert both positive and
negative effects in Gram-negative sepsis, the latter presumably involving overproduction of TNF-
[alpha].


http://www.sciencedirect.com/science/article/B6T1J-4967NBX-5/2/547e12a57790dba51de69c846eeea901

Thromboxane A2 has been implicated as a mediator of bronchial hyperresponsiveness in
asthma. Modulating agents are currently marketed in Japan and under clinical evaluation in the
US, but full characterization of the thromboxane A2 receptor and the signaling pathways that link
it to the proliferative events taking place during airways structural remodeling has not been
achieved. Here, we report that the presence of mRNA for both [alpha] and [beta] isoforms of the
thromboxane A2 receptor in smooth muscle cells from human bronchi correlates with protein
expression evaluated by radioligand binding of the antagonist, SQ29,548 ([1S-
[1[alpha],2[alpha][Z],3[alpha],4[alpha]]-7-[3-[[phenylamino]carbonyl]hydrazino[methyl]-7-
oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic-acid) (Kd=3.4 nM+/-44%CV, coefficient of variation,
Bmax=41 fmol/mg prot+/-38%CV). The receptor is functional, as the agonist, U46619 (9,11-
dideoxy-9[alpha],11[alpha]-methanoepoxy-prosta-5Z,13E-dien-1-oic-acid), induced a
concentration-dependent Ca2+ transient (EC50=0.12 [mu]M+/-27%CV). Furthermore, U46619 concentration dependently increased DNA synthesis and markedly potentiated the epidermal growth factor mitogenic effect. Both events were specifically inhibited by SQ29,548, independently from transactivation of the epidermal growth factor receptor and partially sensitive to pertussis toxin.


http://www.sciencedirect.com/science/article/B6T1J-400WXW3-D/2/34558dbd4dc7821c238b134c7fb97f0c

Recombinant fractalkine possesses both chemoattractive and adhesive properties in vitro. Previous studies have demonstrated an upregulation of this molecule on the membranes of activated human endothelial cells and hypothesised that fractalkine plays a role in the recruitment and adherence of monocytes to the activated endothelium. Here we present data analysing both the adhesive and chemoattractive properties of this chemokine expressed by activated human umbilical vein endothelial cells. We demonstrate that both recombinant fractalkine and endogenously produced fractalkine function as adhesion molecules, tethering monocytes to the endothelium. However, our data demonstrate that although recombinant fractalkine has the potential to function as a potent monocyte chemoattractant, the endogenous fractalkine cleaved from activated human umbilical vein endothelial cells is not responsible for the observed chemotaxis in this model. Instead, we show that monocyte chemoattractant protein-1 (MCP-1), secreted from the activated human umbilical vein endothelial cells, is responsible for the chemotaxis of these monocytes.


http://www.sciencedirect.com/science/article/B6T1J-439VFWF-4/2/f09e8061a7ba36e441eabc229ea22e85

Glucocorticoid-induced apoptosis is a well-recognized physiological regulator of T-cell number and function. Alisol B acetate, a triterpene from Alisma Plantago-aquatica, has a glucocorticoid-like structure, and may have a similar function like glucocorticoid-induced apoptosis in both vascular smooth muscle cell line (A7r5) and human acute lymphoblastic leukemia cell line (CEM cells). For exploring its mechanism, mitochondria membrane potential and apoptosis-related gene expression were discussed. Alisol B (10-6-10-4 M) inhibited serum-stimulated DNA synthesis in a concentration-dependent manner (IC50=4.0+/-0.8 x 10-6 M in A7r5 and 2.1+/-1.2 x 10-6 M in CEM cells). The cell viability was reduced at 10-4 M of alisol B. Similar results were seen in dexamethasone treatment (a synthetic glucocorticoid, 10-6 M, 48 h). Apoptosis was induced after the cells were exposed to 10-5-10-4 M alisol B or 10-6 M dexamethasone for 48 h. The mitochondrial membrane potential ([Delta][Psi]m) was significantly reduced after the alisol B treatment, indicating that the mitochondria might play a role in the alisol B induced cell apoptosis. Alisol B (10-5-10-4 M) increased the levels of c-myc and bax mRNA and proteins, but not on the anti-apoptotic proto-oncogene, bcl-2, in A7r5 and CEM cells. In contrast, dexamethasone (10-6 M) treatment only caused significant increase in c-myc mRNA levels. These results suggest that the increased ratio of Bax/Bcl-2 and the decreased mitochondrial membrane potential might be involved in the mechanisms of alisol B-induced cell apoptosis.

http://www.sciencedirect.com/science/article/B6T1J-3T2H16J-S/2/727e43ed46263b8075ff650d22d70944

Kinin receptors are classified as B1 and B2 based upon agonist and antagonist potencies and cloning and expression studies. Using sequences from human and rat bradykinin B2 receptors, polymerase chain reaction (PCR) was utilized to isolate cDNA from guinea pig lung. The receptor obtained is predicted to have 372 amino acids and shares >80% sequence homology with human, rat, rabbit and mouse B2 receptors. In competition binding experiments in Chinese hamster ovary (CHO-K1) cells in which the guinea pig cDNA was expressed, [3H]bradykinin was displaced by kinin receptor ligands with an order of potency consistent with a B2 subtype. In CHO cells expressing the guinea pig receptor, bradykinin caused a concentration 45Ca2+ efflux. A B1 receptor agonist, desArg9-bradykinin, also caused 45Ca2+ efflux but with a potency several orders of magnitude lower than bradykinin. Curiously, several B1 and B2 receptor antagonists induced 45Ca2+ efflux, indicating that this receptor may be coupled differently in CHO cells than in native tissues.


http://www.sciencedirect.com/science/article/B6T1J-3SJVGWD-13/2/e5c26d8ca38c9356e368f4bdcb2c2d1b

We characterized neutral endopeptidase activity and protein in the three aortic layers and in corresponding cultured primary cells. Neutral endopeptidase was expressed in all three layers of rat aorta with higher protein level and activity in the adventitia than in the media and intimal endothelium. Neutral endopeptidase was also found in primary cultured fibroblasts, smooth muscle and endothelial cells derived from the corresponding layers. Neutral endopeptidase activity and protein were higher in the fibroblasts and smooth muscle cells than in endothelial cells. Neutral endopeptidase inhibition prevented atrial natriuretic peptide (ANP) degradation in endothelial and smooth muscle cells. It potentiated ANP-stimulated cyclic GMP production in these cells. Neutral endopeptidase inhibition also reduced bradykinin degradation and potentiated bradykinin-stimulated release of arachidonic acid in fibroblasts and endothelial cells. Our data demonstrate the presence and functional activity of neutral endopeptidase in all three cell layers of rat aorta as well as in primary cells of the vessel. The data suggest that local concentrations of vasoactive peptides in the vessel wall might be regulated by the neutral endopeptidase cleavage pathway in the immediate vicinity of their target cells.


http://www.sciencedirect.com/science/article/B6T1J-46NYFT4-3/2/419756788c5c946fbdbbda20c550d7d0

TAK-778 [(2R,4S)-(-)-N-(4-Diethoxyphosphorylmethylphenyl)-1,2,4,5-tetrahydro-4-methyl-7,8-methylenedioxy-5-oxo-3-benzothiepin-2-carboxamide: mw 505.52], a novel compound promoting
osteoblast differentiation, promotes osteogenesis in vitro and enhances bone formation during skeletal repair in vivo. In this study, we further evaluated the effects of TAK-778 on the differentiation of cultured bone marrow stromal cells into osteoblasts in the presence of dexamethasone, paying particular attention to the expression of transcription factors involved in regulating osteoblast differentiation. Treatment of TAK-778 (10-7-10-5 M) for 4 h resulted in an increase in the mRNA expression of Msx2, but not Cbfa1 or Dlx5. This transcriptional alteration preceded the changes in other markers related to the osteoblast phenotype, such as alkaline phosphatase and osteocalcin mRNA. The transfection of Msx2-antisense in the cells caused a significant reduction in the levels of alkaline phosphatase mRNA expression induced by TAK-778. These results suggest that TAK-778 promotes osteoblast differentiation partly through the expression of Msx2, a homeobox-related gene.


http://www.sciencedirect.com/science/article/B6T1J-3RSGTV0-X/2/bbd6fc996305673934bf45693cf3d92

Nitric oxide (NO) synthesis may be coupled to the activity of the cellular -arginine transporter, namely the cationic amino acid transporter. The present study examined tumor necrosis factor (TNF)-[alpha]-induced alterations in the gene expression of the cationic amino acid transporter (CAT) and NO production in human umbilical vein endothelial cells. In quiescent endothelial cells, CAT-1 mRNA expression, determined by reverse transcription-polymerase chain reaction, was dominant to that of CAT-2. TNF-[alpha] (10 ng/ml for 1-24 h) induced a time-dependent increase in CAT-2 but not CAT-1 expression. Moreover, TNF-[alpha] (1-30 ng/ml) treatment for 6 h induced a concentration-dependent increase in CAT-2 mRNA expression. The upregulation of CAT-2 expression by TNF-[alpha] was associated with enhanced nitrite accumulation in the culture medium (70% increase compared with vehicle-treated cells at 24 h). Thus, induction of the cationic amino acid transporter may constitute one mechanism for the TNF-[alpha]-induced NO production in human umbilical vein endothelial cells.


http://www.sciencedirect.com/science/article/B6T1J-3W1YHDB-G/2/f34e18193a851079e39fa216f816dd2f

Vascular cell adhesion molecule-1 (VCAM-1) is a mononuclear leukocyte-selective adhesion molecule that is expressed in human vascular endothelial cells at sites of local inflammation. It participates in local endothelial-monocyte interactions during the initiation of atherosclerosis. In the present study, endothelin alone did not induce the surface expression and mRNA accumulation of VCAM-1 in human vascular endothelial cells, but inhibition of endogenous nitric oxide (NO) by NG-nitro-L-arginine enhanced the surface expression and mRNA accumulation of VCAM-1 stimulated by endothelin-1. It is conceivable that in human vascular endothelial cells, stimulation of an endothelin receptor results in the production of nitric oxide (NO), suppressing the expression of VCAM-1. Endothelin-1 enhanced the surface expression and mRNA accumulation of VCAM-1 in cells treated with tumor necrosis factor [alpha] (TNF-[alpha]). The enhancement by endothelin-1 may be explained by the inhibitory effect of TNF-[alpha] on endothelin-induced NO production. Pretreatment with BQ788 (an endothelin ETB receptor antagonist) or inhibitors of nuclear factor kappa B (NF-[kappa]B) activation completely diminished the synergistic enhancement of VCAM-1 expression by endothelin-1 in TNF-[alpha]-stimulated
vascular endothelial cells, both at the protein and mRNA levels. These findings suggest that the synergistic enhancement of VCAM-1 expression by TNF-[alpha] and endothelin ETB receptor stimulation may be augmented by the induction of NF-[kappa]B binding activity in human vascular endothelial cells.


http://www.sciencedirect.com/science/article/B6T1J-4DDRBGW-4/2/3f37c4156f40a035e04a49aa87e6d27d

Central glucocorticoid receptor function may be reduced in depression. In vivo modelling of glucocorticoid receptor underfunctionality would assist in understanding its role in depressive illness. The role of glucocorticoid receptors in modulating 5-HT2A receptor expression and function in the central nervous system (CNS) is presently unclear, but 5-HT2A receptor function also appears altered in depression. With the aid of RNAse H accessibility mapping, we have developed a 21-mer antisense oligodeoxynucleotide (5'-TAAAAACAGGCTTCTGATCCT-3', termed GRAS-5) that showed 56% reduction in glucocorticoid receptor mRNA and 80% down-regulation in glucocorticoid receptor protein in rat C6 glioma cells. Sustained delivery to rat cerebral ventricles in slow release biodegradable polymer microspheres produced a marked decrease in glucocorticoid receptor mRNA and protein in hypothalamus (by 39% and 80%, respectively) and frontal cortex (by 26% and 67%, respectively) 5 days after a single injection, with parallel significant up-regulation of 5-HT2A receptor mRNA expression (13%) and binding (21%) in frontal cortex. 5-HT2A receptor function, determined by DOI-head-shakes, showed a 55% increase. These findings suggest that central 5-HT2A receptors are, directly or indirectly, under tonic inhibitory control by glucocorticoid receptor.


Soluble guanylyl cyclase activity and its stimulation by diethylamineNONOate was measured in aortae from hypertensive TGR(mREN2)27 rats (TGR) and Sprague-Dawley controls. Superoxide dismutase was added in vitro to evaluate the contribution of oxidative breakdown of nitric oxide (NO) by superoxide anions. Expression of soluble guanylyl cyclase was assessed by reverse transcriptase-polymerase chain reaction (RT-PCR). Basal and stimulated soluble guanylyl cyclase activity was significantly reduced in TGR rats, addition of superoxide dismutase had no effect. Expression of soluble guanylyl cyclase subunits was not different between strains. The independent contribution of hypertension and the overactive renin-angiotensin system to soluble guanylyl cyclase subsensitivity was assessed after normalization of TGR's blood pressure by the Ca2+-channel blocker amlodipine or the angiotensin converting enzyme-inhibitor enalapril. Soluble guanylyl cyclase activity in TGR was slightly increased by amlodipine and almost completely restored by enalapril. In conclusion, TGR showed desensitized vascular soluble guanylyl cyclase, depending on their overactive renin-angiotensin system.

Inflammatory gene expression in airway smooth muscle may be influenced by its inflammatory milieu. We analysed the gene expression profile of airway smooth muscle cells cultured from human airways exposed to a pro-inflammatory cytokine, interleukin-1[beta], a T helper-2 cytokine, interleukin-13, and to a growth factor, transforming growth factor (TGF)[beta]1 (10 ng/ml each) after 4 and 24 h using the Affymetrix GeneChip 95A array which detects [not, vert, similar]12,500 genes and expression sequence tags (ESTs). Airway smooth muscle cells were responsive to each cytokine with distinctive patterns of gene expression for cytokines, chemokines, adhesion and signalling proteins, and transcription factors. Interleukin-1[beta] induced the highest number of genes such as cytokines/chemokines including interleukin-8, growth-related oncogene (GRO)-[alpha], -[beta] and -[gamma], epithelial neutrophil activating protein (ENA)-78, monocyte chemotactic protein (MCP)-1, -2 and -3 and eotaxin. Using quantitative real-time reverse transcription-polymerase chain reaction, the expression of GRO-[alpha], -[beta] and -[gamma], interleukin-8 and eotaxin by interleukin-1[beta] was confirmed, with good correlation with microarray data. Transforming growth factor (TGF)[beta]1 induced other growth factors such as connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), insulin growth factor (IGF) and many structural and extracellular matrix proteins. Interleukin-13 was the weakest inducer, with stimulation of eotaxin and genes of unknown function. While many genes were co-regulated at 4 and 24 h, there were also differences in expression patterns. Interleukin-1[beta] induces a predominantly pro-inflammatory profile while TGF[beta]1 can be linked to proliferative and matrix changes. The rich profile of mediators, growth factors and signalling molecules released from airway smooth muscle depends on the inflammatory milieu.


The metabolic response of galanin GAL1 receptor subtype, endogenously expressed in human Bowes melanoma (HBM) cells, was investigated. Cytosensor microphysiometry was used to determine the extracellular acidification rate. A biphasic response, consisting of a rapid increase in the extracellular acidification rate followed by a decrease below the basal level, was observed after perfusion with human galanin. The magnitude and the rate of onset of both phases were dependent on the galanin concentration. The increase in the extracellular acidification rate (maximum of 25% of basal level; -log(EC50)=7.23+/-.14) was transient, whereas the following decrease (maximum of 40% of basal level; -log(EC50)=7.77+/-.23) was sustained. The EC50 values for the increase and decrease were in a similar range. After consecutive galanin administration, the magnitude of the response was the same as for the unexposed cells, indicating the absence of galanin receptor desensitization or internalization in HBM cells. Responses were blocked by pretreatment with pertussis toxin and phorbol-12-myristate-13-acetate (PMA), indicating a G-protein/protein kinase C signalling pathway. Our microphysiometry results show a biphasic response of the extracellular acidification rate mediated by the galanin receptor expressed in HBM cells which has not been described previously for any other endogenously expressed neuropeptide receptor.


http://www.sciencedirect.com/science/article/B6T1J-4B233CJ-2/2/cf9c387690503226eb8ef8c34cac71a4

The aim of this study was to characterise the expression of the melanocortin system in the normal and injured rat visual system. Using real-time polymerase chain reaction and immunohistochemistry, we detected melanocortin MC3, MC4 and MC5 receptors and proopiomelanocortin in adult retina and superior colliculus. Melanocortin MC4 receptor mRNA was the most abundant receptor. Melanocortin MC3, MC4 and MC5 receptors were localised to the ganglion cell and inner nuclear layers and the melanocortin MC3 and MC4 receptors were localised to retinal ganglion cells. Transection of the optic nerve leads to ganglion cell death and both melanocortin receptor and proopiomelanocortin expression decreased in superior colliculus after transection whereas the expression was unchanged or even increased in the retina. [alpha]-Melanocyte-stimulating hormone elicited neurite outgrowth from embryonic retinal explants. Together, these data implicate a role for the melanocortin system in the adult rat retina and that melanocortins can stimulate neurite growth from retinal neurons.


http://www.sciencedirect.com/science/article/B6T1J-47DTGYC-1/2/9c6ba3a0538c559e26c0b1311ad0ccff

Extracellular nucleotides were used to characterise the contractile P2 receptors in the rat basilar artery. The isometric tension was recorded in vitro and receptor mRNA expression was examined by reverse transcriptase polymerase chain reaction (RT-PCR) after endothelium-denudation. Transient vasoconstriction was evoked by [alpha][beta]-methylene-adenosine triphosphate ([alpha][beta]-MeATP), indicating the presence of P2X1 receptors. The P2Y receptors were analysed after P2X receptor desensitisation with 10 [mu]M [alpha][beta]-MeATP. Uridine diphosphate (UDP) and uridine triphosphate (UTP) induced sustained contractions of similar magnitude. The stable nucleotide analogue, uridine 5'-O-thiodiphosphate (UDP[beta]S), was clearly more potent than uridine 5'-O-3-thiotriphosphate (UTP[gamma]S), suggesting prominent contractile effects of P2Y6 receptors. P2Y2 and P2Y4 receptors might also be involved in nucleotide responses, since UTP[gamma]S and adenosine 5'-O-3-thiotriphosphate (ATP[gamma]S) were of similar potency. The P2Y1 selective agonists, adenosine 5'-O-thiodiphosphate (ADP[beta]S) and 2-methylthioadenosine diphosphate (2-MeSADP) did not induce contractions. RT-PCR analysis demonstrated P2X1, P2Y1, P2Y2 and P2Y6 receptor mRNA expression, while the P2Y4 band was weak. In conclusion, extracellular nucleotides induce contractions of cerebral arteries primarily by activation of P2Y6 receptors on smooth muscle cells. with a lesser contribution of P2Y2 and P2X1 receptors. Although mRNA for the P2Y1 receptor was detected by RT-PCR, it does not mediate contraction.


http://www.sciencedirect.com/science/article/B6T1J-47XWTYY-5/2/0a299d48ee1555fa90fe9af94f3e38b7
Adrenomedullin is a hypotensive peptide secreted from various cells. Recently, we found that adrenomedullin, but not calcitonin gene-related peptide (CGRP), stimulates histamine release from rat peritoneal mast cells. In the present studies, we investigated the expression of mRNA for calcitonin-receptor-like receptor (CRLR) and receptor-activity modifying proteins (RAMPs), the components of proposed adrenomedullin receptors, in rat peritoneal mast cells by reverse transcription-polymerase chain reaction (RT-PCR). Results revealed that mRNA for CRLR, RAMP2 and RAMP3 was expressed in rat peritoneal mast cells, whereas mRNA for RAMP1 was not. These data suggest that adrenomedullin might stimulate histamine release via its proposed receptor (CRLR/RAMP2 or 3), rather than via the CGRP receptor (CRLR/RAMP1).


Guanosine 3',5'-cyclic monophosphate (cGMP) has an important role in regulating vascular smooth muscle tone. We examined whether mRNA for multidrug resistance protein (MRP) 4 and MRP5, which were recently identified as ATP-dependent export pumps for cyclic nucleotides, is expressed in the porcine coronary and pulmonary arteries. The results showed that both arteries express mRNA for MRP4 and MRP5, and thus these proteins may be novel targets for the prevention and/or treatment of various cardiovascular diseases.


http://www.sciencedirect.com/science/article/B6T1J-40N7DXF-3/2/6b0442c7eb212f0ecec8d4e81a3555d0

Ferrets (Mustela putorius furo) are useful animals for determining anti-emetic activity via 5-HT3 receptors in vivo. We isolated a cDNA encoding the 5-hydroxytryptamine (5-HT) type 3A receptor subunit (5-HT3A) from ferret colon, expressed it in a human embryonic kidney cell line and determined its pharmacological properties. The open reading frame of the isolated cDNA encoded a 483-amino acid protein, corresponding to the shorter splice variant of 5-HT3A receptors. Splice variants were no longer detected by reverse transcriptase-polymerase chain reaction. The ferret 5-HT3A receptor exhibits a high degree of amino acid sequence identity (>=80%) to that of other species. Binding studies demonstrated the following rank order of potency for agonists: meta-chlorophenylbiguanide (mCPBG)>2-methyl-5-hydroxytryptamine (2-Me-5-HT)=5-HT, and for antagonists: ondansetron=tropisetron>(+)-tubocurarine>metoclopramide. Electrophysiological studies revealed that mCPBG was a partial agonist and 2-Me-5-HT was an almost fully effective agonist compared to 5-HT.


http://www.sciencedirect.com/science/article/B6T1J-3S3M07V-Y/2/7db2a6094c339d5b2e3f82602e28dd6

The aim of the present study was to investigate the level of regulation of the contractile endothelin
ETB receptor which appears spontaneously after organ culture of vascular segments. Endothelin-1 elicited a strong contraction while the selective endothelin ETB receptor agonist, sarafotoxin 6c, had a negligible effect on fresh ring segments of rat mesenteric artery. After organ culture in serum-free Dulbecco's modified Eagle's medium at 37[°]C (for 1 or 2 days) the endothelin-1-induced contraction was unchanged, whereas sarafotoxin 6c induced, after 1 day, a marked contraction which was further increased at day 2. The contraction induced by sarafotoxin 6c was significantly attenuated by the transcriptional inhibitor, actinomycin D, or the translational inhibitor, cyclohexamide, while the endothelin-1-induced contraction was much less affected. mRNA for endothelin ETA and endothelin ETB receptors was present in fresh human omental arteries denuded of endothelium. However, after organ culture, endothelin ETB mRNA was more prominent than endothelin ETA mRNA. Furthermore, the mRNA for both receptors was decreased after treatment with actinomycin D but not with cyclohexamide. This suggests that the endothelin ETA receptor is the dominating contractile receptor in fresh arteries while organ culture induces transcription and subsequent translation of contractile endothelin ETB receptors.


Besides possessing a strong growth hormone (GH)-releasing activity, the gastrointestinal octanoylated peptide ghrelin has been reported to antagonize lipolysis in rat adipocytes. It is not yet clear whether this inhibitory activity on lipolysis is also shared by the major circulating isoform, des-acyl ghrelin, that does not activate the ghrelin receptor, namely the type 1a GH secretagogue-receptor (GHS-R1a) and lacks the endocrine effects of the acylated form. Here we show that des-acyl ghrelin, like ghrelin and some synthetic GHS (hexarelin and MK0677) and carboxy-terminally ghrelin fragments such as ghrelin-(1-5) and ghrelin-(1-10), all significantly reduced, over concentrations ranging from 1 to 1000 nM, the stimulation of glycerol release caused in rat epididymal adipocytes by the nonselective [beta]-adrenoceptor agonist isoproterenol in vitro. The order of potency on stimulated-lipolysis was: des-acyl ghrelin=ghrelin=MK0677=hexarelin=ghrelin-(1-5)=ghrelin-(1-10). This ranking was consistent with the binding experiments performed on membranes of epididymal adipose tissue or isolated adipocytes that did not express mRNA for GHS-R1a. A common high-affinity binding site was recognized in these cells by both acylated and des-acylated ghrelin and also by hexarelin, MK0677, ghrelin-(1-5) and ghrelin-(1-10). In conclusion, these findings provide the first evidence that des-acyl ghrelin, as well as ghrelin, short ghrelin fragments and synthetic GHS, may act directly as antilipolytic factors on the adipose tissue through binding to a specific receptor which is distinct from GHS-R1a.


Using the polymerase chain reaction with degenerate primers to identify novel G-protein-coupled receptors of the rat alveolar Type II cell, we identified sequences expressed by the Type II cell identical to the sequence of the rat brain cannabinoid receptor (CB1). The use of Northern blot analysis to examine expression of CB1 mRNA in rat tissues revealed differences between the
brain and lung. While rat brain expressed a 6.0 kb mRNA as previously described, rat lung expressed mRNA of 4.5 and 6.0 kb. Isolated lung alveolar Type II cells also expressed mRNA of 4.5 and 6.0 kb as determined by Northern analysis. However, only freshly isolated Type II cells contained cannabinoid receptor mRNA. Reverse transcriptase-polymerase chain reaction (RT-PCR) failed to detect CB1 mRNA in Type II cells maintained in culture for 1 or 2 days. We next determined developmental changes in lung CB1 mRNA expression using semi-quantitative RT-PCR. CB1 expression was detected as early as gestational day 16 in rat lung and mRNA levels increased to fetal day 20 before birth, before declining to adult levels. Fetal rat lung explants were utilized to further examine the ontogeny and hormonal effects on CB1 mRNA expression. Hydrocortisone induced a dose-dependent expression in 15-day and 18-day explants, similar to previous results for surfactant-associated proteins. Our results demonstrate expression of CB1 mRNA in rat alveolar Type II cells and rat lung. This expression is ontogenically and hormonally regulated, with maximal expression noted just prior to birth in rat lung. Since CB1 mRNA is only expressed in freshly isolated Type II cells, CB1 may be useful as a Type II cell marker.


http://www.sciencedirect.com/science/article/B6T1J-44NM99X-5/2/c4f201e5c61fe19bc72aa2c6810b0269

Properties of inwardly rectifying K+ channels in small-cell lung cancer (SCLC) cells have not been clarified in detail. Here, we found inwardly rectifying K+ channels in a human SCLC cell line (RERF-LC-MA), which expresses no multidrug resistance-associated protein 1 (MRP1) and multidrug resistance P-glycoprotein (MDR1). Extracellular Ba2+ and Cs+ inhibited inwardly rectifying K+ currents of RERF-LC-MA cells in a concentration-dependent manner, but tetraethylammonium ion and glibenclamide were ineffective. Okadaic acid, an inhibitor of phosphatases 1 and 2A, and phorbol-12,13-dibutyrate, an activator of protein kinase C, significantly decreased the inwardly rectifying K+ current. Lowering the intracellular pH but not the extracellular pH decreased the K+ current. Reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting analysis showed that RERF-LC-MA cells express Kir2.1 mRNA and protein. The inwardly rectifying K+ current is suggested to be generated by Kir2.1 protein in the human small-cell lung cancer cell, and that the K+ channel is negatively regulated by protein kinase C and the intracellular acidic pH.


http://www.sciencedirect.com/science/article/B6T1J-4FD9T1B-2/2/ef9879709fd589d3324afdb58af4e925

To examine the effects of lansoprazole, a proton pump inhibitor, on rhinovirus infection in airways, human tracheal epithelial cells were infected with a major subgroup of rhinoviruses, type 14 rhinovirus. Rhinovirus increased the mRNA expression of intercellular adhesion molecule-1 (ICAM-1) in the cells, the major rhinovirus receptor, and the content of the soluble form of ICAM-1 (sICAM-1) and cytokines in supernatants. Lansoprazole reduced supernatant titers and RNA of rhinovirus, the susceptibility to rhinovirus infection, the ICAM-1 mRNA production, the number and fluorescence intensity of acidic endosomes in the cells, and supernatants sICAM-1 and cytokine concentrations including interleukin-1[beta]. Antibody to interleukin-1[beta] reduced baseline and rhinovirus-induced ICAM-1 production. These results suggest that lansoprazole inhibits rhinovirus infection by reducing ICAM-1 via partly endogenous production of interleukin-1[beta], and by blocking the rhinovirus RNA entry into the endosomes. Lansoprazole may
modulate airway inflammation by reducing the production of cytokines and ICAM-1 in rhinovirus infection.


The affinity, selectivity and agonistic properties of a constrained dopaminergic compound, the benz[e]indole cis-8-hydroxy-3-(n-propyl)1,2,3a,4,5,9b-hexahydro-1H-benz[e]indole (cis-8-OH-PBZI), for the dopamine D3 receptor were evaluated in competition binding experiments with cloned human dopamine receptor subtypes and, to further extend its profile, in vitro radioligand binding assays. The Ki value measured for competition binding of this compound to the dopamine D3 receptor was 27.4±3.1 nM; this was 775-fold, 550-fold, 90-fold and 10-fold higher affinity than that measured at dopamine D1A, D5, D2s and D4 receptors, respectively. Evidence of dopamine receptor activation by cis-8-OH-PBZI was obtained by measuring dose-dependent increases in extracellular acidification rates and decreases in cAMP synthesis. In vivo, cis-8-OH-PBZI potently induced Fos protein immunoreactivity in the rat medial prefrontal cortex and shell region of the nucleus accumbens, but only marginally in the motor dorsolateral striatum, indicating a selective limbic site of action. In conclusion, the present data identify cis-8-OH-PBZI as having preference for the dopamine D3 receptor in vitro, and as having dopamine agonist activity and limbic sites of action in vivo.


The molecular properties of the sulfonylurea receptor 2 (SUR2) subunits of KATP channels expressed in urinary bladder were assessed by polymerase chain reaction (PCR). This showed that SUR2B exon 17- mRNA (72%) was predominant over the SUR2B exon 17+ splice variant (28%). The pharmacological properties of both of these isoforms stably expressed in mouse Ltk-cells (L-cells) with KIR 6.2 were determined by measuring changes in membrane potential responses evoked by K+ channel openers using bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)) fluorescence. The rank order potency of a variety of structurally distinct K+ channel openers was found to be the same in both stable cell lines and compared well with guinea pig bladder cells. The potency of these compounds in the SUR2B exon 17- cells more closely resembled the potency measured in guinea pig bladder unlike the cell line containing the SUR2B exon 17+ subtype. Analysis of the displacement of [125I]A-312110 binding with the same K+ channel openers to the SUR2B exon 17- cells showed excellent correlation to those measured in guinea pig bladder. This study supports the notion that KATP channels containing SUR2B exon 17- represent a major splice variant expressed in urinary bladder smooth muscle.

We previously reported that tumor necrosis factor-[alpha] (TNF-[alpha])/cachectin suppresses lipoprotein lipase activity and its gene expression in brown adipocytes differentiated in culture. Recent evidence suggests that the effect of TNF-[alpha] over various cells is related to the enhanced production of nitric oxide (NO). The present study examined whether the suppressive effect of TNF-[alpha] on lipoprotein lipase activity is mediated by production of NO in the brown adipocytes. A reverse transcription-polymerase chain reaction (RT-PCR) assay revealed that TNF-[alpha] caused a concentration- and time-dependent expression of inducible NO synthase in brown adipocytes. Increasing concentrations of TNF-[alpha] (0.5-50 ng/ml) for 24 h resulted in a concentration-dependent decrease in lipoprotein lipase activity with reciprocal increase in nitrite production in the medium. The suppressive effect of TNF-[alpha] on lipoprotein lipase activity was significantly prevented by NO synthase inhibitors, NG-nitroarginine methyl ester (-NAME) and aminoguanidine, but not by -NAME, an inactive isomer. Furthermore, 8-bromoguanosine 3',5'-cyclic monophosphate, cell permeant cGMP, suppressed lipoprotein lipase activity and 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one, a selective inhibitor for soluble guanylate cyclase, restored the TNF-[alpha]-suppressed lipoprotein lipase activity. These results suggest that TNF-[alpha] stimulates brown adipocytes to express inducible NO synthase, followed by production of NO, which in turn mediates the suppressive effect of TNF-[alpha] on lipoprotein lipase activity. The effect of NO is mediated, at least partly, through production of cGMP.


Activation of cardiac [alpha]1-adrenoreceptors has a number of physiological effects. Ascribing these effects to a specific [alpha]1-adrenoreceptor subtype first requires the elucidation of the subtypes that are present in the tissue of interest. In the present study, mRNA transcripts for the [alpha]1A, [alpha]1B and [alpha]1D-adrenoreceptor subtypes were detected in cultured neonatal rat cardiac myocytes, using reverse transcriptase-polymerase chain reaction analysis. However, binding sites for only the [alpha]1A and [alpha]1B-adrenoreceptor subtypes were detected in cultured neonatal rat cardiac myocytes, using competition binding analysis with a variety of [alpha]1-selective receptor antagonists. Phenylephrine-stimulated phosphatidylinositol hydrolysis was inhibited by [alpha]1-selective receptor antagonists with affinities consistent with the [alpha]1A-adrenoreceptor subtype, whereas phenylephrine-induced activation of the mitogen activated protein kinase cascade was inhibited by these same antagonists with affinities more closely resembling the [alpha]1B-adrenoreceptor subtype. In the case of both signaling pathways, the [alpha]1D-selective receptor antagonist, BMY 7378, exhibited affinities suggestive of the relative absence of a [alpha]1D-adrenoreceptor subtype. Thus, despite the presence of mRNA transcripts for all three [alpha]1-adrenoreceptor subtypes, only the [alpha]1A and [alpha]1B-adrenoreceptor subtypes were expressed and functionally coupled at detectable levels in neonatal rat cardiac myocytes. Of particular interest, phenylephrine-induced activation of the mitogen activated protein kinase cascade appears to be mediated by a subtype resembling most closely the pharmacological profile of the [alpha]1B-adrenoreceptor subtype.

http://www.sciencedirect.com/science/article/B73F5-47S65Y3-1R/2/dbc01adb13578c67cefacce68cda4130

Expression of human cytochrome P450 (CYP) in heterologous cells is a means of specifically studying the role of these enzymes in drug metabolism. The complete cDNA encoding CYP3A4 (PCN1) was inserted into an expression vector containing the strong myeloproliferative sarcoma virus promoter in combination with the enhancer of the cytomegalovirus and stably expressed in V79 Chinese hamster cells. The presence of genomically integrated CYP3A4 cDNA cell clones was confirmed by polymerase chain reaction analysis. Transcription was detected by reverse transcribed polymerase chain reaction analysis. Functional expression could be demonstrated by conversion of testosterone to the specific 6[beta]-hydroxylated product. In recombinant V79 cells expressing CYP3A4 about 6% of the substrate was converted to 6[beta]-hydroxytestosterone. The metabolism of two dopaminergic ergot derivatives was investigated in live recombinant V79 cells. Both lisuride and terguride were monodeethylated.

European Journal of Pharmacology: Molecular Pharmacology (5)


http://www.sciencedirect.com/science/article/B73F6-478BPR3-F/2/181aacf2a0ff2d0be5e82c25a8d8d3d9

The expression of the [alpha]1C-adrenoceptor subtype in human and rabbit blood vessels has been analyzed using the reverse transcriptase/polymerase chain reaction technique (RT/PCR). The 20 bp primers employed were designed from the bovine [alpha]1C-adrenoceptor and flank a least conserved region -- the putative third cytoplasmic loop. RT/PCR products generated from rabbit and human brain mRNA both had 93% homology to the bovine [alpha]1C-adrenoceptor and were used as species and subtype specific probes in Southern blot analysis of vascular RT/PCR products. Poly A+ RNA was purified from the human saphenous vein and rabbit aorta, renal, pulmonary and central ear arteries and amplified by RT/PCR. Size analysis by agarose gel electrophoresis, together with Southern hybridization of the resulting cDNA products confirm the expression of the [alpha]1C-adrenoceptor in these vessels.


http://www.sciencedirect.com/science/article/B73F6-47STGW-94/2/15e4bafada4783ef385b1e3bc9976b8c
The possible mechanisms of antiproliferative effect of baicalein were studied in human T-lymphoid leukemia cells (CEM cells) and compared with those of esculetin. Baicalein, esculetin and related compounds, baicalin, wogonin, esculine and scoparone, inhibited CEM cell proliferation. Baicalein exhibited the greatest antiproliferative activity with an IC50 of 4.7 +/- 0.5 [mu]M and the maximal suppression of 91.5 +/- 1.4% in CEM cells. The protein tyrosine kinase activity in the CEM cells was significantly reduced by baicalein (10-6 - 10-4 M) and esculetin (10-4 M). Baicalein exhibited a greater inhibitory activity on the protein tyrosine kinase than did esculetin (74.1 +/- 3.3% vs. 64.6 +/- 2.8% inhibition at 10-4 M). On the other hand, the protein kinase C activity stimulated by phorbol-12-myristate 13-acetate was reduced by directly incubating with baicalein (10-6 - 10-4 M) and esculetin (10-4 M). However, the inhibitory activities on protein kinase C did not show a dose-dependency. The reverse transcription-polymerase chain reaction analysis of platelet-derived growth factor-A (PDGF-A) and transforming growth factor-[beta]1 (TGF-[beta]1) messenger RNA levels demonstrates that baicalein and esculetin reduced the PDGF-A mRNA level, but less affected the TGF-[beta]1 mRNA. Baicalein exhibited the greater reduction on the expression of PDGF-A mRNA than did esculetin. It is suggested that baicalein and esculetin may affect cell proliferation by direct inhibition of growth-related signal, protein tyrosine kinase, as well as reduction of mRNA expression of growth factor, platelet-derived growth factor.


http://www.sciencedirect.com/science/article/B73F6-47STG39-1P/2/a7f7de9f8056974e59d0ec508b27f03b

Rat glutamate receptors have been shown to be expressed as two developmentally regulated, alternatively spliced isoforms. We have investigated the expression of these isoforms of GluRA and GluRB in the human hippocampus. The expression pattern of the mRNAs coding for these subunits does not correspond to that in the rat hippocampus, both isoforms being preferentially expressed in the dentate gyrus and CA1 regions, with lower expression in CA3, with the exception of GluRB flop, where hybridization in CA3 is only lower than in dentate gyrus. Cloning of cDNA from human frontal cortex has also revealed that the two isoforms of human GluRB have virtual nucleotide sequence identity with the alternative exons in the rat, confirming the usefulness of oligonucleotides complementary to the rat cDNAs as probes for these receptor subunits in human neuropsychiatric disorders.


http://www.sciencedirect.com/science/article/B73F6-47STG5J-2T/2/6c8e0609e74e5a639b2fe67e9cc04c0

Chronic administration (twice a day for three days and on the morning of the fourth day) of SR 46349B (trans-4-[(3Z)3-(2-dimethylaminoethyl)oxyimino-3-(2-fluorophenyl)propen-1-yl]phenol hemifumarate) (10mg/kg, orally), a selective 5-HT2 receptor antagonist, caused 24 h later a marked increase (+ 42%) of the maximum binding capacity of [3H]ketanserin in rat brain cortical membranes without change in its affinity constant. Further, administration of the 5-HT2 receptor agonist, (+/-)-DOI((+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) (1 mg/kg, i.p.), produced in chronic SR 46349B treated rats a significant increase in the amount of [3H]-inositol phosphate compared to corresponding controls. In addition, subacute administration of SR
46349B caused a 2-fold increase in the head-twitch response to (+/-)-DOI (0.5 mg/kg, i.p.). This enhanced response was blocked by an acute administration of ritanserin (6-{2-[bis(4-fluorophenyl)methylene]-1-piperidinyl}ethyl)-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one) (10 mg/kg). Finally, a significant enhancement (+ 29%) of 5-HT2 receptor mRNA levels was observed in the cortex. Taken together, these data showed that an up-regulation of 5-HT2 receptors occurred in rats following repeated treatment with a selective 5-HT2 receptor antagonist. The effects of SR 46349B on 5-HT2 receptors might implicate pre-translational regulation.


http://www.sciencedirect.com/science/article/B73F6-47S63NN-1W2/77800af4a4249ba508413efa378cb4cf

Benzodiazepines modulate [gamma]-aminobutyric acid (GABA)-evoked chloride currents through a specific binding site at the GABAA receptor-chloride channel complex. The heterogeneity of diazepam-sensitive benzodiazepine binding sites (type I and type II) has been identified by pharmacological approaches both with native receptors and recombinant receptors coexpressing [alpha], [beta] and [gamma] subunits. In addition, two distinguishable diazepam-insensitive benzodiazepine sites are found, spatially distributed between cerebral cortical and cerebellar regions. Coexpression of [alpha]6 with [beta]2 and [gamma]2L subunits creates a pharmacologically similar benzodiazepine receptor to the diazepam-insensitive site observed in cerebellum, however, there is no evidence regarding the possible subunit combination forming the DI site in cerebral tissues. Here we report the cloning of the human [alpha]4 cDNA and its pharmacology by coexpression of this [alpha]4 subunit with [beta]2 and [gamma]2L subunits. This recombinant receptor complex showed a high affinity for the previously described benzodiazepine partial agonist bretazenil, the pyrazoloquinoline compounds CGS-9895 and CGS-9896, as well as the inverse agonists DMCM (methyl 6,7-dimethoxy 4-ethyl-[beta]-carboline-3-carboxylate) and Ro15-4513 as determined by [3H]Ro15-4513 binding. However, it is insensitive to the benzodiazepine type I selective compounds CL218,872 (3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-b]pyridazine) and zolpidem as well as the benzodiazepine full agonists diazepam, halazolam and midazolam. In addition, the benzodiazepine receptor ligands DMCM, [beta]-CCE ([beta]-carboline-3-carboxylate ethyl ester), [beta]-CCM ([beta]-carboline-3-carboxylate methyl ester), FG-7142, CGS-9895 and CGS-9896 showed 7 to 10 times higher affinity for [alpha]4[beta]2[gamma]2L than for [alpha]6[beta]2[gamma]2L. The pharmacology of the [alpha]4[beta]2[gamma]2L receptor complex appears to resemble those of the diazepam-insensitive site found in the cerebral cortex. Our study thus suggests that this subpopulation of diazepam-insensitive GABAA receptors may be composed of [alpha]4[beta]2[gamma]2L subunits.

European Journal of Vascular and Endovascular Surgery (2)

Objective
Serological studies have suggested that one of the risk factors for aneurysm development is C. pneumoniae infection. The purpose of this study was to evaluate whether there is an association between the presence of C. pneumoniae DNA in aneurysms and ruptured Abdominal Aortic Aneurysms.

Methods
Aortic walls were collected consecutively from 30 patients with intact AAA, 16 patients with ruptured AAA and 19 healthy organ donors (control). Purified DNAs from all aortas were analyzed for the presence of C. pneumoniae DNA in parallel by polymerase chain reaction-enzyme immunoassay (PCR-EIA) and agarose gel electrophoresis. PCR-EIA has a high sensitivity in detecting low DNA copy number in clinical atherosclerotic samples.

Results
C. pneumoniae DNA was detected more frequently in patients with aneurysms, particularly with ruptured aneurysms. The incidence of positive C. pneumoniae DNA was 73.3% in intact AAA and 10.5% in control aortas, with the highest frequency in ruptured AAA (100%).

Conclusion
Giving the high specificity and sensitivity of PCR-EIA, these findings support the association of C. pneumoniae in the pathogenesis of aneurysm development, growth and rupture.


Objectives
Photodynamic therapy (PDT) is a promising strategy to limit restenosis. PDT depletes the resident cells from the vessel wall without adventitial cell ingrowth. This study was undertaken to further explore the mechanisms by which PDT of matrix acts on key mechanisms in the development of restenosis.

Materials and Methods
Control and PDT-treated collagen type-I matrix gels were prepared. Thereafter, untreated human fibroblasts were seeded on matrix gels (n=12). Fibroblast proliferation and invasive migration were quantified by calibrated phase contrast microscopy. Fibroblast bFGF and TGF-β1 mRNA expression were analyzed using a quantitative real-time reverse transcription polymerase chain reaction.

Results
Fibroblast proliferation on PDT-treated matrix gels was reduced by 30 and 76% after 3 and 7 days, respectively (3 days: P<0.01, 7 days: P<0.001). PDT of matrix gels led to a 47% reduction of migration after 3 days and 51% after 7 days (P<0.001). PDT led to a 77% reduction of fibroblast TGF-β1 mRNA (P<0.02) and to a 79% reduction of bFGF mRNA (P<0.03). Conclusions
PDT of matrix-induced reduction of bFGF and TGF-β1 mRNA levels may be important mechanisms of reducing fibroblast proliferation and invasive migration and thus the development of restenosis. These newly identified mechanisms highlight PDT’s pleiotropic effects on the vessel wall and its potential clinical value.

European Neuropsychopharmacology (1)

The symptoms of attention deficit hyperactivity disorder (ADHD) can be treated with methylphenidate (MPH), a potent blocker of dopamine transporter (DAT). The homozygosity of the 10-repeat allele at the DAT gene (DAT1) seems to be associated with a poor response to MPH in children with ADHD. In the present study, we investigated the association between DAT density using I-123-N-(3-iodopropen-2-yl)-2[beta]-carbomethoxy-3beta-(4-chlorophenyl)tropane [123I]IPT single photon emission computed tomography (SPECT) and the homozygosity for 10-repeat allele at DAT1 and response to MPH in Korean children with ADHD. Eleven drug-naive children with ADHD were included in the study and treated with MPH for about 8 weeks. After the genotyping and SPECT were performed, we compared DAT density between ADHD children with and without the homozygosity for 10-repeat allele at DAT1 and investigated the correlation between the homozygosity for 10-repeat allele and response to MPH. ADHD children with 10/10 genotype (n=7) had a significantly greater increase of the DAT density in basal ganglia than the children without 10/10 genotype (n=4). We found that while only 28.6% (2/7) of the subject with 10/10 genotype showed good response to MPH treatment, 100% (4/4) of the subjects without 10/10 genotype showed good response to MPH treatment. Our findings support an association between homozygosity for 10-repeat allele at DAT1 and the DAT density assessed in vivo and correlation between the homozygosity for 10-repeat allele and poor response to MPH.


Objectives: Alpha-1-antitrypsin ([alpha]1-antitrypsin) is a major protease inhibitor controlling tissue degradation. Reduced [alpha]1-antitrypsin levels could result in a change of collagen metabolism. Previous studies have described decreased [alpha]1-antitrypsin levels in patients with Peyronie's disease. However, only a small number of patients were analyzed, and the reason for the decreased [alpha]1-antitrypsin levels remained unclear. This study investigated prospectively the levels of [alpha]1-antitrypsin in patients with Peyronie's disease, as well as genetic variation in the coding region of the [alpha]1-antitrypsin gene. Methods: [alpha]1-antitrypsin levels were determined prospectively in 94 patients with Peyronie's disease and compared to healthy controls. Analysis of the [alpha]1-antitrypsin gene (S, Z variants; single nucleotid polymorphisms [SNPs]: T-395A, M2, M3, G6118A) was done in 141 Peyronie's patients including 43 patients with investigated [alpha]1-antitrypsin serum levels and compared to healthy controls. Results: In patients with Peyronie's disease, the [alpha]1-antitrypsin levels seemed to be decreased significantly compared to healthy controls. However, in the age matched approach no significant differences occurred. Moreover, a significant (p Conclusions: The results of this study do not indicate a significant association between Peyronie's disease and decreased [alpha]1-antitrypsin levels. Low [alpha]1-antitrypsin levels in Peyronie's patients are, rather, an age-related phenomenon, as revealed by the comparison with aged matched healthy controls. The decrease of the [alpha]1-antitrypsin serum level with increasing age has not been described before.

http://www.sciencedirect.com/science/article/B6X10-479M4FH-2/2/a8c457119184bf55a9f56441a172325

Objectives: Osteocalcin is a vitamin-K dependent protein which is related to the metabolism of bone and calcium. The formation or progression of prostate cancer is presumed to be associated with the osteocalcin gene. The most frequently seen polymorphism is HindIII which is located at the promoter region. HindIII is therefore a possible genetic marker in the search for the association between prostate cancer and normal control subjects.

Methods: In our study, a normal control group of 132 healthy people and 96 patients with prostate cancer were examined. The polymorphism was seen following polymerase chain reaction (PCR) based restriction analysis.

Results: The result revealed significant differences between normal individuals and cancer patients (p=0.034) and the distribution of the "CC" homozygote in the control group was higher than that in the patient group. No statistical differences were found in clinical staging and grading. The 54 patients who received hormone therapy were further categorized into response and non-response groups, statistical differences between these two groups were revealed (p=0.007, Fisher's exact test).

Conclusions: Based on our results, we conclude that the HindIII polymorphism of the osteocalcin gene is a suitable genetic marker of prostate cancer which can be used in the prediction of the outcome of patients who receive hormone therapy.


http://ecam.oupjournals.org/cgi/content/abstract/neh077v1

We have established an allergic dermatitis model in NC/Nga mice by repeated local exposure of mite antigen for analyzing atopic dermatitis. We examined how four Kampo medicines, Juzen-taiho-to, Hochu-ekki-to, Shofu-san and Oren-gedoku-to, on the dermatitis model to obtain basic information on their usefulness for treating atopic dermatitis. Mite antigen (Dermatophagoides farinae crude extract) solution at a concentration of 10 mg/ml was painted on the ear of NC/Nga mice after tape stripping. The procedure was repeated five times, at 7 day intervals. An apparent biphasic ear swelling was caused after the fourth and fifth antigen exposures with elevated serum IgE levels and accumulation of inflammatory cells. In the cervical lymph nodes and ear lobes, the five procedures of antigen exposure induced interleukin-4 mRNA expression but reduced interferon-\(\gamma\) mRNA expression. Oral administration of all four Kampo medicines inhibited the formation of ear swelling and inflammatory cell accumulation. Juzen-taiho-to and Hochu-ekki-to apparently prevented the elevation of serum IgE level. Furthermore, the four Kampo medicines showed a tendency to prevent not only the increase in interleukin-4 mRNA expression but also the decrease in interferon-\(\gamma\) mRNA expression. The present results indicate that Juzen-taiho-to, Hochu-ekki-to, Shofu-san and Oren-gedoku-to may correct the Th1/Th2 balance skewed to Th2, and this activity helps inhibit dermatitis in NC/Nga mice. The ability of the Kampo medicines to correct the Th1/Th2 balance seems to underlie their effectiveness in treating atopic dermatitis.
We have investigated the incidence of the C677T and A1298C methylene tetrahydrofolate reductase (MTHFR) gene single nucleotide polymorphisms (SNPs) in the South Indian Tamil Nadu population with a total number of 72 individuals. The MTHFR genotyping was performed using the polymerase chain reaction followed by restriction enzyme analysis. Homozygosity for the MTHFR A1298C SNP was detected in 15.3% (11/72) of the individuals tested, and 47.2% (34/72) were heterozygous for this SNP. Homozygosity for the C677T MTHFR SNP was detected in 1.38% (1/72), and the frequency of the C677T heterozygotes was 18.1% (13/72). When we analyzed the combined frequency of the two SNPs, the frequency of double heterozygosity was 19.6%, and the frequency of double homozygosity was completely absent among the study group. The ‘C’ allele frequency for MTHFR A1298C was 0.389, and the ‘T’ allele frequency for C677T mutation was 0.104. Out of the 72 individuals included in the study, 52 were acute myocardial infarction (AMI) patients and 20 were healthy individuals with no documented history of heart disease. The results of this study indicate that the MTHFR A1298C SNP is more prevalent among the Tamilians when compared to the MTHFR C677T SNP, suggesting a possible role of MTHFR A1298C in the pathogenesis of heart diseases.

Dendritic cells (DCs) consist of a heterogeneous population of hematopoietic cells characterized by their unique dendritic morphology, their efficient antigen-presenting capability to activate naive CD4+ and CD8+ T cells, and their lack of lineage specific markers. Functional properties comparing umbilical cord blood monocyte-derived and umbilical cord blood stem cell-derived DCs have not yet been investigated. CD14+ monocytes and CD34+ stem cells were isolated from human umbilical cord blood and were induced to differentiate into dendritic cells using 100 ng/mL granulocyte-macrophage colony stimulating factor (GM-CSF), 25 ng/mL IL-4, 2.5 ng/mL tumor necrosis factor-α (TNF-α), 100 ng/mL GM-CSF, 25 ng/mL stem cell factor, and 2.5 ng/mL TNF-α, respectively. Flow cytometric analysis revealed that the 14-day-old dendritic cells were CD80+, CD86+, CD83+, CD54+, CD1a+, CD11b+, CD11c+, HLA-DR+, CD34−, CD3−, CD19−, CD14−, and CD16−. Reverse transcription polymerase chain reaction was employed to detect expression of mRNA for CD80 and CD86. Differentiating monocytes initially expressed CD86 while CD80 appeared on day 2. Differentiating stem cells expressed CD80 and CD86 on day 2 of culture. The surface expression of CD80 and CD86 was studied over the course of differentiation. Mixed lymphocyte reaction was employed to evaluate the two types of lineage-derived DCs. Prior to the functional assay, CD14+ and CD34+ derived DCs were stimulated for 18 h with 0.1 mg/mL and 1.0 mg/mL E. coli lipopolyssacharide, respectively. Monoclonal
antibodies (mabs) to CD80 and CD86 were employed to assess their costimulatory roles. A decrease of stimulation as depicted by decreased T cell activation was significant with mabs to both CD80 and CD86 on monocyte-derived DCs while only mabs to CD86 induced decreased T cell activation by stem cell-derived DCs. The varied functional role of CD80 and CD86 costimulatory molecules is associated with DC differentiation from distinct cord blood isolated hematopoietic lineages. These studies demonstrate that DC association with distinct hematopoietic lineages is of relevance in transplantation and vaccine therapies.


http://www.sciencedirect.com/science/article/B6WFB-49H1JWG-1/2/ef3e5e05b82f674896d346334774ac02

Dendritic cells (DCs) consist of a heterogeneous population of hematopoietic cells characterized by their unique dendritic morphology, their efficient antigen-presenting capability to activate naive CD4+ and CD8+ T cells, as well as their lack of lineage-specific markers. Functional properties comparing umbilical cord blood monocyte-derived and umbilical cord blood stem cell-derived DCs have not yet been investigated. Human umbilical cord blood CD14+ monocytes and CD34+ stem cells were induced to differentiate into dendritic cells using 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF), 25 ng/mL interleukin (II)-4, 2.5 ng/mL tumor necrosis factor [alpha] (TNF-[alpha]) and 100 ng/mL GM-CSF, 25 ng/mL stem cell factor, and 2.5 ng/mL TNF-[alpha], respectively. Differentiated dendritic cells were CD80+, CD86+, CD83+, CD54+, CD1a+, CD11b+, CD11c+, HLA-DR+, CD34-, CD3-, CD19-, CD14-, and CD16-. Reverse transcription polymerase chain reaction revealed that differentiating monocytes initially expressed CD86 mRNA while CD80 mRNA appeared on Day 2. Differentiating stem cells expressed both CD80 and CD86 mRNA on Day 2 of culture. Mixed lymphocyte reaction was employed to evaluate the two types of lineage-derived DCs. Monoclonal antibodies (mabs) to CD80 and CD86 were employed to assess their costimulatory roles. CD14 and CD34 derived DCs prior to the functional assay were stimulated for 18 h with 0.1 and 1.0 mg/mL Escherichia coli lipopolyssacharide, respectively. A decrease in stimulation as depicted by decreased T-cell activation was significant with mabs to both CD80 and CD86 on monocyte-derived DCs while only mabs to CD86 induced decreased T-cell activation by stem cell-derived DCs. The varied functional role of CD80 and CD86 costimulatory molecules is associated with DC differentiation from distinct cord blood-isolated hematopoietic lineages. These studies demonstrate that DC association with distinct hematopoietic lineages is of relevance in transplantation and vaccine therapies.


http://www.sciencedirect.com/science/article/B6WFB-4BMC81C-5/2/a846c00b2a2ee8726e469cc50c5506f3

Whole blood samples of known methylene tetrahydrofolate reductase (MTHFR) genotypes from 24 individuals were examined at site C677T. Their amplified DNA products were assessed by two-color fluorescence cross-correlation measurements and agarose gel electrophoresis/capillary gel electrophoresis. DNA subpopulations were identified which were not associated with the proper genotype by primer combinations and cycling conditions called multiplexes. We confirmed that DNA analysis by two-color fluorescence cross-correlation measurements allowed the detection of fluorescence signals specifically associated with the proper genotypes in a mixture of
amplified nontarget DNA molecules without DNA sizing. The measurement approach does not require complex, follow-up mathematical analysis and is applicable to any single nucleotide polymorphisms. The simple immunogenetic model showed how the approach works to reveal specific DNA target by preventing detection of nontarget DNA. Under those experimental conditions, a new ultrasensitive, and specific method for clinical immunologists is born.


http://www.sciencedirect.com/science/article/B6WFB-4C47KRR-1/2/d7abc59ca8a861de01d7afcf237ffd

Members of the inhibitor of apoptosis protein (IAP) family, including survivin, have been reported to be expressed in many tumors. However, their expression in esophageal cancer has not been clarified completely. We investigated the expression of mRNA for IAP family proteins in samples from esophageal cancers and their adjacent normal mucosa tissues by real-time quantitative RT-PCR. The survivin expression in esophageal cancer was significantly higher than that in normal mucosa (P P P < 0.05). Immunohistochemical staining demonstrated cytoplasmic as well as nuclear expression of survivin in esophageal cancer, and further, in situ hybridization analysis demonstrated cytoplasmic expression of mRNA for survivin. The results suggest that the expression of IAP family proteins, especially survivin, may be associated with the biological character of esophageal cancer, such as apoptosis.

Experimental and Toxicologic Pathology (1)


Brown Norway (BN) and Fischer 344 (F344) rats were exposed to aerosol of 1% ovalbumin (OVA) solution for 30 min at 1 week after the second sensitization with 1 mg of OVA at 2-week intervals. Changes in the histology and expression of cytokines and chemokines in the lung were examined for up to 96 h after the exposure. The lung weight significantly increased in BN rats but not in F344 rats. Histologically, in the lung of BN rats, multiple foci of hemorrhage in the alveolar space with infiltration of eosinophils and macrophages in the surrounding alveolar septa were first observed. Thereafter, granulomatous lesions developed in the preexisting hemorrhagic foci, finally resulting in formation of multiple eosinophilic granulomas. On the other hand, in F344 rats, infiltration of eosinophils and macrophages was observed around the vessels and bronchi. Thereafter it progressed gradually, resulting in mild thickening of alveolar septa. The levels of Th1- (interferon-[gamma] and interleukin 2 (IL-2)) and Th2-related cytokines (IL-4 and IL-5) and chemokines (eotaxin and monocyte chemoattractant protein-1) mRNAs measured by reverse transcription-polymerase chain reaction method were elevated in the lung of both strains, and the levels were higher in BN rats than in F344 rats. These results suggest that BN rats are more sensitive to OVA-sensitization/inhalation than F344 rats and that the difference in the severity of lung lesions between BN and F344 rats may reflect the difference in the expression levels of cytokines and chemokines between these two strains.
Reproduction and development are highly dependent on apoptosis to balance the proliferation that necessarily occurs during these processes. How the absence of two apoptotic factors in mice would affect reproduction and development was examined. Given previous reports of increased neural tube defects in p53-/- female fetuses, decreased fertility in gld female mice, and altered spermatogenesis in both p53 and gld male mice, the possibility that these phenotypes might be enhanced by the elimination of a second apoptotic factor was investigated. The reproductive vigor and the health of offspring were monitored during the production of the new double-deficient strain (FasL-/-p53-/-) for any changes from the reported phenotypes. Thus, any unusual phenotypes that could lead to new models for studying mechanisms of health and disease would be identified. Double-deficient male offspring appeared healthy and occurred at expected frequencies. Additionally, spermatogenesis and male fertility were unaffected by the gene deficiencies. On the other hand, FasL+/+p53-/- and FasL-/-p53-/- female mice were susceptible to increased malformations and post-natal death. These abnormalities were consistent with previous reports of neural tube defects in p53-/- female mice. Fertility rates were also significantly decreased in p53-/- female mice that lived to be adults, an observation not previously reported. Finally, the absence of both Fasl and p53 led to dystocia in pregnant female mice, suggesting that the two genes play complementary roles in parturition. Therefore, although male mouse development and reproduction remained unaffected by p53 and FasL deficiencies, female mouse development was adversely affected by the absence of p53, and no live litters were born to female mice with the combined absence of both Fasl and p53. In this report, we suggest a potential mechanism involving corpora luteal regression to explain this defect in parturition in FasL-/-p53-/- female mice.


Flavonoids isolated from cocoa have biological activities relevant to oxidant defenses, vascular health, tumor suppression, and immune function. The intake of certain dietary flavonoids, along with other dietary substances such as tocopherols, ascorbate, and carotenoids, is epidemiologically associated with a reduced risk of cardiovascular disease. Flavonoids have also been shown to modulate tumor pathology in vitro and in animal models. We took advantage of the conserved sequences found in tyrosine kinases to study the influence of cocoa fractions and controls on gene expression. We report that the pentameric procyanidin (molecular weight of 1442 daltons) fraction isolated from cocoa was a potent inhibitor of tyrosine kinase ErbB2 expression, a receptor important in angiogenesis regulation. Consistent with this primary observation, the cocoa flavonoid fraction also suppressed human aortic endothelial cell (HAEC) growth and decreased expression of two tyrosine kinases responsive to ErbB2 modulation,
namely VEGFR-2/KDR and MapK 11/p38(beta)2. These inhibitory effects were observed when HAECs were treated with the flavonol fraction (molecular weight 280 daltons) isolated from cocoa, which comprise the structural subunits from which the procyanidin flavonoid subclass is biosynthetically constructed. Down-regulation of ErbB2 and inhibition of HAEC growth by cocoa procyanidins may have several downstream implications, including reduced vascular endothelial growth factor (VEGF) activity and angiogenic activity associated with tumor pathology. These results suggest specific dietary flavonoids are capable of selectively inhibiting ErbB2 and therefore may offer important insight into the design of therapeutic agents that target tumors overexpressing ErbB2.


http://www.ebmonline.org/cgi/content/abstract/228/3/261

Traumatic brain injury (TBI) causes excess release of neurotransmitters, such as glutamate, and increases intracellular calcium levels. Elevated levels of calcium, and perhaps other intracellular second messengers, as a result of TBI can alter the expression of many genes. The protein products of some of these genes may be signals for TBI-associated memory dysfunction. Therefore, identification of genes whose expression is altered after TBI in the hippocampus, a structure in the medial temporal lobe that plays a critical role in memory formation and storage, and elucidation of the role(s) of their protein products may shed light on the molecular mechanisms underlying TBI-elicited memory dysfunction. The prodynorphin gene is expressed in hippocampal granule cells, and its expression has been reported to be enhanced as a result of elevated intracellular calcium. The prodynorphin protein is proteolytically cleaved to generate multiple dynorphin peptides, which can modulate neurotransmitter release through the activation of presynaptic (kappa) opioid receptors. In this study, we report that 1) TBI transiently increases prodynorphin mRNA in the hippocampus, 2) dynorphin peptide immunoreactivity is enhanced for up to 24 hr after TBI and 3) intracerebroventricular infusion of the (kappa) receptor antagonist nor-binaltorphimine (nor-BNI) impairs subsequent performance in a spatial memory task. These results suggest that dynorphin action may serve a beneficial role after TBI.


http://www.ebmonline.org/cgi/content/abstract/227/1/26

Metallothioneins (MT) are low-molecular-weight, heat-stable, cysteine-rich proteins with four isoforms. MT-I and MT-II are ubiquitous and are induced by oxidative, physical, and chemical stress. MT-I is an efficient scavenger of superoxide (*O2) and hydroxyl ion (OH-). We have demonstrated that *O2 and hypohalous acid can cause an increase in glomerular albumin permeability (Palb) in vitro. The purpose of this study was to document the protective effect of MT gene product on the *O2-mediated increase in Palb. Glomeruli from Sprague-Dawley rats in 4% BSA medium were incubated for 4 hr at 37(degrees)C in duplicate tubes. Each set contained glomeruli alone or with 5 {micro}M Cd++, 0.3 mM Spermine-NONOate (NO donor), 0.3 mM Sulfo-NONOate (nitrous oxide donor), 0.6 mM SNP (nonspecific NO donor) and SNP + carboxy-PTIO (10 mg/ml). After incubation, one set of tubes was used to isolate total RNA for the measurement of the mRNA levels of MT-I by reverse transcriptase polymerase chain reaction (RT-PCR). Duplicate tubes were incubated for an additional 10 min with 10 nM of *O2, and Palb was measured using video microscopy. RT-PCR of total RNA from Cd++ and Spermine-NONOate treated glomeruli revealed a 2-fold induction of MT-I expression at the mRNA level. *O2 caused a
significant increase in Palb (0.8 {+/-} 0.06 vs. control 0.0 {+/-} 0.12, P < 0.05) and induction of MT-I in glomeruli by Cd++ or by Spermine-NONOate blocked this effect (0.21 {+/-} 0.12 and 0.24 {+/-} 0.19, respectively, P < 0.05 vs. *O2). In contrast, Sulfo-NONOate and SNP did not induce mRNA for MT-I in glomeruli and did not provide protection against *O2-mediated increase in Palb. We conclude that MT-I gene products may play an important role in protecting the glomerular filtration barrier from the injury induced by reactive oxygen species in immune and/or nonimmune renal diseases.

http://www.ebmonline.org/cgi/content/abstract/228/5/550

Monocytes play key roles both in innate and adaptive antigen-specific immunity and they constitute critical components of the immune responses. Although most of the monocyte-derived cytokines exhibit proinflammatory functions in vivo, heme oxygenase-1 (HO-1), an inducible heme-degrading enzyme, exerts potent anti-inflammatory effect through production of carbon monoxide and bilirubin. We compared HO-1 production by monocytes in vivo in various acute inflammatory illnesses and in normal controls. Freshly isolated monocytes produced little HO-1 as detected by immunohistochemistry, but it was rapidly induced in vitro upon stimulation. HO-1 production by monocytes was selective because it was not induced in other leukocyte populations, including granulocytes and lymphocytes. Monocytes from acute inflammatory illnesses, such as Kawasaki disease and acute infectious diseases, viral or bacterial, produced significant levels of HO-1, as detected by flow cytometry, immunohistochemistry, and reverse transcription polymerase chain reaction. Quantitative analysis of HO-1 mRNA expression by real-time polymerase chain reaction revealed that monocytes from controls exhibited low, but significant levels of HO-1 mRNA, indicating that circulating monocytes produce HO-1 constantly, in response to basal level of oxidative stress encountered daily. Significantly elevated HO-1 mRNA levels seen in acute inflammatory illnesses suggest that monocyte HO-1 production serve as potent anti-inflammatory agent to control excessive cell or tissue injury in the presence of oxidative stress and cytokinemia.

http://www.sciencedirect.com/science/article/B6WFC-4DPGWYJ-1/2/39ceae41ca1f1deb9f7a1c57d5d6d5c4

The protein dlk, encoded by the Dlk1 gene, belongs to the Notch epidermal growth factor (EGF)-like family of receptors and ligands, which participate in cell fate decisions during development. The molecular mechanisms by which dlk regulates cell differentiation remain unknown. By using the yeast two-hybrid system, we found that dlk interacts with Notch1 in a specific manner. Moreover, by using luciferase as a reporter gene under the control of a CSL/RBP-Jk/CBF-1-dependent promoter in the dlk-negative, Notch1-positive Balb/c 14 cell line, we found that
addition of synthetic dlk EGF-like peptides to the culture medium or forced expression of dlk decreases endogenous Notch activity. Furthermore, the expression of the gene Hes-1, a target for Notch1 activation, diminishes in confluent Balb/c14 cells transfected with an expression construct encoding for the extracellular EGF-like region of dlk. The expression of Dlk1 and Notch1 increases in 3T3-L1 cells maintained in a confluent state for several days, which is associated with a concomitant decrease in Hes-1 expression. On the other hand, the decrease of Dlk1 expression in 3T3-L1 cells by antisense cDNA transfection is associated with an increase in Hes-1 expression. These results suggest that dlk functionally interacts in vivo with Notch1, which may lead to the regulation of differentiation processes modulated by Notch1 activation and signaling, including adipogenesis.


Activity of the independently regulated human c-myc P0 promoter has been associated with the undifferentiated status of leukemia cells as well as the hormone-independent proliferation of breast cancer cells. The P0 transcript is distinguished from the predominant P1 and P2 c-myc mRNAs by an ~639-nucleotide extension of the 5'-untranslated region. We hypothesized that this complex 5'-untranslated RNA sequence unique to the P0 transcript may contribute significantly to the composite regulation of the c-myc locus and that enforced intracellular synthesis of the isolated P0 5'-UTR, out of its native sequence context, might amplify or dominantly interfere with its normal regulatory function. Human tumor (HeLa) cells in which the isolated P0 5'-UTR was ectopically expressed displayed a dramatic decrease in anchorage-independent proliferation. Furthermore, P0 5'-UTR-expressing HeLa cells failed to form tumors when inoculated into SCID mice. This loss of tumorigenicity was associated with increases in levels of the c-Myc1 (p67) and c-Myc2 (p64) proteins and a 3- to 5-fold elevation of spontaneous apoptotic index. These results demonstrate that an isolated 5'-untranslated RNA sequence can be attributed potent in trans gene-regulatory and phenotype-altering capabilities and that extrinsic alterations in c-myc regulation can be utilized to reestablish the natural proapoptotic (tumor suppressor) activities associated with this protooncogene.


Among the more than 30 different human proteins of the cytokeratin (CK) group of intermediate filament (IF) proteins, the significance of the epidermal polypeptide CK 2 (Moll et al., 1982, Cell 31, 11-24) has been repeatedly questioned in the literature. Here, we show, by in vitro translation and protein gel electrophoresis, that human epidermis from various body sites does indeed contain relatively large amounts of mRNA encoding a distinct polypeptide comigrating with native epidermal CK 2. We also report the isolation of a cDNA clone encoding the complete sequence of CK 2, which is a type II CK different from--but related to--epidermal CKs 1 and 5 on the one hand and corneal CK 3 on the other. The mRNA of ~2.6 kb encodes a polypeptide of 645 amino acids and Mr 65,852, in good agreement with the value of 65.5 kDa previously estimated from gel electrophoresis. This human CK, the largest so far known, displays several features typical of CKs of stratified epithelia, including numerous repeats of glycinerich tetrapeptides in the head and tail domains. Northern blot and in situ hybridizations have shown that CK 2 is expressed
strictly suprabasally, usually starting in the third or fourth cell layer of epidermis, and this was confirmed at the protein level by immunohistochemistry using CK 2-specific antibodies. The protein has been detected as a regular epidermal component in skin samples from different body sites, albeit as a minor CK in "soft skin" (e.g., breast nipple, penile shaft, axilla), but not in foreskin epithelium and in other epithelia, in squamous metaplasias and carcinomas, or in cultured cell lines derived therefrom. We propose that CK 2 is a late cytoskeletal IF addition synthesized during maturation of epidermal keratinocytes which probably contributes to terminal cornification.


http://www.sciencedirect.com/science/article/B6WFC-4B3NKY2-3/2/8466b51d030c5c8694f466b1536cbf18

HIPK2 is a member of a novel family of nuclear serine-threonine kinases identified through their ability to interact with the Nkx-1.2 homeoprotein. The physiological role of these kinases is largely unknown, but we have recently reported on the involvement of HIPK2 in the induction of apoptosis of tumor cells after UV stress through p53 phosphorylation and transcriptional activation. Here, we demonstrate that the chemotherapeutic drug cisplatin increases HIPK2 protein expression and its kinase activity, and that HIPK2 is involved in cisplatin-dependent apoptosis. Indeed, induction of HIPK2 and of cell death by cisplatin are efficiently inhibited by the serine-threonine kinase inhibitor SB203580 or the transduction of HIPK2-specific RNA-interfering molecules. HIPK2 gene silencing efficiently reduces the p53-mediated transcriptional activation of apoptotic gene promoters as well as apoptotic cell death after treatment with cisplatin. These findings, along with the involvement of p53 phosphorylation at serine 46 (Ser46) in the transcriptional activation of apoptotic gene promoters, suggest a critical role for HIPK2 in triggering p53-dependent apoptosis in response to the antineoplastic drug cisplatin.


http://www.sciencedirect.com/science/article/B6WFC-4DXRXW1-7X/2/2b20c832e342add91d0a39ed03f42100

The laminin-like protein merosin was purified from human placenta in intact form and as pepsin fragments and compared to laminin in heparin affinity chromatography and cell binding assays. Intact merosin and a small fragment of merosin comprising the last two repeats of the heavy chain g domain bind to heparin. Intact merosin and large pepsin fragments of merosin, but not the small C-terminal fragment, mediate the attachment and spreading of several types of cells and promote neurite outgrowth from neuronal cells similar to laminin and its corresponding fragments. Cells with various integrin-type receptors for laminin attached equally well to merosin and laminin, suggesting that several of the known laminin binding receptors also bind to merosin. Antibodies to the [beta]1 subunit of integrins inhibited neurite outgrowth on merosin as well as on laminin, confirming the involvement of integrin-mediated interaction of cells with both merosin and laminin. Schwannoma cells, which have previously been shown to produce a laminin-like, neurite-promoting factor, synthesize merosin in vivo and in vitro as shown by protein and mRNA analysis. The results suggest that merosin, which is the more abundant basement membrane protein in the laminin family, has properties very similar to laminin despite differences in the structure of the heavy chain. Furthermore, merosin may be identical to or a component of the neurite-promoting factors previously reported from heart, muscle, and Schwann cells.

http://www.sciencedirect.com/science/article/B6WFC-4D1V75F-2/2/936be1b8235bdf76566f31a3baf3445

The presence of many laminin receptors of the \([\beta]1\) integrin family on most cells makes it difficult to define the biological functions of other major laminin receptors such as integrin \([\alpha]6[\beta]4\) and dystroglycan. We therefore tested the binding of a \([\beta]1\) integrin-null cell line GD25 to four different laminin variants. The cells were shown to produce dystroglycan, which based on affinity chromatography bound to laminin-1, -2/4, and -10/11, but not to laminin-5. The cells also expressed the integrin \([\alpha]6[\beta]4A\) variant. GD25 \([\beta]1\) integrin-null cells are known to bind poorly to laminin-1, but we demonstrate here that these cells bind avidly to laminin-2/4, -5, and -10/11. The initial binding at 20 min to each of these laminins could be inhibited by an integrin \([\alpha]6\) antibody, but not by a dystroglycan antibody. Hence, integrin \([\alpha]6A[\beta]4A\) of GD25 cells was identified as a major receptor for initial GD25 cell adhesion to three out of four tested laminin isoforms. Remarkably, cell adhesion to laminin-5 failed to promote cell spreading, proliferation, and extracellular signal-regulated kinase (ERK) activation, whereas all these responses occurred in response to adhesion to laminin-2/4 or -10/11. The data establish GD25 cells as useful tools to define the role integrin \([\alpha]6A[\beta]4A\) and suggest that laminin isoforms have distinctly different capacities to promote cell adhesion and signaling via integrin \([\alpha]6A[\beta]4A\).


http://www.sciencedirect.com/science/article/B6WFC-4C604NK-3/2/5de6b31a4856bedd5013a403abf1bed9

The critical factors in the regulation of telomere length are not yet clearly defined. Telomerase is a key player in telomere elongation, although previous studies have shown that telomeres are differentially elongated after telomerase reconstitution. Moreover, a clear relation between the level of telomerase activity and telomere length was not observed. To investigate which factors are critical in telomere length regulation, we generated 24 telomerase-reconstituted primary human fibroblast clones. In these clones, in vitro telomerase activity level is clearly related to telomere length. High levels of telomerase activity are associated with longer telomeres and better telomere maintenance over time. The correlation coefficient, however, indicates that the level of telomerase activity is not the only factor in the regulation of telomere length. Clearly, factors that are not measured in an in vitro telomerase activity assay are involved in telomere length regulation in vivo. To investigate which telomerase components are critical in regulating telomerase activity levels, we studied expression levels of \(hTERT\) mRNA and \(hTR\). Expression is highly variable between individual clones, but not related to the level of telomerase activity or telomere length. Our results indicate that expression levels of \(hTERT\) mRNA and \(hTR\) do not regulate the activity level of the telomerase complex, suggesting posttranscriptional modification of \(hTERT\) or the presence of additional proteins that modulate telomerase enzyme activity.

Tsujie, M., S. Nakamori, et al. (2003). "Thiazolidinediones inhibit growth of gastrointestinal, biliary, and

Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]), a ligand-activated transcription factor, forms a heterodimer with retinoid X receptor [alpha] (RXR[alpha]), and its transcriptional activity is thought to be maximal in the presence of both PPAR[gamma] and RXR[alpha] ligands. Although previous studies suggested that thiazolidinediones (TZDs), known as PPAR[gamma] ligands, inhibit the growth of several types of tumor cells, the precise mechanism still remains obscure. The present study was designed to examine the effects of PPAR[gamma]/RXR[alpha] transcriptional activation on cell growth in cancer cells. We compared the effects of six types of TZDs (troglitazone, RS-1303, RS-1330, RS-1455, and RS-1456) and 9-cis RA, an RXR[alpha] ligand, on the activation of PPAR[gamma]/RXR[alpha] and the growth inhibition of six types of adenocarcinoma cell lines (MKN45, HT-29, HCT116, HuCCT1, KMP-2, and BxPC3) established from abdominal malignancies. PPAR[gamma] was expressed in all six tumor cell lines and transcriptionally functional in five of the six lines. The stronger PPAR[gamma] activator showed the stronger growth inhibitor in these five cell lines. However, no significant growth inhibitory effect of six types of PPAR[gamma] activators was observed in BxPC3 cells, which showed no significant PPAR[gamma] transactivation by these activators. Simultaneous addition of troglitazone and 9-cis RA enhanced both activation of PPAR[gamma]/RXR[alpha] and growth inhibition in several types of cancer cells. The degree of PPAR[gamma]/RXR[alpha] activation correlated with the extent of growth inhibition (r > 0.70, P < 0.05). This growth inhibition was associated with G1 cell cycle arrest and cell differentiation. These findings suggest that activation of the PPAR[gamma]/RXR[alpha] pathway plays an important role in the growth inhibition of tumor cells and that this nuclear hormone receptor may be a possible novel molecular target for treatment of tumors in humans.


Activation of fatty acid synthase (FAS) expression and fatty acid synthesis is a common event in human breast cancer. Sterol regulatory element binding proteins (SREBPs) are a family of transcription factors that regulate genes involved in lipid metabolism, including FAS. SREBP-1c expression is induced in liver and adipose tissue by insulin and by fasting/refeeding and is critical for nutritional regulation of lipogenic gene expression. In contrast, upregulation of fatty acid metabolism during in vitro transformation of human mammary epithelial cells and in breast cancer cells was driven by increased MAP kinase and PI 3-kinase signaling, which increased SREBP-1 levels. SREBP-1a was more abundant than SREBP-1c in many proliferative tissues and cultured cells and was thus a candidate to regulate lipogenesis for support of membrane synthesis during cell growth. We now show that SREBP-1c and FAS mRNA were both increased by H-ras transformation of MCF-10a breast epithelial cells and were both reduced by exposure of MCF-7 breast cancer cells to the MAP kinase inhibitor, PD98059, or the PI 3-kinase inhibitor, wortmannin, while SREBP-1a and SREBP-2 showed less variation. Similarly, the mRNA levels for FAS and SREBP-1c in a panel of primary human breast cancer samples showed much greater increases than did those for SREBP-1a and SREBP-2 and were significantly correlated with each other, suggesting coordinate regulation of SREBP-1c and FAS in clinical breast cancer. We conclude that regulation of FAS expression in breast cancer is achieved through modulation of SREBP-1c, similar to the regulation in liver and adipose tissue, although the upstream regulation of lipogenesis differs in these tissues.
Myopia is a condition in which the eye is too long for the focal length of cornea and lens. Analysis of the messengers that are released by the retina to control axial eye growth in the animal model of the chicken revealed that glucagon-immunoreactive amacrine cells are involved in the retinal image processing that controls the growth of the sclera. It was found that the amount of retinal glucagon mRNA increased during treatment with positive lenses and pharmacological studies supported the idea that glucagon may act as a stop signal for eye growth. Glucagon exerts its regulatory effects by binding to a single type of glucagon receptor. In this study, we have sequenced the chicken glucagon receptor and compared its DNA and amino acid sequence with the human and mouse homologues. After sequencing about 80% of the receptor, we found a homology between 79.4 and 75.6% on cDNA level. At the protein level, about 73% of the amino acids were identical. Moreover, the cellular localization and regulation of the glucagon receptor in the chick retina was studied. In situ hybridization studies showed that many cells in the ganglion cell layer and inner nuclear layer, and some cells in the outer nuclear layer, express the receptor mRNA. Injection of the glucagon agonist Lys17,18,Glu21-glucagon induced a down-regulation of glucagon receptor mRNA content. Since the mouse would be an attractive mammalian model to study the biochemical and genetic basis of myopia, and because recent studies have demonstrated that form deprivation myopia can be induced, the expression of preproglucagon and glucagon receptor genes were also studied in the mouse retina and were found to be expressed.


Ocular neovascularisation is the leading cause of blindness in developed countries and the most potent angiogenic factor associated with neovascularisation is vascular endothelial growth factor (VEGF). We have previously described a sense oligonucleotide (ODN-1) that possesses anti-human and rat VEGF activity. This paper describes the synthesis of lipid-lysine dendrimers and their subsequent ability to delivery ODN-1 to its target and mediate a reduction in VEGF concentration both in vitro and in vivo. Positively charged dendrimers were used to deliver ODN-1 into the nucleus of cultured D407 cells. The effects on VEGF mRNA transcription and protein expression were analysed using RT-PCR and ELISA, respectively. The most effective dendrimers in vitro were further investigated in vivo using an animal model of choroidal neovascularisation (CNV). All dendrimer/ODN-1 complexes mediated a significant reduction in VEGF expression during an initial 24 hr period (40-60%). Several complexes maintained this level of VEGF
reduction during a subsequent, second 24 hr period, which indicated protection of ODN-1 from the effects of endogenous nucleases. In addition, the transfection efficiency of dendrimers that possessed 8 positive charges (x=81[middle dot]51%) was significantly better (P=0[middle dot]0036) than those that possessed 4 positive charges (x=56[middle dot]8%). RT-PCR revealed a correlation between levels of VEGF protein mRNA. These results indicated that the most effective structural combination was three branched chains of intermediate length with 8 positive charges such as that found for dendrimer 4. Dendrimer 4 and 7/ODN-1 complexes were subsequently chosen for in vivo analysis. Fluorescein angiography demonstrated that both dendrimers significantly (P<0[middle dot]0001) reduced the severity of laser mediated CNV for up to two months post-injection. This study demonstrated that lipophilic, charged dendrimer mediated delivery of ODN-1 resulted in the down-regulation of in vitro VEGF expression. In addition, in vivo delivery of ODN-1 by two of the dendrimers resulted in significant inhibition of CNV in an inducible rat model. Time course studies showed that the dendrimer/ODN-1 complexes remained active for up to two months indicating the dendrimer compounds provided protection against the effects of nucleases.


http://www.sciencedirect.com/science/article/B6WFD-49N0DVH-1/2/3205ab62a89cfca4feb6c75eeab11ee3

Endotoxin-induced uveitis (EIU) is an animal model of acute ocular inflammation. Cytokines, chemokines, and nitric oxide (NO) have been reported to play important roles. We have determined whether heme oxygenase (HO)-1, a heat shock protein, can suppress EIU. EIU was induced by a footpad injection of lipopolysaccharide (LPS) in male Lewis rats. Hemin, an inducer of HO-1, was injected intraperitoneally 1 hr prior to the LPS injection. HO-1 and HO-2 expression in the iris-ciliary body (ICB) was studied by real time PCR and Western blot analysis. The number of infiltrating cells and the protein concentration in the aqueous humor (AqH) were evaluated by microscopy and by protein assay. The expression of inducible nitric oxide synthase (iNOS), interleukin (IL)-6, tumor necrosis factor (TNF)-[alpha], and IL-1[beta] mRNA was determined by real time PCR. The concentration of nitrate plus nitrite, and levels of IL-6 and TNF-[alpha] in the AqH were also evaluated by Griess reagents and by enzyme-linked immunosorbent assay, respectively. The expression of HO-1 mRNA and protein, induced by LPS, was enhanced significantly by pre-injection of hemin (PPP<0.001). Hemin is effective in inducing HO-1 and in reducing the ocular inflammation induced by LPS probably by down-regulating NO and pro-inflammatory cytokine expression.


http://www.sciencedirect.com/science/article/B6WFD-47YH4V8-1/2/da477e65bb8c0fbc4518feefee4befeaaba

Purpose. Previous studies in our laboratory have shown that 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE), a product of 12-lipoxygenase (12-LOX) activity, is the predominant metabolite formed in rabbit corneas after injury. The present study was undertaken to investigate the effects of epidermal growth factor (EGF), hepatocyte growth factor (HGF), and keratinocyte growth factor (KGF) on 12-LOX expression and activity. We also investigated whether 12(S)-HETE mediated the growth factor-induced proliferation of corneal epithelial cells. Methods. Rabbit corneas were stimulated with EGF, HGF, and KGF (10 ng ml-1) for different times. 12-LOX activity was assayed by incubating corneal microsomal preparations with radiolabeled arachidonic acid (AA)
as substrate. For inhibitor studies, the microsomes were pretreated with 12-LOX-specific inhibitors baicalein (BC) or cinnamyl 3,4-dihydroxy-(alpha)-cyanocinnamate (CDC). Lipid extracts were injected onto an Ultramex 5 [mu]m C18 column and radioactivity was monitored online by a Radiomatic Flo-One Beta detector. Stereochemical analysis of 12-HETE product was determined by chiral-phase HPLC. To evaluate the effects of growth factors on 12-LOX mRNA expression, mRNA was extracted at several time points (12, 24, 36, 48 hr) and subjected to real-time PCR. For 12-LOX protein expression, micromolar preparations from 24- and 48-hr incubations were analyzed by Western blot. In cell-proliferation studies, epithelial cells treated with EGF, HGF, or KGF for 24, 48, and 72 hr were measured with a CyQUANT cell-proliferation assay kit. To determine the role of growth factor-induced 12(S)-HETE synthesis on corneal epithelial cell proliferation, cells were pretreated with 12-LOX-specific inhibitors BC or CDC prior to growth-factor supplementation.

Results. Stimulation with EGF, HGF, or KGF for 12 hr induced 12-LOX mRNA expression in rabbit corneal epithelial cells. This gene induction was followed by an increase in protein expression at 24 and 48 hr and a marked increase in 12(S)-HETE synthesis when compared to untreated controls. At 24-hr incubations, KGF showed a greater capacity than did EGF and HGF to stimulate microsomal 12-LOX activity, while at 48 hr 12(S)-HETE synthesis was significantly greater in EGF-treated cells as compared to that of HGF- and KGF-treated cells. Pretreatment with 12-LOX inhibitors blocked the growth factor-induced increase in 12(S)-HETE synthesis. Stimulation with growth factors or 12(S)-HETE for 24, 48, and 72 hr produced a significant increase in corneal epithelial proliferation, which was partially inhibited by pretreatment of cells with 12-LOX-specific inhibitors.

Conclusion. These findings suggest that EGF, HGF, and KGF stimulate 12(S)-HETE production in rabbit corneal epithelial cells through gene induction of 12-LOX. Furthermore, 12(S)-HETE may play a role in regulating epithelial cell proliferation and the rate of corneal re-epithelialization following an injury.


http://www.sciencedirect.com/science/article/B6WFD-4D3B124-1/2/c6d3184a75fe087d7001bac8d59c7180

Neovascularization stimulated by IGF-1 mediated induction of vascular endothelial growth factor (VEGF) is one of the leading causes of blindness in humans. It plays a central role in the pathogenesis of proliferative diabetic retinopathy (DR), neovascular glaucoma, exudative age-related macular degeneration (AMD) and retinopathy of prematurity. Neovascularization is a multi-step process that involves complex interactions of a variety of mitogenic factors such as VEGF and IGF-I which are produced locally in the human eye by a variety of cells including retinal pigment epithelial (RPE) cells, retinal capillary pericytes, endothelial cells, Mueller cells and ganglion cells. We hypothesized that somatostatin would inhibit the IGF-1 signal transduction pathway in RPE cells, resulting in decreased VEGF production. We have observed expression of somatostatin receptor protein in retinal pigment epithelial (RPE) cells of the human eye using immunohistochemistry and have confirmed expression of somatostatin receptors in cultured human RPE cells using reverse transcriptase-PCR. IGF-1 induced a dose dependent increase in IGF-1R phosphorylation and in VEGF mRNA levels in cultured human RPE cells. Somatostatin and octreotide, a somatostatin analogue, inhibited IGF-1 receptor (IGF-1R) phosphorylation and decreased VEGF production. Both IGF-1R phosphorylation and accumulation of VEGF mRNA were inhibited by physiological levels of somatostatin and octreotide (1 nM). These results demonstrate somatostatin and octreotide mediated attenuation of both IGF-1R signal transduction and VEGF mRNA accumulation via somatostatin receptor type 2 (sst2). Furthermore, these data suggest a rationale for the use of octreotide as a prophylactic and therapeutic option in disease states that cause ocular neovascularization.

http://www.sciencedirect.com/science/article/B6WFD-47K2MTC-2/2/819cd9dfe246fe0c9c7e75f31f577f1

The aim of this study was to elucidate the expression of chemokines, their role and regulation in bacterial corneal infection using three bacterial strains (*Pseudomonas. aeruginosa* - invasive, cytotoxic and contact lens induced acute red eye strains) which have been shown to produce three distinct patterns of corneal disease in the mouse. The predominant chemokine expressed in response to all three strains was MIP-2. Prolonged expression of high levels of MIP-2 was associated with increased severity of corneal inflammation. Significantly reduced disease severity upon administration of anti-MIP-2 antibodies suggested that MIP-2 may play an important role in the pathogenesis of *Pseudomonas* keratitis at least in part by being a major chemoattractant for polymorphonuclear leukocytes (PMN) recruitment. Interestingly, the numbers of bacteria in eyes with neutralized MIP-2 activity did not decrease even though the severity of the disease was decreased. This implies PMNs as the major destructive factor in microbial keratitis. Further, neutralization of IL-1[beta] activity alone using monoclonal antibodies resulted in significant reduction of both MIP-2 and KC activity indicating that chemokine levels were regulated by IL-1[beta]. These studies demonstrate that the regulation of MIP-2 activity may be beneficial in reducing corneal damage during microbial keratitis in rodents and perhaps that regulation of the human homologue of MIP-2, IL-8, may be useful for controlling keratitis in humans.

*Experimental Gerontology*  (10)


http://www.sciencedirect.com/science/article/B6T6J-48HXT1W-3/2/0a720d1a61ba4af09f3d8bb3c2c6d951c

Adenosine (Ado), a naturally occurring autacoid, exerts cardioprotective effects against myocardial ischemia and reperfusion injury, through activation of its receptors type 1 (A1) and 2A (A2A). Since ageing involves a complex change in these effects, we evaluated A1 and A2A gene expression in left (LV) and right ventricle (RV) from 2-, 5-, 12-, and 21-month-old Sprague-Dawley rats. LV end-diastolic (EDD) and end-systolic (ESD) internal dimensions (mm) and LV fractional shortening (FS, %) were measured by M-mode echocardiography. Senescence was associated with a reduction in FS (42+/,-1, 38+/,-2, 39+/,-2 and 35+/,-2, in 2-, 5-, 12- and 21-month-old rats; ppp=0.002). Ado A1 mRNA levels were highest in 12 and 21-month-old animals in both ventricles (LV: pp=0.001). By contrast, Ado A2A gene expression was lower in the aged LV (ppThese modifications of Ado receptor gene expression and especially the increase in A1 receptor mRNA may partially explain the stronger antiadrenergic effects of Ado in the senescent heart.


http://www.sciencedirect.com/science/article/B6T6J-3V4XPND-
In this study, we have used the mRNA differential display technique to investigate the changes in gene expression that occur in the process of cellular aging. A number of cDNAs whose corresponding mRNAs are either increasingly or decreasingly expressed in senescent cells were thereby isolated. Through DNA sequencing, one of these differentially displayed mRNAs was identified as mitochondrial ADP/ATP translocase. The altered expression of ADP/ATP translocase in different stages of senescent fibroblasts was further confirmed by Northern blots and semiquantitative RT-PCR. Our results demonstrate that expression of ADP/ATP translocase is progressively decreased during the process of in vitro cellular senescence. Further analyses with MTT assays indicate that the decreased expression of ADP/ATP translocase in senescent cells is in parallel with the decline of mitochondrial functions, suggesting that altered expression of this important mitochondrial enzyme might play an active role in the process of cellular senescence.


Since there is still debate about the ability of the aged liver to regenerate, we compared some aspects of this response in young, adult and old rodents. 2, 6, 12 and 19-month-old rats were intraperitoneally injected with CCl4 (3 mg/kg) or left untreated (CT) and killed either 2 h (group A) or 24 h (group B) after intoxication. Liver injury was checked histologically and by assaying transaminases. mRNA levels of albumin (Alb), c-fos, c-myc, hepatocyte growth factor (HGF), transforming growth factor (TGF)-[alpha] and TGF-[beta]1 were also analyzed. Heat shock protein (HSP)70 gene expression was evaluated, and liver GSH content. Transaminases and histology show more damage in aged rats. Alb mRNA was reduced starting at 12 months in group A and at all ages in group B; c-fos and c-myc mRNAs reached the highest levels in 6-month-old rats and the lowest in those aged 12 and 19 months of group A. In group B, c-fos was detectable only in 6-month animals, but c-myc at all ages. HGF, TGF-[alpha] and TGF-[beta]1 mRNAs were up-regulated in treated rats, but to a lesser extent in the aged. HSP70 mRNA, absent in CT, was significantly increased at the age of 6 months, undetectable in the oldest rats in group A; in group B it was only visible in 6-month animals. GSH content was reduced with aging. In conclusion, during aging the liver regenerative machinery is preserved but its activation is reduced and delayed.


Neurons express proteins of the classical complement pathway, including C9. Both the mRNA and protein levels for C9 are sharply upregulated in brain areas affected by Alzheimer's disease (AD). Since little is known about the signals that are responsible for this upregulation, we evaluated in human SH-SY5Y neuroblastoma cells the factors which stimulate C9 production. Interferon-[gamma], phorbol myristate acetate and interleukin-6 all stimulated C9 mRNA
expression but the inflammatory cytokines tumor necrosis factor-[alpha], interleukin-1[beta], as well as the anaphylatoxin C5a and the bacterial lipopolysaccharide, were ineffective. Immunohistochemical analysis of postmortem human brains for C9 protein demonstrated its presence in many cortical pyramidal neurons in AD, Down's syndrome, the parkinsonism dementia complex of Guam and pallido-ponto-nigral degeneration, as well as in thalamic neurons of progressive supranuclear palsy and ballooned neurons of Pick's disease. Since C9 is required for the membrane attack complex of complement to become functional, interfering with signaling pathways that stimulate its production could offer new therapeutic strategies for treating various neurodegenerative disorders.


http://www.sciencedirect.com/science/article/B6T6J-46RCVF9-1/2/25e7f11d8f2d8db7e187699f34fc6e88

The aim of our study was to monitor the protein expression profile in pituitary glands of healthy C57BL/6J mice during aging. Pituitary glands of 4-week old (immature), 3-month old (mature), and >25-month old mice were analysed by proteomic tools such as two-dimensional electrophoresis and N-terminal micro-sequencing. A change was detected in the expression of growth hormone after sexual maturation. Our particular interest, however, was directed against up-regulated proteins in the old pituitary glands, which are proposed to be involved in the process of neuroendocrine aging. Among these proteins, the expression of glutathione-S-transferase (GST) and apolipoprotein A-1 were increased in old pituitaries. Furthermore, ubiquitin carboxyl-terminal hydrolase (UCH-L1) was significantly up-regulated in senescent C57BL/6J mouse pituitaries. Since only the rat homologue was known, we isolated and analysed the mouse UCH-L1 sequence. Since GST is involved in antioxidative defence and UCH-L1 is part of the ubiquitin/proteasome system, which is responsible for the removal of damaged proteins, these results suggest increased oxidative burden and an increased activity of the ubiquitin system.


http://www.sciencedirect.com/science/article/B6T6J-44JD70N-8/2/74c6449e6641ac1d44b01c9d6af1a92b

Aging alters the vascular response to extracellular nucleotides. However, the molecular mechanisms that underlie the effect of aging remain unclear. We investigated the mRNA expression of P2X1, P2Y1, P2Y2 subtypes of the nucleotide receptors (P2) in the basilar artery, aorta and carotid artery from male Sprague-Dawley rats, 2-months and 19-months old. In the basilar arteries of 19-month old rats, as compared to the 2-month old rats, the P2X1 receptor transcripts were reduced and the P2Y1 and P2Y2 receptor mRNA was increased. In the aorta and carotid arteries, P2Y1 receptor mRNA was decreased in the 19-month old rats when compared to the 2-month old rats. There were no marked changes of P2X1 and P2Y2 receptor mRNA between the two age groups in the aorta or carotid artery. In endothelial cells, P2Y1 and P2Y2 receptor mRNA was reduced with age. We concluded that, down-regulation of P2X1 and up-regulation of P2Y1, P2Y2 receptor mRNA in smooth muscle cells and down-regulation of P2Y1 and P2Y2 receptor mRNA on vascular endothelial cells might underlie the changes of cerebral vascular tone in aging.

http://www.sciencedirect.com/science/article/B6T6J-49TRGHM-1/2/533c25021ad64e421747db5575b08240

Mice in which the p66SHC specific region of the SHC gene is deleted live 30% longer without apparent disease. These mice have lower levels of oxidative stress and apoptosis, both of which have been linked to old age survival in man. This makes SHC1 an important candidate gene for longevity in humans. We found no variations in the p66 specific region of the SHC1 gene in 30 young and 30 extreme long-lived subjects. Thus in man, no common sequence variations occur in p66 specific region of the SHC1 gene. In two independent cohorts of respectively 730 and 563 subjects aged 85 and over, we tested the only known non-synonymous polymorphism, Met410Val, for association with longevity using a prospective follow-up design. In the first cohort, we found increasing valine allele frequency in three strata of increasing age at death (2.8-5.2%). Moreover, compared to Met/Met carriers, mortality rate was a factor of 0.71 (95% CI 0.45-1.13) reduced for Met/Val carriers in the combined cohorts, with similar risk estimates in both cohorts. Low valine allele frequency resulted, however, in low power to detect statistical significance. These data suggest that an association between the Met410Val polymorphism and longevity in humans may exist.


http://www.sciencedirect.com/science/article/B6T6J-4BMJC5X-3/2/22020f63a8f5c6753f1e0dcfdfc91a6d

Repair of mismatches in mammalian cell DNA is mediated by a complex of proteins that constitute the so-called mismatch repair system (MMR), the main post-replicative pathway for the correction of replication errors. Loss of MMR (as exemplified by germline mutations in some MMR genes, leading to hereditary non-polyposis colorectal cancer) results in increased mutation rates at both coding sequences and in non-coding regions such as microsatellites. In order to evaluate possible functional alterations of this repair system during ageing that could affect immune system efficiency, we studied microsatellite instability at five different loci interspersed in the genome (CD4, VWA31, Tpox, Fes/FPS and p53) in total DNA from T lymphocyte clones derived from hematopoietic stem cells, or peripheral T cells of young or elderly subjects. In addition, these clones had been maintained for different periods in vitro to represent a culture model of ageing. We observed increasing instability accumulating with increasing passages in culture, particularly in CD34+cell-derived clones, but no clear donor age relationship.


http://www.sciencedirect.com/science/article/B6T6J-3WRJNGY-8/2/4e041ba504ec766cb4a6aff35d6c8d16

The effect of aging on gingival fibroblasts in response to bacterial infection was studied. Rat gingival fibroblast (rGF) cells were cultured from gingival tissue removed from young (6 weeks old) and old (20 months old) rats. Both types of rGF cells were challenged with lipopolysaccharide (LPS) from the periodontal pathogen Campylobacter rectus. The levels of prostaglandin E2 (PGE2) and interleukin 1[beta] (IL-1[beta]) released into the cultured medium were measured by
a specific radioimmunoassay. LPS stimulated PGE2 and IL-1[beta] production in a dose- and
time-dependent manner in rGF cells from both young and old rats was seen. Production of PGE2
and IL-1[beta] by rGF cells from the old rats was higher than those from the young in response to
LPS. This greater ability from the older rGF cells to produce PGE2 and IL-1[beta] was due to
higher mRNA levels of cyclooxygenase 2 and IL-1[beta], respectively. In contrast,
cyclooxygenase-1 and IL-1[beta] converting enzyme gene mRNA levels remained unchanged.
Because LPS-stimulated PGE2 and IL-1[beta] production was enhanced by in vivo cellular aging,
aging of GF may affect the severity of inflammation and bone resorption by producing a large
amount of PGE2 and IL-1[beta] in response to bacterial infection.


http://www.sciencedirect.com/science/article/B6T6J-4CXKN7M-1/2/2f29fe81c4ba6f464e8de42b0f1672e5

Aging is associated with an impaired capacity of the immune system to respond properly to
danger signals, such as infection and cancer. Here, we provide evidence that an impaired innate
immune response, as measured by a low production capacity of pro- and anti-inflammatory
cytokines upon ex vivo standardized danger signalling with bacterial LPS, is predictive for frailty in
elderly people: participants who at age 85-year produced low levels of LPS-induced IL-1[beta], IL-
6, TNF-[alpha] and IL-1Ra and IL-10, were found to have a more than 2-fold elevated overall
mortality risk, independent of chronic illnesses (relative risk is 2.21, 95% confidence interval 1.27-
3.82, P=0.005), compared to peers with a higher production of any of the pro- and/or anti-
inflammatory cytokines. A significant genetic association with the IL-10 promoter gene was found,
indicating that people who are genetically predisposed low cytokine producers are at a higher risk
of losing the capacity to respond properly to danger signals with aging. We conclude that a
malfunctioning innate immune response predicts frailty in old age and is under specific (immuno-)
genetic control.

Experimental Hematology (29)

cells." Experimental Hematology 31(11): 1051.

http://www.sciencedirect.com/science/article/B6VP8-49V4XXY-C/2/a6de57b70ed0a54af24386f2e08045b2

ObjectiveThe gene BAALC (Brain And Acute Leukemia, Cytoplasmic), a novel molecular marker
involved in leukemia, is highly expressed in a subset of patients with acute leukemia and
predictive of clinical outcome in patients with acute myeloid leukemia and normal karyotype.
The role of BAALC in hematopoiesis and leukemogenesis is unknown.

Material and methodsWe used real-time RT-PCR to show that BAALC is strongly expressed in CD34+ cells from the bone
marrow and blood and only weakly expressed in total normal bone marrow and blood
cells.

ResultsExpression analyses of FACSorted cells revealed high BAALC transcript levels in
CD34+ bone marrow cells including CD34+/CD38-, CD34+/CD33+, as well as
CD34+/CD19+/CD10+, CD34+/CD7+, and CD34+/CD71+/CD45- cell fractions. Expression was
significantly lower in all CD34- fractions. In vitro differentiation of CD34+ bone marrow cells showed downregulation of BAALC and CD34 transcripts as early as day 4 in suspension cultures supplemented with lineage-specific cytokines (G-CSF, M-CSF, or EPO). In cultures with only lineage-unspecific cytokines (IL-3, SCF, GM-CSF), BAALC transcripts persisted up to day 20, while CD34 transcripts disappeared earlier. These observations suggest that expression of BAALC is stage specific.

Conclusions
BAALC expression is restricted to progenitor cells, and downregulation of BAALC occurs with cell differentiation. We postulate that BAALC represents a novel marker of an early progenitor cell common to the myeloid, lymphoid, and erythroid pathways.


http://www.sciencedirect.com/science/article/B6VP8-40MT25MD2/2/d4be8ac50cedbb3af9fa3af503e3bb1b

Objective
Bone marrow stromal cells (BMSC) are an attractive target for novel strategies in the gene/cell therapy of hematologic and skeletal pathologies, involving BMSC in vitro expansion/transfection and reinfusion. We investigated the effects of in vitro expansion on BMSC pluripotentiality, proliferative ability, and bone-forming efficiency in vivo.

Materials and Methods
BMSC from three marrow donors were cultured to determine their growth kinetics. At each passage, their differentiation potential was verified by culture in inductive media and staining with alizarin red, alcian blue, or Sudan black, and by immunostaining for osteocalcin or collagen II. First passage cells were compared to fresh marrow for their bone-forming efficiency in vivo. Stromal cell clones were isolated from five donors and characterized for their multidifferentiation ability. The lifespan and differentiation kinetics of five of these clones were determined.

Results
After the first passage, BMSC had a markedly diminish proliferation rate and gradually lost their multiple differentiation potential. Their bone-forming efficiency in vivo was reduced by about 36 times at first confluence as compared to fresh bone marrow. Experiments on the clones yielded comparable results.

Conclusions
Culture expansion causes BMSC to gradually lose their early progenitor properties. Both the duration and the conditions of culture could be crucial to successful clinical use of these cells and must be considered when designing novel therapeutic strategies involving stromal mesenchymal progenitor manipulation and reinfusion.


http://www.sciencedirect.com/science/article/B6VP8-49V4XXYF2/8odef8a89621ac94b93e6dabf9639bb3

Objective
The expression of an MDS1-EVI1-like-1 (MEL1) gene is reported in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with translocation t(1;3)(p36;q21). MEL1 (at chromosome band 1p36.3) is thought to be transcriptionally activated as a result of juxtaposition to the RPN1 gene at 3q21. It is not known whether MEL1 expression is restricted to cases with this particular translocation.

Materials and Methods
Using real-time polymerase chain reaction, we measured MEL1 expression levels, normal bone marrow, and distinct blood cell fractions in 162 de novo AML patients. We also investigated the existence of an EVI1-like gene (EL1) by applying the same method. The existence of these transcripts was confirmed by Northern blot analysis.

Results
MEL1 expression was detected in 87% (141/162) of de novo AML patients. The EL1 transcript also was detected in the majority of the patients. EL1 expression
levels highly correlated with MEL1 expression levels in AML cases. Variable MEL1/EL1 expression levels were observed. However, all the patients with favorable-risk karyotypes, i.e., with t(15;17), t(8;21), or inv(16), showed low MEL1/EL1 expression levels. Expression analysis of MEL1/EL1 compared with MDS1-EVI1/EVI1 in distinct normal marrow or blood cell fractions revealed that 1) all four gene products are expressed in CD34+ progenitor cell fractions; 2) both MEL1 and EVI1 are turned down in neutrophils and monocytes/macrophages; while 3) MDS1-EVI1 and EL1 remain expressed in mature blood cell fractions. Conclusion Our data suggest that simultaneous low MEL1/EL1 expression in AML is abnormal and that favorable disease is highly associated with this abnormal phenotype.


transcribed factors such as Myb, Tie-1, and VEGF, there was a small Rh123lowCD34+
subpopulation that transcribed significant levels of several members of the GATA family of
transcription factors. The morphology of the Rh123lowCD34+ (also expressing the P-
glycoprotein) was different compared to the Rh123highCD34- population. Mesenchymal
differentiation into glial fibrillary acidic protein (GFAP)+ glial cells could be shown from the entire
CD34-CD105+ cell population.ConclusionsThe findings provide evidence that it is possible to
isolate CD34-CD105+ mesenchymal stem cell lines from human peripheral blood cells that
contain a small subpopulation of CD34+ and GATA-transcribing cells. Those cells are potential
hematopoietic progenitors and can be recruited from the CD34- stem cell pool. The plasticity of
stem cells seems to require essential molecular tools, such as a panel of transcription factors, to
respond to the environmental demand within a biologic system.

without secreting identifiable colony-stimulating factors and without engagement of host T cells." Experimental Hematology 27(12): 1757.

Tumor growth is associated with neutrophilia, thrombocytosis, and extramedullar hematopoiesis. The mechanism(s) accounting for these phenomena is unclear, although granulocyte-
macrophage colony-stimulating factor (GM-CSF) and/or granulocyte colony-stimulating factor (G-
CSF) released by tumor cells have been involved. We studied whether CSF released by Ehrlich
tumor (ET) may play a role. A comparative study was performed with two cell variants (ET and
ET/0) growing in euthymic, nude, and SCID mice. Extramedullar hematopoiesis was assessed in
the spleen by scoring organ enlargement, wheat germ agglutinin ve+ cells, and interleukin 3-
dependent granulocyte-macrophage colony-forming unit (GM-CFU). Both cell lines showed the
same cytokine profile by reverse transcriptase polymerase chain reaction, including GM-CSF, G-
CSF, and macrophage colony-stimulating factor (M-CSF); yet, only ET cells produced detectable
colony-stimulating activity in vitro, mainly due to GM-CSF. No differences in tumorigenicity were
noted between ET and ET/0 cells inoculated to normal or immunodeficient mice. An increase in
extramedullar hematopoiesis, accompanied by neutrophilia and thrombocytosis, was associated
with tumor progression irrespective of the cell line. A strong correlation was obtained between the
increase in splenic GM-CFU and tumor mass (r = 0.96, p 0.0001) that was independent on the
tumor cell line, strain of mice, or stage of tumor development. The results point against CSF
released by tumor cells and/or reactive host T cells as the only factors involved in the
extramedullar hematopoiesis in this tumor model. The remarkable correlation between splenic
GM-CFU and the tumor mass still suggests that a factor(s) of tumor origin may play a critical role.

Dror, Y., P. Durie, et al. (2002). "Clonal evolution in marrows of patients with Shwachman-Diamond

ObjectivesShwachman-Diamond syndrome (SDS) is characterized by varying degrees of marrow
failure. Retrospective studies suggested a high propensity for malignant myeloid transformation
into myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The study's aims
were to determine the cellular and molecular characteristics as well as the clinical course of
malignant myeloid transformation and clonal marrow disease in patients with SDS.MethodsThis is
a longitudinal prospective study of 14 patients recruited for annual hematological evaluations.
Results of baseline and serial hematological assessments for up to 5 years are
Clonal marrow cytogenetic abnormalities (CMCA) were detected in 4 patients (29%) on first testing or at follow-up. The abnormalities were del(20q) in two patients, i(7q) in one, and combined del(20q) and i(7q) in one. The following tests did not distinguish patients with CMCA from other SDS patients: severity of peripheral cytopenia, fetal hemoglobin levels, percentage of marrow CD34+ cells, colony growth from marrow CD34+ cells, cluster-to-colony ratio, marrow stromal function, percentage of marrow apoptosis cells, and granulocyte colony-stimulating factor receptor expression. RAS and p53 mutation analysis and AML blast colony assays were uniformly negative. No patients showed progression into more advanced stages of MDS or into AML. In one patient, the abnormal clone became undetectable after 2 years of follow-up.

Conclusions
We conclude that although CMCA in SDS is high, progression into advanced stages of MDS or to overt AML may be slow and difficult to predict. Treatment should be cautious since some abnormal clones can regress.


Objective
This study investigated the effect of interleukin-9 (IL-9) on the proliferation and differentiation of human colony-forming unit megakaryocytic progenitor cells (CFU-Meg). Materials and Methods
Peripheral blood-derived CD34+IL-6R- cells were sorted and cultured in the presence of IL-9, erythropoietin (Epo), stem cell factor (SCF), and thrombopoietin (TPO) alone or in combination. The number of pure and mixed megakaryocyte colonies, the size of pure megakaryocyte colonies, the ploidy distribution of megakaryocytes, and proplatelet formation were investigated.

Results
Apart from TPO, no single factor could support CFU-Meg-derived colony formation, but each two-factor combination among IL-9, Epo, and SCF supported a few CFU-Meg colonies. Interestingly, the combination of Epo+SCF+IL-9 induced four to six times as many CFU-Meg colonies as any of the two-factor combinations. Neutralizing monoclonal antibodies (mAbs) for IL-9 receptor and c-kit completely abolished this synergistic effect. In contrast, addition of neutralizing anti-c-Mpl or anti-CXCR4 Abs did not influence colony formation, indicating that this synergistic effect was independent of TPO or SDF-1. Moreover, the endogenous production of TPO by cultured CD34+IL-6R- cells in the presence of Epo+SCF+IL-9 was ruled out by reverse transcriptase polymerase chain reaction for TPO mRNA. Interestingly, the combination of TPO, Epo, SCF, and IL-9 supported the largest number of pure and mixed megakaryocyte colonies, suggesting that this combination of cytokines might recruit primitive megakaryocytic as well as multipotential progenitors. This combination also potently enhanced proplatelet formation compared with TPO alone or a combination of Epo, SCF, and IL-9.

Conclusion
This study demonstrated for the first time that human IL-9 can potentiating human megakaryocytopoiesis in the presence of Epo and/or SCF.
antibody. RNA and protein expression were confirmed in different cell lines by Northern and Western blot analysis. Fluorescence-activated cell sorting (FACS) analysis was applied to examine cell viability. Nuclear factor \( \kappa \)B (NF\( \kappa \)B) pathways were blocked using a specific inhibitor. Results We found strongly enhanced expression of the cellular inhibitor of apoptosis cIA\( P \)P1 and cIA\( P \)P2 in Karpas 299 cells stimulated with anti-CD30. Furthermore, we showed that CD30-regulated expression of cIA\( P \)P1 and cIA\( P \)P2 was mediated by NF\( \kappa \)B. Induction of NF\( \kappa \)B, cIA\( P \), and cIA\( P \)2 correlated with partial protection from apoptotic cell death caused by etoposide. Correspondingly, inhibition of the NF\( \kappa \)B pathway not only prevented the prevalent antiapoptotic effects mediated by CD30, but even led to CD30-induced apoptosis. Finally, we found enhanced expression of cIA\( P \)P1 and cIA\( P \)P2 in several other ALCL cell lines and the HD-derived cell line HDLM-2 upon CD30 stimulation. Conclusions Our results indicate that CD30-mediated protection from apoptosis is a common feature of CD30+ cells. Therefore, CD30-induced signaling may have a significant impact on the clinical outcome of patients with ALCL.


We have evaluated the in vivo amplification potential of purified murine hematopoietic stem cells, identified as Wheat Germ Agglutinin+ (WGA+), 15-1.1-, Rhodamine 123 Dull (Rho-dull) cells, by serial transplantation into stem cell defective nonmyeloablated W/Wv mice. C57BL Rho-dull cells (250/500 cells/mouse) permanently engrafted nonablated W/Wv mice as defined by the presence of > 95% red and > 20% white donor-derived circulating cells for at least 1.5 years following transplantation. At this time, approximately 61% of Rho-dull cells and all the Rho-bright progenitor and colony forming cells of the engrafted mice were found to be donor-derived by c-Kit genotyping and by their response to stem cell factor (SCF). Retransplantation of 250-1000 Rho-dull cells from primary into secondary W/Wv recipients generated C57BL hematopoiesis in 40%-64% of animals revealing the presence of donor derived hematopoietic stem cells (HSC) in the bone marrow of the primary recipients. One and half years after transplantation, the bone marrow of the secondary engrafted animals contained C57BL Rho-dull cells (cong 51% by genotype), which were capable of reconstituting tertiary W/Wv recipients. In this respect, 25% of tertiary mice expressed C57BL hematopoiesis when transplanted with 250-1000 Rho-dull cells purified from secondary W/Wv recipients. On the basis of the number of Rho-dull cells purified from a single mouse, we calculate that approximately 7.3 x 104 Rho-dull cells, which are genotypically and functionally defined as C57BL long-term repopulating stem cells, were generated in the marrow of reconstituted primary W/Wv recipients transplanted 1.5 years earlier with 250-500 C57BL Rho-dull cells. We conclude that murine HSC have extensive amplification capacity in nonmyeloablated animals.


Objective. Genetic alterations, including p53 mutations, have been identified in the stroma of solid tumors and are thought to be involved in the induction of tumor growth and metastasis. We tested the hypothesis that somatic molecular alterations in bone marrow stromal cells provide a favorable growth environment for leukemic cells. Materials and Methods. We established an in
vitro model consisting of stroma expressing mutant p53 (Cys135Ser) to study its ability to support growth of cells from a pre-B acute lymphoblastic leukemia (ALL) cell line. Normal and leukemic bone marrow stromal cells were screened for p53 mutations by mutant-specific ELISA, SSCP, and direct sequencing. Secretion of vascular endothelial growth factor (VEGF) was measured by quantitative ELISA. Results. Transfection of stromal cells with mutant p53 increased synthesis of VEGF and supported the growth of leukemic cells. An ELISA-based assay suggested the occurrence of in vivo p53 alterations in bone marrow stromal cells from 2 of 12 ALL patients screened. Direct sequencing of one of these samples revealed a somatic heterozygous p53 gene mutation (Asp49His). This sample secreted more VEGF and provided increased growth support to leukemic cells. The ability of Asp 49His-p53 to increase the expression of VEGF was confirmed with transfection experiments in a p53-null cell line. Conclusion. Our findings indicate that genetic alterations, such as p53 mutations, in stromal cells can increase stromal-derived support of leukemia growth. Increased synthesis of pro-angiogenic cytokines, such as VEGF, may constitute one possible pathway by which this process is mediated.


Objective The aim of this study was to assess the gene transfer efficiency of an in situ administration protocol for hematopoietic stem/progenitor cells in the rhesus macaque (Macaca mulatta) animal model. Materials and Methods Moloney murine leukemia virus amphotropic vector producer cells (1-2 x 10^8 cells/animal) were transplanted into the femoral bone marrow cavities of six macaques. To determine if the levels of gene transfer could be increased, a second injection at the same dose of producer cells was performed into the iliac crest in three of the six macaques. Results We demonstrated that 0.02-0.1% of peripheral blood mononuclear cells contained the vector transgene for up to 12 months following the initial administration of producer cells. Hematopoietic progenitor cell assays indicated that the neomycin phosphotransferase gene was detected in 10-30% of progenitor cell colonies. A humoral immune response directed toward viral particles was demonstrated in all animals. Additionally, we demonstrated that an increase in the levels of transduced cells, up to 1% of circulating peripheral blood mononuclear cells and granulocytes, contain the transgene following producer cell readministration. Conclusions These data demonstrate the successful in situ gene transfer to hematopoietic stem/progenitor cells and circulating peripheral blood mononuclear cells that persists as long as 12 months postinjection, in the absence of any preconditioning.


Objective Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia that is characterized by a deficiency of glycosylphosphatidylinositol-anchored membrane proteins due to phosphatidylinositol glycan-class A (PIG-A) gene abnormalities in various lineages of peripheral blood cells and hematopoietic precursors. The purpose of our study was to clarify the distribution of PIG-A gene abnormalities among various cell lineages during differentiation and maturation in PNH patients. Patients and Methods The expression of CD16b or CD59 in peripheral blood
granulocytes or cultured erythroblasts from three Japanese PNH patients was analyzed using flow cytometry. PIG-A gene abnormalities in both cell types, including glycophorin A+ bone marrow erythroblasts, were examined using nucleotide sequence analysis. The expression study of PIG-A genes from each patient was also performed using JY-5 cells. ResultsFlow cytometry revealed that the erythroblasts consisted of negative, intermediate, and positive populations in Cases 1 and 3 and negative and intermediate populations in Case 2. The granulocytes consisted of negative and positive populations in all three cases. DNA sequence analysis indicated that all the PNH cases had two or three types of PIG-A gene abnormalities, and that a predominant clone with an abnormal PIG-A gene was different in granulocytes and erythroblasts from Cases 2 and 3. Expression studies showed that all the mutations from the patients were responsible for the null phenotype. Conclusion PIG-A gene abnormalities result in deficiencies of glycosylphosphatidylinositol-anchored proteins in PNH erythroblasts and granulocytes. The distribution of predominant PNH clones with PIG-A gene abnormalities is often heterogeneous between the cell types, suggesting that a clonal selection of PIG-A gene abnormalities occurs independently among various cell lineages during differentiation and maturation.


http://www.sciencedirect.com/science/article/B6VP8-445G7M5-B/2/c44a838f6f02c9979ff4b45a78323723

Objective To support immune reconstitution after cord blood transplantation, immunotherapy using gene-modified dendritic cells (DCs), the most potent antigen-presenting cells, can be a powerful strategy for preventing infection and recurrence. To investigate the applicability of lentiviral vector-transduced DCs compared to retroviral vectors, we transduced umbilical cord blood (CB) CD34+ cells, then expanded and differentiated them into DCs. Materials and Methods We transduced CB CD34+ cells by vesicular stomatitis virus G-protein pseudotyped self-inactivating lentiviral vector or retroviral vectors carrying the enhanced green fluorescent protein gene. The cells were expanded in the stroma-dependent culture system and transferred to the culture condition for developing DCs. The efficiency of transduction and expression of the transgene in severe combined immunodeficiency (SCID) mice-repopulating cells (SRCs) and DCs were compared between lentiviral vector and retroviral vectors. Induced DCs were characterized by SRCs assay and clonogenic assay of bone marrow cells from the transplanted mice. DCs derived from these cells expressed green fluorescent protein and surface markers CD1a, CD80, and HLA-DR and showed potent allostimulatory activity as well as nontransduced DCs did. On the other hand, we did not detect transgene expression in SRCs and DCs transduced by retroviral vectors. Conclusion Gene-modified DCs derived from ex vivo expanded CB CD34+ cells transduced by lentiviral vector will be useful in future immunotherapy protocols.


http://www.sciencedirect.com/science/article/B6VP8-46Y0K0Y-F/2/4f3321f3ae810a9321965e44c43d2626

Objective Bone marrow stromal cells provide the microenvironment for self-renewal and differentiation of hematopoietic stem/progenitor cells through complex cell-cell interaction. To elucidate the regulatory mechanisms of hematopoiesis by stromal cells, we established a novel
stroma-dependent hematopoietic cell line and explored the phenotypic changes regulated by the two stromal cells. Materials and Methods DFC-28 cells clonally established from long-term bone marrow culture of C57BL/6 mice were sustained by coculture on MSS62 cells (mouse spleen stromal cell line). When DFC-28 cells were transferred to TBR31-1 cells (mouse bone marrow stromal cell line), their phenotypic changes were analyzed by flow cytometry and reverse transcriptase polymerase chain reaction. Results DFC-28 cells on MSS62 cells exhibited surface phenotypes of the immature hematopoietic progenitor cells (Lin-AA4.1+c-kit+Sca-1-). By stroma-replacement from MSS62 cells to TBR31-1 cells, DFC-28 cells were differentiated into very early B-lymphoid stage characterized by c-kit down-regulation and induction of BP-1 and B-lymphoid-associated genes (Pax-5, CD19, TdT, Rag-1, and Rag-2). In addition, the differentiation phenotypes reverted to the immature state characterized by c-kit induction and down-regulation of BP-1 and B-lymphoid-associated genes by replacing stroma back to MSS62 from TBR31-1. Interleukin-7 stimulation and conditioned medium of TBR31-1 cells were ineffective in converting the differentiation phenotypes of DFC-28 cells. Conclusion The results demonstrate that the differentiation phenotypes and growth potential of stroma-dependent hematopoietic progenitor cells we established could be reversibly controlled via direct contact with stromal cells in the microenvironment.


http://www.sciencedirect.com/science/article/B6VP8-40MT25M-6/2/430580064f29a23a73c607b3c4820715

Objective To understand regulation of myeloid development, it is necessary to obtain the myeloid progenitor cell lines with self-renewal and differentiation capacities. Because prolonged hematopoiesis occurs with the production of myeloid cells at all stages of differentiation in the Dexter-type long-term bone marrow cultures, we tried to obtain stroma-dependent myeloid progenitor cells starting from the long-term bone marrow culture. Materials and Methods Murine cobblestone areas generated in long-term bone marrow cultures were serially passaged every 10 days. After 4 months, the resultant hematopoietic cells, designated as DFC, were passaged on a monolayer of established spleen stromal cell line, MSS62. After 10-12 passages of DFC cells on MSS62, several clones were obtained by colony formation on MSS62 cell layer. Among these clones, DFC-a cells could be maintained for a long period by coculturing with the established stromal cell line, MSS62. Results DFC-a cells proliferated by forming cobblestones and contained blast cells, granulocytes, and macrophages. Cell sorting and coculture experiments indicated that the blast type cells exhibiting c-Kit+ Gr-1- Mac-1-, stroma-dependently self-renewed, and spontaneously differentiated toward granulocytes (c-Kit+ Gr-1+ Mac-1+) and macrophages (c-Kitlow/+ Gr-1- Mac-1high). Although most of DFC-a cells expressed c-Kit, SCF-c-Kit interaction was not always necessary for their growth. In the presence of stromal cells, growth and differentiation of DFC-a cells were stimulated by GM-CSF or IL-3. Without stromal cells, DFC-a was transiently expanded by GM-CSF or IL-3 but could not be maintained constantly by these cytokines. Conclusion The present study demonstrated that DFC-a is a novel bipotent myeloid progenitor cell clone as a simple model system of stroma-dependent myeloid development. It may reflect distinct properties for the earliest myeloid progenitor cells in vivo. It is of interest to know what signals are provided by MSS62 stromal cells to maintain the myeloid progenitor cells.


http://www.sciencedirect.com/science/article/B6VP8-40MT25M-
Objective
We investigated whether gene transfer into hematopoietic cells could be achieved by direct injection of retroviral vector supernatant into the bone marrow space of newborn sheep.

Methods
Six sheep (5 weeks old) were injected bilaterally with either 1 mL of G1nBgSvNa8.1 vector supernatant (titer: 1 x 10^7) in each hip (n = 5) or with 3 mL of the same vector preparation/hip (n = 1). In addition, one 3-month-old sheep was injected unilaterally with 1 mL of the same vector preparation. Blood and marrow of these animals were analyzed for the transgene before injection and at intervals thereafter.

Results
At 1 week postinjection, an average of 11.6% of the lymphocytes and 25.5% of the granulocytes/monocytes in the marrow, and an average of 0.9% of the lymphocytes and 1.8% of the granulocytes/monocytes in the blood contained and expressed the LacZ gene. The presence/expression of the transgene has persisted for at least 13 months within the blood and bone marrow of these animals.

Conclusions
These findings demonstrate that the direct injection of small volumes of high-titer retroviral supernatant into the bone marrow of newborn sheep results in transduction of hematopoietic cells that persists for at least 13 months postinjection.


Objective
Quantitative assessment of gene expression in stem cells is essential for understanding the molecular events underlying normal and malignant hematopoiesis. The aim of the present study was to develop a method for precise quantitation of gene expression in small subsets of highly purified CD34+CD38- stem cell populations.

Methods.
Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantitate housekeeping and drug resistance gene expression in cDNA obtained from 300 CD34+CD38- cells without cDNA amplification or nested PCR techniques.

Results
Validation experiments in cell lines showed efficient, representative and reproducible gene amplification using 300-cell real-time quantitative RT-PCR. Sensitivity was confirmed in dilutional experiments and by detection of the low-copy gene PGD. GAPDH was found to be a useful reference gene in normal and leukemic CD34+CD38- cells. In contrast, 18S rRNA content varied 100-fold to 1000-fold in these populations. Moreover, expression of 18S rRNA was significantly lower in leukemic CD34+CD38+ cells compared to normal CD34+CD38+ cells (p = 0.002). Expression of MDR-1 (18-fold, p p +CD38- compared to CD34+CD38+ cells.

Conclusions
Real-time quantitative RT-PCR is a valuable tool for precise quantitation of gene expression in small subsets of hematopoietic cells. Using this method, we showed the inappropriateness of 18S as a reference gene in these progenitors and the down-regulation of drug-resistance-related genes early in hematopoiesis.

we studied the dependence of CMML on granulocyte-macrophage colony-stimulating factor (GM-CSF). Materials and Methods We used in vitro colony assays in methylcellulose where CMML cells were tested in the presence or absence of the specific GM-CSF antagonist E21R. We also developed an in vivo model in which CMML cells were tested for their ability to engraft into immunodeficient mice transgenic for human GM-CSF. Results Bone marrow cells from seven of seven patients with CMML formed spontaneous colonies that were sensitive to E21R treatment, with reduction in colony growth by up to 92%. E21R also inhibited colony formation by CMML patient cells stimulated by exogenously added GM-CSF but not interleukin-3. In in vivo experiments we observed engraftment of CMML cells (but not normal cells) in immunodeficient mice transgenic for human GM-CSF. None engrafted in nontransgenic mice. Cell dose escalation showed that the optimal number was 0.5 to 1 x 10^8 peripheral blood mononuclear cells per mouse, which is equivalent to an infusion of 0.2 to 3.6 x 10^6 CD34+ cells. Time course experiments showed that maximal engraftment occurred 6 weeks after injection. Conclusions These results demonstrate that in some CMML patients, GM-CSF produced by either autocrine or paracrine mechanisms is a major growth determinant. The results suggest that therapies directed at blocking this cytokine could control the growth of some CMML patients in vivo.


http://www.sciencedirect.com/science/article/B6VP8-48KTW4D-6/2/4a72acf7a3b6cd888f542176914b364f

Objective. Megakaryopoiesis is characterized by two major processes, acquisition of lineage-specific markers and polyploidization. Polyploidy is a result of endomitosis, a process that is characterized by continued DNA replication in the presence of abortive mitosis. Stathmin is a major microtubule-regulatory protein that plays an important role in the regulation of the mitotic spindle. Our previous studies had shown that inhibition of stathmin expression in human leukemia cells results in the assembly of atypical mitotic spindles and abnormal exit from mitosis. We hypothesized that the absence of stathmin expression in megakaryocytes might be important for their abortive mitosis. Materials and Methods. The experimental models that we used were human K562 and HEL cell lines that can be induced to undergo megakaryocytic differentiation and primary murine megakaryocytes generated by in vitro culture of bone marrow cells. The megakaryocytic phenotype was evaluated by flow cytometry and light microscopy. The DNA content (ploidy) was analyzed by flow cytometry. Stathmin expression was analyzed by Western and Northern blotting and by RT-PCR. Results. Our studies showed an inverse correlation between the level of ploidy and the level of stathmin expression in megakaryocytic cell lines and in primary cells. More importantly, inhibition of stathmin expression in K562 cells enhanced the propensity of these cells to undergo endomitosis and to become polyploid upon induction of megakaryocytic differentiation. In contrast, inhibition of stathmin expression interfered with the ability of the cells to acquire megakaryocyte-specific markers of differentiation. Conclusion. Based on these observations, we propose a model of megakaryopoiesis in which stathmin expression is necessary for the proliferation and differentiation of early megakaryoblasts and its suppression in the later stages of megakaryocytic maturation is necessary for polyploidization.


http://www.sciencedirect.com/science/article/B6VP8-3WWKMPH-4N/2/96e2d7e433eca0442f82559bf701902
Fabry disease is a lysosomal storage disorder that is due to a deficiency in \([\alpha]-\text{galactosidase A} ([\alpha]-\text{gal A}). Previously we have shown that a recombinant retrovirus synthesized for the transfer of the human \([\alpha]-\text{gal A} coding sequence was able to engineer enzymatic correction of the hydrolase deficiency in fibroblasts and lymphoblasts from Fabry patients. The corrected cells secreted \([\alpha]-\text{gal A} that was taken up and utilized by uncorrected bystander cells, thus demonstrating metabolic cooperativity. In separate experiments we used transduced murine bone marrow cells and successfully tested and quantitated this phenomenon in vivo. In the present studies, which were designed to bring this therapeutic approach closer to clinical utility, we establish that cells originating from the bone marrow of numerous Fabry patients and normal volunteers can be effectively transduced and that these target cells demonstrate metabolic cooperativity. Both isolated CD34\(^+\)-enriched cells and long-term bone marrow culture cells, including nonadherent hematopoietic cells and adherent stromal cells, were transduced. The transferred gene generates increased intracellular \([\alpha]-\text{gal A} enzyme activity in these cells. Further, it causes functional correction of lipid accumulation and provides for long-term \([\alpha]-\text{gal A} secretion. Collectively, these results indicate that a multifaceted gene transfer approach to bone marrow cells may be of therapeutic benefit for patients with Fabry disease.


http://www.sciencedirect.com/science/article/B6VP8-458WBCC-3/2/fe794f219599fb6684c990062bb97c80

Objective CD22 is believed to be restricted to normal and neoplastic B cells. Human basophils were found to express CD22 molecules. Among the antibodies against CD22, Leu14, which recognized the ligand binding domain, reacted to basophils, and B3 and 4KB128, which recognized the amino terminus side and carboxy terminus side of the ligand binding epitope, respectively, did not. To clarify the difference of CD22 antigenicity in human B cells and basophils, we investigated RNA sequence and structures of CD22 molecules. Materials and Methods Purified B cells and basophils were obtained from normal human volunteers by using a MACS magnetic cell sorting system and anti-CD19 and anti-Fc[\epsilon]RI antibodies, respectively. RT-PCR and sequencing of CD22 mRNA were performed in the exons 3 to 8. Western blotting analysis of CD22 was also performed. Results The sequence of CD22 mRNA extracted from the basophils was the same as that of B cells in exons 3 to 8 (epitopes recognized by Leu14, B3, and 4KB128 were translated from exons 4 and 5). Reduced CD22 peptide extracted from the basophils reacted to Leu14 as well as B3 and 4KB128, and the molecular size of the reduced and nonreduced products was 130 kDa as expected. Conclusion Disulfide bonds and the resulting 3D conformation of the CD22 molecules may have important roles in the difference of antigenicity of CD22[\beta] in B cells (CD22[\beta]1) and basophils (CD22[\beta]2). The difference in molecular structure surrounding the ligand-binding domain of CD22 may imply a specialization of the conformational forms of CD22 according to the ligand isoforms.


http://www.sciencedirect.com/science/article/B6VP8-445G7M5-8/2/f854e9406f925c07a5a266df8a7b6896

Objective The CD36 molecule is expressed in platelets, monocytes, erythroblasts, and other different tissues. The two types of platelet CD36 deficiency, types I and II, are associated with the absence and presence of CD36 on monocytes, respectively. To clarify the involvement of the erythroid lineage in CD36 deficiency, we investigated the phenotype and RNA expression of
CD36.Materials and Methods

CD36 expression was examined in 296 patients with several cardiovascular diseases in our outpatient clinic. There were 12 patients with type I deficiency and 16 with type II CD36 deficiency. A bone marrow sample was examined in five type I and four type II patients. Expression of CD36 mRNA was examined in burst-forming unit-erythroid (BFU-E). The sequences of reverse transcriptase polymerase chain reaction (RT-PCR) products of the CD36 mRNA from monocytes were examined.

Results

As expected, CD36 was deficient in erythroblasts from all five patients with type I deficiency. CD36 was present in erythroblasts from three of the four with type II deficiency, suggesting that their abnormality is restricted to platelets (type IIa). CD36 was unexpectedly absent from erythroblasts of a single type II patient (type IIb). CD36-specific mRNA was identified in BFU-E from each of two normals, six type I, and six type II patients, including type IIb. The sequences of RT-PCR products of the CD36 mRNA in a patient with type IIa and another with type IIb showed homozygous wild alleles.

Conclusion

The findings provide evidence for further heterogeneity among CD36-deficient individuals and the existence of a basic principle mechanism of type II, such as glycosylation abnormality.


http://www.sciencedirect.com/science/article/B6VP8-426H6SJ-F/2/0433a57d8780a51e98c2b6943854bf47

Objective

The rae28 gene (rae28) is a murine homologue of the Drosophila polyhomeotic gene, which is a member of the Polycomb-group genes. In this study, we examined the role of rae28 in lymphocyte development.

Materials and Methods

Because homozygous rae28-deficient (rae28-/-) mice died in the perinatal period, we examined lymphocyte development by generating chimeric mice reconstituted with green fluorescence protein-labeled mutant fetal liver cells as well as in in vitro culture systems. We further examined RAE28 expression by reverse transcriptase polymerase chain reaction assay in human leukemic cells with B-lineage acute lymphoblastic leukemia (ALL).

Results

Severe B-cell maturation arrest was observed in rae28-/- between pro- and pre-B lymphocyte stages. B-cell development was also delayed in heterozygous neonates. Furthermore, interleukin-7-dependent colony-forming ability was impaired not only in homozygous lymphocytes but also in heterozygotes. Its human homologue, RAE28, is located on chromosome 12p13, which frequently is associated with chromosomal abnormalities and loss of heterozygosity in patients with hematologic malignancies. To determine whether a link exists between RAE28 and leukemia, we examined RAE28 expression in leukemic cells from pediatric patients with B-lineage ALL. RAE28 expression was not detected in four B-cell precursor ALL cases of a total of 43 examined, although RAE28 is normally expressed constitutively during the process of B-cell maturation as assessed in isolated cell populations.

Conclusions

rae28 plays an important role in the early B-cell developmental stage in a gene dosage-dependent manner. Furthermore, the human RAE28 locus may provide a candidate gene causing the molecular pathogenesis of childhood B-cell precursor ALL.


http://www.sciencedirect.com/science/article/B6VP8-4F8SXDF-D/2/9835e3c2f757bf6c1bee3c4bc664e5f1

Objective

Circadian genes have recently been characterized in many tissues, but not in hematopoietic stem cells. These cells are rare in the bone marrow (BM), which makes it difficult to collect enough cells for detailed molecular analysis in a short period of time without reduced RNA quality. The aim was to improve methodology and reliability of clock gene expression...
analysis in purified mouse hematopoietic stem cells. Methods: Stem cells were highly enriched by high-speed flow cytometric cell sorting of the side population (SP) cells from Hoechst 33342 (Hoechst)-stained mouse BM. Total RNA was isolated from sorted SP and whole BM cells and exposed to DNase treatment. The relative mRNA levels of major clock genes mPer1, mPer2, mBmal1, mCry1, mClock, and mRev-erb [alpha] were measured with real-time quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) and normalized to m36B4, used as a reference gene. The clonogenity of sorted SP cells and whole BM; cells taken before and after sorting, were tested in colony-formation assay. Results: Clock gene activity in sorted SP cells showed pronounced relative differences compared with whole BM for mPer1 and mCry1. The high-speed sorting procedure did not influence clock gene expression or cell clonogenity, even when this was performed with a delay period up to 24 hours. Conclusions: We demonstrated expression of six clock genes in mouse hematopoietic stem cells. A combination of high-speed flow cytometric sorting and Q-RT-PCR was shown to be useful and reliable for analysis of clock gene activity in small stem cell fractions.


http://www.sciencedirect.com/science/article/B6VP8-4F8SXDF-8/2/ab8528c05881b60240a52439bf30ff89

Objective: Ceramide, an intermediate of apoptosis induction in response to chemotherapy, can be detoxified by glycosylation at the cytoplasmic surface of the Golgi membrane. P-glycoprotein (p-gp) might augment ceramide glycosylation by translocating glucosylceramide (GC) across the Golgi membrane. We aimed to show that glucosylceramide synthase (GCS) activity is linked to p-gp expression and resistance to ceramide-induced apoptosis in acute myeloid leukemia (AML). Methods: Apoptosis and cell-cycle analysis were measured using propidium iodide staining and flow cytometry. Fluorescent microscopy assessed p-gp expression in, and rhodamine 123 uptake by, the Golgi. P-gp interaction with GC was assessed by modulation of rhodamine accumulation. The GCS activity assay was based upon the transfer of UDP-3H-glucose to C8-ceramide to form radiolabeled GC, by rate-limiting cell-derived GCS. TLC and fluorimetry were used to measure the metabolites of fluorescent ceramide. Cell viability was measured using 7-amino-actinomycin D staining and flow cytometry with an internal standard for cell enumeration. Results: P-gp+ cell lines (KG1a, TF-1) were resistant to C8-ceramide-induced apoptosis compared to p-gp- cell lines (HL-60, U937). P-gp inhibitors GF120918 and cyclosporin A enhanced ceramide-induced apoptosis in the p-gp expressing cells. P-gp expression was identified in the Golgi of these cells. Pgp's efflux function in TF-1 but not KG1a cells was inhibited by glucosylceramide. In the presence of p-gp inhibitors, R123 accumulation in the Golgi of TF-1 cells was lost, and GCS activity and lactosylceramide formation were downregulated. Intact cells were necessary for the involvement of p-gp in the regulation of GCS activity. Conclusion: Our data suggests that ceramide induces apoptosis in AML cells and that p-gp confers resistance to ceramide-induced apoptosis, with modulation of the ceramide-glucosylceramide pathway making a marked contribution to this resistance in TF-1 cells.


http://www.sciencedirect.com/science/article/B6VP8-428FKDM-F/2/4d72eb835879c26bce01c9f7f3984e7
Objective
The aim of this study was to determine the molecular basis of p47-phox-deficient chronic granulomatous disease (CGD), the most common autosomal recessive form of the disease. CGD is an inherited condition characterized by defective oxygen radical production due to defects in the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Mutational analysis of p47-phox-deficient CGD patients previously demonstrated that the majority of patients have a GT dinucleotide ([Delta]GT) deletion at the start of exon 2, a signature sequence also observed in the highly homologous pseudogenes of NCF1.

Methods
We performed genetic analysis of NCF1 and its pseudogenes using genomic DNA in 29 p47-phox-deficient CGD patients from 22 separate families. First-strand cDNA analysis was performed in 17 of the 29 patients.

Results
We confirmed the significance of the [Delta]GT mutation; in 27 of 29 patients, only the [Delta]GT sequence was detectable. All but one of the 27 had at least one additional signature sequence, specific to the pseudogene, in either intron 1 and/or intron 2. We extended our analysis to look at signature sequence differences in exons 6 and 9 and detected both the wild-type and pseudogene sequences in all patients tested.

Conclusions
Although detection of only [Delta]GT sequence accounts for over 85% of affected patients, the molecular basis is most likely due to partial cross-over events between the wild-type and pseudogene(s) of p47-phox at different recombination sites. Our results suggest that complete gene conversion or deletion of the p47-phox gene (NCF1) occurs rarely, if it all.


http://www.sciencedirect.com/science/article/B6VP8-49NR2WN-C/2/1359f5ab61922f0195b18b849c91e443

Objective
Nonmyeloablative conditioning regimens for allogeneic stem cell transplantation are now commonly used in the treatment of patients with hematologic malignancies. Since this treatment often results in the establishment of mixed hematopoietic chimerism, this approach may also prove to be useful in the treatment of nonmalignant disorders, such as sickle cell disease and thalassemia major. To apply this approach to these diseases, it will be necessary to determine the levels of donor erythropoiesis required to correct hemolysis and ameliorate disease symptoms. Current methods for measuring hematopoietic chimerism are based on DNA polymorphisms that distinguish recipient from donor. These methods accurately measure donor leukocyte engraftment but do not quantify the relative contributions of recipient and donor erythropoiesis following transplant.

Methods
To specifically measure erythroid-lineage chimerism, we used pyrosequencing of the sickle cell mutation to quantify the relative levels of normal and sickle [beta]-globin mRNA in patient samples. Results of [beta]-globin RNA chimerism were compared to assessment of [beta]-globin DNA chimerism as well as analysis of short tandem repeat (STR) polymorphisms, cytogenetics, and hemoglobin electrophoresis.

Results
Donor engraftment was measured in two adult patients following nonmyeloablative stem cell transplant for sickle cell disease. In Patient 1, 25 to 30% of peripheral leukocytes were donor derived after day 41. In contrast, more than 55% of peripheral blood [beta]-globin mRNA was of donor origin, and these results correlated with posttransplant clinical improvement. Patient 2 achieved 40 to 50% donor leukocyte engraftment from day 33 onward. This was associated with 70 to 100% peripheral blood donor [beta]-globin mRNA.

Conclusions
These studies demonstrate that relatively low levels of donor leukocyte engraftment can be associated with higher levels of donor erythropoiesis and with significant clinical improvement. Pyrosequencing of lineage-specific mRNA directly measures functional reconstitution of donor cells and provides valuable information that can affect clinical decisions in patients with nonmalignant diseases following allogeneic transplant.

Several lines of evidence suggest that overexpression of interferon [gamma] (IFN-[gamma]) in the marrow microenvironment may play a role in the pathogenesis of marrow suppression in aplastic anemia. We previously showed that overexpression of IFN-[gamma] by marrow stromal cells inhibits human long-term culture initiating cell activity assayed in vitro to a much greater degree than the addition of soluble IFN-[gamma]. The effect of IFN-[gamma] on true repopulating stem cells assayed in vivo has not been studied previously. We compared the effect of co-culture of murine marrow cells in the presence of stromal cells transduced with a retroviral vector expressing murine IFN-[gamma] vs stromal cells transduced with a control neo vector. Using a murine congenic competitive repopulation assay, there was significantly less long-term repopulating stem cell activity remaining after culture on mIFN-[gamma]-expressing stroma as compared to control stroma. We also investigated the effect of directly transducing murine bone marrow cells with the mIFN-[gamma] or control vector. Marrow cells transduced with either vector were transplanted into W/Wv recipient mice. The percentage of vector-containing cells in the mIFN-[gamma] mice was significantly lower than in the control mice, suggesting that mIFN-[gamma]-transduced primitive cells may not have survived culture, or that mIFN-[gamma] directly decreases gene transfer into repopulating cells. Despite no significant differences in white or red blood cells in the mice transplanted with the mIFN-[gamma]-transduced cells, the number of bone marrow colony-forming unit-C 16 weeks after transplantation was significantly lower in the IFN-[gamma] group. These data indicate that ectopic or overexpression of mIFN-[gamma], especially by marrow microenvironmental elements, may have a marked effect on primitive hematopoiesis as assayed in vivo. Published by Elsevier Science Inc.


Bone marrow (BM) is a rich source of stem cells and may represent a valid alternative to neural or embryonic cells in replacing autologous damaged tissues for neurodegenerative diseases. The purpose of the present study is to identify human adult BM progenitor cells capable of neuro-glial differentiation and to develop effective protocols of trans-differentiation to surmount the hematopoietic commitment in vitro. Heterogeneous cell populations such as whole BM, low-density mononuclear and mesenchymal stem (MSCs), and several immunomagnetically separated cell populations were investigated. Among them, MSCs and CD90+ cells were demonstrated to express neuro-glial transcripts before any treatment. Several culture conditions with the addition of stem cell or astroblast conditioned media, different concentrations of serum, growth factors, and supplements, used alone or in combinations, were demonstrated to alter the cellular morphology in some cell subpopulations. In particular, MSCs and CD90+ cells acquired astrocytic and neuron-like morphologies in specific culture conditions. They expressed several neuro-glial specific markers by RT-PCR and glial fibrillary acid protein by immunocytochemistry after co-culture with astroblasts, both in the absence or presence of cell contact. In addition, floating neurosphere-like clones have been observed when CD90+ cells were grown in neural
specific media. In conclusion, among the large variety of human adult BM cell populations analyzed, we demonstrated the in vitro neuro-glial potential of both the MSC and CD90+ subset of cells. Moreover, unidentified soluble factors provided by the conditioned media and cellular contacts in co-culture systems were effective in inducing the neuro-glial phenotype, further supporting the adult BM neural differentiative capability.


Abnormal formation or loss of myelin is a distinguishing feature of many neurological disorders and contributes to the pathobiology of neurotrauma. In this study we characterize the functional and molecular changes in CNS white matter in Long Evans Shaker (LES) rats. These rats have a spontaneous mutation of the gene encoding myelin basic protein which results in severe dysmyelination of the central nervous system (CNS), providing a unique model for demyelinating/dysmyelinating disorders. To date, the functional and molecular changes in CNS white matter in this model are not well understood. We have used in vivo somatosensory evoked potential (SSEP), in vitro compound action potential (CAP) recording in isolated dorsal columns, confocal immunohistochemistry, Western blotting and real-time PCR to examine the electrophysiological, molecular and cellular changes in spinal cord white matter in LES rats. We observed that dysmyelination is associated with dispersed labeling of Kv1.1 and Kv1.2 K+ channel subunits, as well as Caspr, a protein normally confined to paranodes, along the LES rat spinal cord axons. Abnormal electrophysiological properties including attenuation of CAP amplitude and conduction velocity, high frequency conduction failure and enhanced sensitivity to K+ channel blockers 4-aminopyridine and dendrotoxin-I were observed in spinal cord axons from LES rats. Our results in LES rats clarify some of the key molecular, cellular and functional consequences of dysmyelination and myelin-axon interactions. Further understanding of these issues in this model could provide critical insights for neurological disorders characterized by demyelination.


Green tea polyphenol is known to act as a buffer, reducing biological responses to oxidative stress. Several effects of polyphenol have been reported, such as protection of tissue from ischemia, antineoplastic and anti-inflammatory effects, and suppression of arteriosclerosis. In this study, we investigated whether peripheral nerve segments could be kept viable in a polyphenol solution for 1 month. Sciatic nerve segments, 20 mm long, were harvested from Lewis rats and treated in three different ways before transplanting to recipient Lewis rats to bridge sciatic nerve gaps created by removal of 15-mm-long nerve segments. Group F: nerve segments were transplanted immediately after harvesting. Group P: nerve segments were transplanted after they had been stored in Dulbecco's Modified Eagle's Medium (DMEM) containing polyphenol for 7 days at 4[deg]C and then in DMEM for 21 days at 4[deg]C. Group M: nerve segments were stored in DMEM solution alone for 28 days at 4[deg]C. Viability of the nerve segments was assessed by vital staining (calcein-AM/ethidium homodimer), by electron microscopy and by genomic studies before transplantation. Nerve regeneration was evaluated using electrophysiological and morphological studies 12 and 24 weeks after transplantation. Neural cell viability of the preserved nerve segments was confirmed in group P, in which the nerve
regeneration was similar to that in group F and superior to that in group M. Peripheral nerve segments can be successfully preserved for 1 month using green tea polyphenol.


http://www.sciencedirect.com/science/article/B6WFG-49KWMS-1/2/e1819cd1b98f66deee409dd4781d84d3

The neuropilins, NP-1 and NP-2, are coreceptors for Sema3A and Sema3F, respectively, both of which are repulsive axonal guidance molecules. NP-1 and NP-2 are also coreceptors for vascular endothelial growth factor (VEGF). The neuropilins and their ligands are known to play prominent roles in axonal pathfinding, fasciculation, and blood vessel formation during peripheral nervous system (PNS) development. We confirmed a prior report (Exp. Neurol. 172 (2001) 398) that VEGF mRNA levels rise during Wallerian degeneration in the PNS and herein demonstrate that NP-1, NP-2, Sema3A, and Sema3F mRNA levels increase in peripheral nerves distal to a transection or crush injury. In a sciatic nerve crush model, in which axonal regeneration is robust, the highest levels of Sema3F mRNA below the injury site are in the epi- and perineurium. Our results suggest the possibility that the neuropilins and their semaphorin ligands serve to guide, rather than to impede, regenerating axons in the adult PNS.


http://www.sciencedirect.com/science/article/B6WFG-4C8H7KS-9J/2/ddee22a10d94211d246e58ba61c16d08

The adrenal gland is a well-demonstrated source for different neurotrophic factors. The presence of the [beta]-nerve growth factor ([beta]-NGF) mRNA in the adrenal tissue used for grafting in a Parkinsonian patient is reported here. Adrenal samples were obtained on the day of implantation, and a specific cDNA was synthesized after the extraction of total RNA using a synthetic oligonucleotide as a reverse transcription primer. A 168-bp portion of the cDNA was amplified using two other oligonucleotides as Taq polymerase primers in a polymerase chain reaction. Thirty-two cycles of amplification were performed. The amplification products were identified by agarose gel electrophoresis and Southern blot analysis as a single DNA band hybridizing with a third [beta]-NGF specific oligonucleotide. The identity of the fragment was confirmed by DNA sequencing. Quantitative analysis demonstrated a [beta]-NGF mRNA concentration exceeding 5 fg/[mu]g of total adrenal RNA. These findings add NGF to the other neurotrophic factors produced by the gland (i.e., basic fibroblast growth factor) and demonstrate the retained functional capacity of the Parkinsonian adrenal to express the [beta]-NGF mRNA. All these data may assume relevant meaning for neurotransplantation research.


http://www.sciencedirect.com/science/article/B6WFG-49Y3XT1-8/2/ef8ceadf4eee3ea8aedb56d13c386860
Bone marrow stromal cells (BMSC) have been shown to generate neural cells under experimental conditions in vitro and following transplantation into animal models of stroke and traumatic CNS injury. Hastened recovery from the neurological deficit has not correlated with structural repair of the lesion in the stroke model. Secretory functions of BMSC, such as the elaboration of growth factors and cytokines, have been hypothesized to play a role in the enhanced recovery of neurological function. Using gene expression arrays, real time RT-PCR and radioimmunoassay, we have found that brain natriuretic peptide (BNP) is synthesized and released by BMSC at physiologically relevant levels in vitro. BNP, like its close homolog atrial natriuretic peptide (ANP), exerts powerful natriuretic, diuretic and vasodilatory effects. We speculate that transplanted BMSCs facilitate recovery from brain and spinal cord lesions by releasing BNP and other vasoactive factors that reduce edema, decrease intracranial pressure and improve cerebral perfusion.

**Experimental Parasitology** (5)


http://www.sciencedirect.com/science/article/B6WFH-45TDHV0-7/2/ce01ef4158c85f4f014650cf6e888693


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The sporocyst stage of trematode development plays the crucial role of establishing a successful infection in the molluscan intermediate host. Due to the small size and presence of this stage within the tissues of the host, much of our current knowledge of sporocyst biology relies on cultured specimens. To gain insight into the transcriptional patterns of early sporocysts, suppression subtractive hybridization was employed to identify 69 unique expressed sequence tags likely to be upregulated in cultured sporocysts of Echinostoma paraensei, a trematode parasite of the planorbid snail, Biomphalaria glabrata. Upwards of 70% of the unique sequences were not identified by homology to known genes. However, one transcript may encode an inhibitor of nitric oxide synthase, indicating a possible role in protection against host defense mechanisms. An array containing the majority of the sequenced clones was probed with in vivo-derived cDNA, confirming for the first time in vivo expression of putative sporocyst genes. However, qPCR quantification demonstrated significant reductions in transcription rates in cultured versus in vivo sporocysts for three of six transcripts tested. Additionally, five of the six tested transcripts demonstrated significant variation in expression over the entire life cycle, with the significant upregulation occurring during early intramolluscan development or in the free-living stages immediately preceding snail penetration, confirming the efficacy of the SSH technique.

The rapid emergence of multidrug-resistant Plasmodium falciparum is a worldwide concern. Despite the magnitude of the problem, the mechanisms involved in this phenomenon are not well understood. One current proposal suggests that toxic heme molecules are degraded by glutathione (GSH), and that anti-malarial drugs, such as chloroquine (CQ), inhibit this degradation, thus implicating GSH in drug resistance. Furthermore, in some strains of Plasmodium berghei and P. falciparum, chloroquine resistance is accompanied by an increase in glutathione levels and increased activity in GSH-related enzymes. We are investigating the relationship between the [gamma]-glutamylcysteine synthetase (ggcs) gene, the rate-limiting enzyme in de novo synthesis of GSH, and drug resistance in P. berghei at the molecular level. In this report, we have demonstrated an increase in pbggcs mRNA levels associated with CQ and mefloquine (MFQ) resistance. In addition, the pbggcs gene locus structure was shown to be similar and localized to chromosome 8 in four parasite lines of P. berghei with different drug resistance profiles. This work suggests a link between increased GSH levels and drug resistance in Plasmodium.

Index Descriptors and Abbreviations: Apicomplexa; Malaria; Plasmodium berghei; Drug resistance; [gamma]-Glutamylcysteine synthetase; Glutathione; Chloroquine; MDR, multidrug resistance phenotype; AMO, amodiaquine; MFQ, mefloquine; CQ, chloroquine; GSH, glutathione; ggcs, [gamma]-glutamylcysteine synthetase; GB, GenBank; RBC, red blood cell; BSO, buthionine sulfoximine; pfggcs, Plasmodium falciparum ggcs gene; pbggcs, Plasmodium berghei ggcs gene; RPA, ribonuclease protection assay.


In this study, the mature domains of type I (CPB) and type II (CPA) cysteine proteinases (CPs) of Leishmania infantum were expressed and their immunogenic properties defined using sera from active and recovered cases of human visceral leishmaniasis and sera from infected dogs. Immunoblotting and ELISA analysis indicated that a freeze/thaw extract of parasite antigens showed similar and intensive recognition in both active cases of human and dog sera but lower recognition in recovered human individuals. The total IgG of actively infected human sera was higher than in recovered cases when rCPs were used as antigen. In contrast to dog sera, both active and recovered human cases have higher recognition toward rCPB than rCPA. Furthermore, the asymptomatic dogs in contrast to the symptomatic cases exhibited specific lymphocyte proliferation to both crude antigens and rCPs.


The objective of this study was to analyse the modulatory effect of proteins released by cultured Leishmania infantum promastigotes on the cellular immune response of infected susceptible
(BALB/c) and more resistant (C57BL/6) mice strains after 30 and 45 days of infection. One month after parasite inoculation, L. infantum released protein fractions (High, Inter, and Low according to molecular weight) stimulated C57BL/6 mice spleen cells to proliferate and to express cytokines. Following the decrease of parasite load only the Low protein fraction induced a considerable release of IL-4. In BALB/c mice, specific immune response to protein fractions was only observed at the higher parasitic level, with the fraction Inter promoting the production of IL-4 and fractions High and Low inducing high levels of IL-12. These results point out to a role of these proteins fractions in the modulation of host immunity, that depending on the host genetic background and parasite magnitude, seem to be critical in the control of parasite replication levels, thus avoiding premature host death.

FASEB J (12)


http://www.fasebj.org/cgi/content/abstract/01-0602fjev1

A pharmacological approach to neoplasia by differentiation therapy relies on the availability of cytodifferentiating agents whose antitumor efficacy is usually assayed first on malignant cells in vitro. Using murine erythroleukemia cells (MELCs) as the model, we found that WEB-2086, a triazolobenzodiazepine-derived PAF antagonist originally developed as an anti-inflammatory drug, induces a dose-dependent inhibition of MELC growth and hemoglobin accumulation as a result of a true commitment to differentiation. MELCs treated for 5 days with 1 mM WEB-2086 show greater than or equal to 85% benzidine-positive cells, increased expression of a- and b-globin genes, and down-regulation of c-Myb. This differentiation pattern, which does not involve histone H4 acetylation and is abrogated by the action of phorbol 12-myristate 13-acetate, recalls the pattern induced by hexamethylene bisacetamide (HMBA). In addition to MELCs, human erythroleukemia K562 and HEL and myeloid HL60 cells are massively committed to maturation by WEB-2086 and, with some differences, by its analog, WEB-2170. This suggests that WEB-2086, structurally distant from other known inducers, might be a member of a new class of cytodifferentiation agents active on a broad range of transformed cells in vitro and useful, prospectively, for anticancer therapy due to their high tolerability in vivo. Key words: inducer · PAF receptor · maturation · neoplasia · differentiation therapy


http://www.fasebj.org/cgi/content/abstract/02-0883fjev1

Hydrogen sulfide (H2S), produced by commensal sulfate-reducing bacteria, is an environmental insult that potentially contributes to chronic intestinal epithelial disorders. We tested the hypothesis that exposure of nontransformed intestinal epithelial cells (IEC-18) to the reducing agent sodium hydrogen sulfide (NaHS) activates molecular pathways that underlie epithelial hyperplasia, a phenotype common to both ulcerative colitis (UC) and colorectal cancer. Exposure of IEC-18 cells to NaHS rapidly increased the NADPH/NADP ratio, reduced the intracellular redox environment, and inhibited mitochondrial respiratory activity. The addition of 0.2-5 mM NaHS for 4
h increased the IEC-18 proliferative cell fraction (P<0.05), as evidenced by analysis of the cell cycle and proliferating cell nuclear antigen expression, while apoptosis occurred only at the highest concentration of NaHS. Thirty minutes of NaHS exposure increased (P<0.05) c-Jun mRNA concentrations, consistent with the observed activation of mitogen activated protein kinases (MAPK). Microarray analysis confirmed an increase (P<0.05) in MAPK-mediated proliferative activity, likely reflecting the reduced redox environment of NaHS-treated cells. These data identify functional pathways by which H2S may initiate epithelial dysregulation and thereby contribute to UC or colorectal cancer. Thus, it becomes crucial to understand how genetic background may affect epithelial responsiveness to this bacterial-derived environmental insult. Key words: IEC-18 colorectal cancer ulcerative colitis epithelial hyperproliferation microarray analysis


http://www.fasebj.org/cgi/content/abstract/01-0564fjev1

A role for choline during early stages of mammalian embryogenesis has not been established, although recent studies show that inhibitors of choline uptake and metabolism, 2-dimethylaminoethanol (DMAE), and 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (ET-18-OCH3), produce neural tube defects in mouse embryos grown in vitro. To determine potential mechanisms responsible for these abnormalities, choline metabolism in the presence or absence of these inhibitors was evaluated in cultured, neurulating mouse embryos by using chromatographic techniques. Results showed that 90%-95% of 14C-choline was incorporated into phosphocholine and phosphatidylcholine (PtdCho), which was metabolized to sphingomyelin. Choline was oxidized to betaine, and betaine homocysteine methyltransferase was expressed. Acetylcholine was synthesized in yolk sacs, but 70 kDa choline acetyltransferase was undetectable by immunoblot. DMAE reduced embryonic choline uptake and inhibited phosphocholine, PtdCho, phosphatidylethanolamine (PtdEtn), and sphingomyelin synthesis. ET-18-OCH3 also inhibited PtdCho synthesis. In embryos and yolk sacs incubated with 3H-ethanolamine, 95% of recovered label was PtdEtn, but PtdEtn was not converted to PtdCho, which suggested that phosphatidylethanolamine methyltransferase (PeMT) activity was absent. In ET-18-OCH3treated yolk sacs, PtdEtn was increased, but PtdCho was still not generated through PeMT. Results suggest that endogenous PtdCho synthesis is important during neurulation and that perturbed choline metabolism contributes to neural tube defects produced by DMAE and ET-18-OCH3. Key Words: neurulation · dimethylaminoethanol · ET-18-OCH3 · embryo culture


http://www.fasebj.org/cgi/content/abstract/01-0655fjev1

Substance P (SP), a potent modulator of neuroimmunoregulation, is expressed in human immune cells. We observed elevated plasma SP levels in HIV-infected men compared with uninfected subjects. In the present study, we investigated the possible cellular source of the increased SP level caused by HIV infection. Using real-time reverse transcriptase-polymerase chain reaction, we demonstrated that monocyte-derived macrophages (MDM) and lymphocytes from both placental cord blood and adult peripheral blood expressed SP mRNA, which was significantly increased by HIV infection. HIV-induced SP expression was positively related to virus replication in the infected MDM. Purified recombinant HIV envelope glycoprotein 120 (gp120) derived from both the macrophage-tropic strain (MN) and the T lymphocyte-tropic strain (IIIb), when added to MDM cultures, enhanced SP mRNA expression. The gp120-induced SP expression was
abrogated by pretreating the cells with soluble CD4. Furthermore, the activation of HIV in the latently infected promonocytic cell line (U1) and T-cell line (ACH-2) up-regulated SP mRNA expression. These data support the hypothesis that interaction of HIV and SP may have significant in vivo relevance to the immunopathogenesis of HIV infection and AIDS. Key words: lymphocytes · gp120 · tachykinin · neuroimmunoregulation · monocyte-derived macrophages


http://www.fasebj.org/cgi/content/abstract/01-0908fjev1

Junctin is a 26-kDa integral membrane protein, colocalized with the ryanodine receptor (RyR) and calsequestrin at the junctional sarcoplasmic reticulum (SR) membrane in cardiac and skeletal muscles. To elucidate the functional role of junctin in heart, transgenic (TG) mice overexpressing canine junctin (24-29 folds) under the control of mouse a-myosin heavy chain promoter were generated. Overexpression of the junctin in mouse heart was associated with heart enlargements, bradycardia, atrial fibrillation, and increased fibrosis. Many ultrastructural alterations were observed in TG atria. The junctional SR cisternae facing transverse-tubules contained a dense matrix of calsequestrin in TG heart. According to echocardiography, TG mice showed enlarged left ventricles, dilated right atriums, and ventricles with paradoxical septal motion and impaired left ventricular systolic function. Overexpression of junctin led to down-regulation of triadin and RyR but to up-regulation of dihydropyridine receptor. The L-type Ca2+ current density and action potential durations increased, which could be the cause for the bradycardia in TG heart. This study provides an important example of pathogenesis leading to substantial cardiac remodeling and atrial fibrillation, which was caused by overexpression of junctin in heart. Key words: excitation-contraction coupling · sarcoplasmic reticulum · ryanodine receptor · dihydropyridine receptor


http://www.fasebj.org/cgi/content/abstract/01-0354fjev1

The effect of uni-axial cyclic mechanical stretch on the activation of the transcription factor nuclear factor kB (NF-kB) was investigated in a human fibroblast cell line (TIG-1). In response to uni-axial cyclic stretch, NF-kB was found to be translocated into the nucleus. The NF-kB was first detectable 2 min after the onset of stretch and then peaked at 4 min and returned to the basal level within 10 min. To investigate whether NF-kB is activated following the translocation into the nucleus, we measured the luciferase activity in the cells transfected with pNF-kB-luciferase. The activity of luciferase increased 4 min after the initiation of cyclic stretch, peaked at 15 min (6.4-fold increase), and decreased gradually. We examined the involvement of the stretch-activated (SA) channel in the stretch-induced NF-kB activation. The application of Gd3+, a blocker of the SA channel, or the removal of extracellular Ca2+ inhibited both the translocation into the nucleus and the activation of NF-kB, which suggests that NF-kB is activated by uni-axial cyclic stretch via SA channel activation in human lung fibroblasts. Key words: stretch-activated channel · gadolinium · calcium · fibroblast

Nuclear factor (NF)-B p50 protein is involved in promoting survival in hippocampal neurons after trimethyltin (TMT)-injury. In the current study, hippocampal NF-B activity was examined and quantitated from transgenic B-lacZ reporter mice after chemical-induced injury. NF-B activity was localized primarily to hippocampal neurons and significantly elevated over that in saline-treated mice between 4 and 21 days after TMT injection. Seven days after TMT injection, a timepoint of elevated NF-B activity, gene expression in the hippocampus was studied by microarray analysis through comparison of expression profiles between treated nontransgenic and p50-null mice with their saline-injected controls. Seventeen genes increased in nontransgenic TMT-treated mice relative to saline-treated as well as showing no increase in p50-null mice, indicating a role for p50 in their regulation. One of these genes, the Na+, K+-ATPase- subunit, was detected in brain for the first time. Several of the genes modulated by NF-B are potentially related to neuroplasticity, providing additional evidence that this transcription factor is a neuroprotective signal in the hippocampus. Key words: signal transduction Na+, K+-ATPase neurodegeneration NF-B p50 transcription factors


Proprotein convertases (PCs) are evolutionarily conserved enzymes responsible for processing the precursors of many bioactive peptides in mammals. The invertebrate homologues of PC2 play important roles during development that makes the enzyme a good target for practical applications in pest management. Screening of a plant nematode Heterodera glycines cDNA library resulted in isolation of a full-length clone encoding a PC2-like precursor. The deduced protein (74.2 kD) exhibits strong amino acid homology to all known PC2s, including human, and shares the main structural characteristics: signal peptide; prosegment; catalytic domain, with D/H/S catalytic triad, PC2-specific residues, and 7B2 binding sites; P domain (with RRGDT pentapeptide); and carboxyl terminus. Comparative analysis of PC2s from 15 species discloses the presence of an insert in the catalytic domain unique to nematodes. Expression of PC2-like mRNA found in eggs and juveniles was undetectable in adult stages of *H. glycines*. Nucleotide analysis reveals distinctive differences in base composition and codon usage between *H. glycines* and Caenorhabditis elegans PC2s. The *H. glycines* cDNA clone encoding PC2 is the first one isolated from plant-parasitic nematodes. Key words: cDNA cloning · (G+C) content · plant-parasitic nematode · Heterodera glycines


Glucose-dependent insulinoergic polypeptide (GIP) is secreted postprandially and acts in concert with glucose to stimulate insulin secretion from the pancreas. Here, we describe a novel pathway for the regulation of GIP receptor (GIPR) expression within clonal b-cell lines, pancreatic islets, and in vivo. High (25 mM) glucose was able to significantly reduce GIPR mRNA levels in INS(832/13) cells after only 6 h. In contrast, palmitic acid (2 mM) and WY 14643 (100 mM) stimulated approximate doublings of GIPR expression in INS(832/13) cells under low (5.5 mM), but not high (25 mM), glucose conditions, suggesting that fat can regulate GIPR expression via PPARa in a glucose-dependent manner. Both MK-886, an antagonist of PPARa, and a dominant
negative form of PPARα transfected into INS(832/13) cells caused a significant reduction in GIPR expression in low, but not high, glucose conditions. Finally, in hyperglycemic clamped rats, there was a 70% reduction in GIPR expression in the islets and a 71% reduction in GIP-stimulated insulin secretion from the perfused pancreas. Thus, evidence is presented that the GIPR is controlled at normoglycemia by the fatty acid load on the islet; however, when exposed to hyperglycemic conditions, the GIPR is down-regulated, which may contribute to the decreased responsiveness to GIP that is observed in type 2 diabetes. Key words: PPARα · type 2 diabetes · Zucker rats · INS(832/13) cells · gastric inhibitory polypeptide


http://www.fasebj.org/cgi/content/abstract/16/9/1077

Reactive oxygen species such as superoxide and hydroxyl radicals have been implicated in the pathogenic growth of various cell types. The molecular mechanisms involved in redox-sensitive cell growth control are poorly understood. Stimulation of cultured vascular smooth muscle cells (VSMC) with xanthin/xanthin oxidase (X/XO) increases proliferation, whereas stimulation with hydrogen peroxide and Fe3+NTA (H-Fe) causes growth arrest of VSMC. Differential Display led to the identification of two novel, differentially regulated redox-sensitive genes. The dominant negative helix-loop-helix protein Id3 is induced by X/XO and down-regulated by H-Fe. The transcription factor gut-enriched Kruppel-like factor (GKLF) is induced by H-Fe but not by X/XO. Induction of GKLF and inhibition of Id3 via transfection experiments leads to growth arrest, whereas overexpression of Id3 and inhibition of GKLF cause cell growth. Id3 down-regulation is induced via binding of GKLF to the Id3 promoter and concomitantly reduced Id3 gene transcription rate. GKLF induction by H-Fe is mediated through hydroxyl radicals, p38MAP kinase-, calcium-, and protein synthesis-dependent pathways. Id3 is induced by X/XO via superoxide, calcium, p38, and p42/44 MAP kinase. GKLF induces and Id3 depresses expression of p21WAF1/Cip1, p27KIP1, p53. Induction of Id3 is accomplished by angiotensin II via superoxide release. A vascular injury mouse model revealed that Id3 is overexpressed in proliferating vascular tissue in vivo. These findings reveal novel mechanisms of redox-controlled cellular proliferation involving GKLF and Id3 that may have general implications for our understanding of vascular and nonvascular growth control.--Nickenig, G., Baudler, S., Muller, C., Werner, N., Welzel, H., Strehlow, K., Bohm, M. redox-sensitive vascular smooth muscle cell proliferation is mediated by GKLF and Id3 in vitro and in vivo.


http://www.fasebj.org/cgi/content/abstract/16/12/1497

Interleukin 1β (IL-1β) is a proinflammatory cytokine that maintains thermal hyperalgesia and facilitates the release of calcitonin gene-related peptide from rat cutaneous nociceptors in vivo and in vitro. Brief applications of IL-1β to nociceptive neurons yielded a potentiation of heat-activated inward currents (Iheat) and a shift of activation threshold toward lower temperature without altering intracellular calcium levels. The IL-1β-induced heat sensitization was not dependent on G-protein-coupled receptors but was mediated by activation of protein kinases. The nonspecific protein kinase inhibitor staurosporine, the specific protein kinase C inhibitor bisindolylmaleimide BIM1, and the protein tyrosine kinase inhibitor genistatin reduced the sensitizing effect of IL-1β whereas negative controls were ineffective. RT-PCR and in situ hybridization revealed IL-1RI but not RII expression in neurons rather than surrounding satellite
cells in rat dorsal root ganglia. IL-1β acts on sensory neurons to increase their susceptibility for noxious heat via an IL-1RI/PTK/PKC-dependent mechanism.--Obreja, O., Rathee, P. K., Lips, K. S., Distler, C., Kress, M. IL-1β potentiates heat-activated currents in rat sensory neurons: involvement of IL-1RI, tyrosine kinase, and protein kinase C.


http://www.fasebj.org/cgi/content/abstract/17/10/1277

Consumption of red meat is associated with increased colon cancer risk. Our previous work indicated that this association might be due to the heme content of red meat. In rat studies, dietary heme increased colonic cytotoxicity and epithelial cell turnover, carcinogenesis biomarkers. Here we apply DNA microarray technology to examine effects of heme on colonic gene expression. A rat colon-specific microarray was constructed and hybridized in duplicate to RNA extracts from colon scrapings of rats fed diets with or without heme (n=6-7). We were able to reproducibly identify changes in colonic mRNA abundance in response to heme. Most striking was a >10-fold down-regulation of a single rat gene, an unprecedented gene-modulating effect of a dietary component. Based on homology, the novel gene encodes a pentraxin, the first identified in colon. Pentraxins are postulated to be involved in dealing with dying cells. Quantitative PCR confirmed the strong heme-induced down-regulation of this gene, which we named mucosal pentraxin (Mptx). Overall, our data support the efficacy of cDNA array expression profiling to investigate effects of specific nutrients in an in vivo system and may provide an approach to establishing markers for diet-induced stress of mammalian colonic mucosa.--van der Meer-van Kraaij, C., van Lieshout, E. M. M., Kramer, E., van der Meer, R., Keijer, J. Mucosal pentraxin (Mptx), a novel rat gene 10-fold down-regulated in colon by dietary heme.

FEBS J. (2)


http://content.febsjournal.org/cgi/content/abstract/272/4/942

Planarians are one of the simplest animal groups with a central nervous system. Their primitive central nervous system produces large quantities of a variety of neuropeptides, of which many are amidated at their C terminus. In vertebrates, peptide amidation is catalyzed by two enzymes [peptidylglycine {alpha}-hydroxylating monooxygenase (PHM) and peptidyl-{alpha}-hydroxylglycine {alpha}-amidating lyase] acting sequentially. In mammals, both enzymatic activities are contained within a single protein that is encoded by a single gene. By utilizing PCR with degenerate oligonucleotides derived from conserved regions of PHM, we succeeded in cloning a full-length cDNA encoding planarian PHM. The deduced amino acid sequence showed full conservation of five His residues and one Met residue, which bind two Cu atoms that are essential for the activity of PHM. Northern blot analysis confirmed the expression of a PHM mRNA of the expected size. Distribution of the mRNA was analyzed by in situ hybridization, showing specific expression in neurons with two morphologically distinct structures, a pair of the
ventral nerve cords and the brain. The distribution of PHM was very similar to that of cytochrome b561. This indicates that the ascorbate-related electron transfer system operates in the planarian central nervous system to support the PHM activity and that it predates the emergence of Plathelminthes in the evolutionary history.


http://content.febsjournal.org/cgi/content/abstract/272/6/1440

The upp gene, encoding uracil phosphoribosyltransferase (UPRTase) from the thermoacidophilic archaeon Sulfolobus solfataricus, was cloned and expressed in Escherichia coli. The enzyme was purified to homogeneity. It behaved as a tetramer in solution and showed optimal activity at pH 5.5 when assayed at 60 (degrees)C. Enzyme activity was strongly stimulated by GTP and inhibited by CTP. GTP caused an approximately 20-fold increase in the turnover number kcat and raised the Km values for 5-phosphoribosyl-1-diphosphate (PRPP) and uracil by two- and >10-fold, respectively. The inhibition by CTP was complex as it depended on the presence of the reaction product UMP. Neither CTP nor UMP were strong inhibitors of the enzyme, but when present in combination their inhibition was extremely powerful. Ligand binding analyses showed that GTP and PRPP bind cooperatively to the enzyme and that the inhibitors CTP and UMP can be bound simultaneously (KD equal to 2 and 0.5 (micro)M, respectively). The binding of each of the inhibitors was incompatible with binding of PRPP or GTP. The data indicate that UPRTase undergoes a transition from a weakly active or inactive T-state, favored by binding of UMP and CTP, to an active R-state, favored by binding of GTP and PRPP.

FEBS Letters (157)


http://www.sciencedirect.com/science/article/B6T36-3S0F2K8-D/2/8001c6f5ba11652a714af6e2b17cad62

Solitary long terminal repeats (LTRs) of the human endogenous retroviruses, scattered in several thousand copies throughout the human genome, are potentially capable of affecting the expression of closely located genes. To assess their regulatory potential, the LTR sequences of one of the most abundant HERV families (HERV-K) were screened for the presence of binding sites for the host cell nuclear factors using mobility shift and UV-crosslinking assays. It was shown that the LTR sequences of two subfamilies harbor a specific binding site for a complex consisting of at least three proteins, ERF1, ERF2 and ERF3 of 98, 91 and 88 kDa apparent molecular mass, respectively. This binding site is located in the 5' region of the LTR U3 element. The preservation of the specific protein binding site in different HERV-K LTR sequences suggests their possible role in regulation of nearby located genes.

http://www.sciencedirect.com/science/article/B6T36-45K11DT-12/2/ce2cc1ed36c9c1dad8c5bbc411e03b13

To gain an understanding of the mechanism by which the subcellular distribution of cytosolic epoxide hydrolase (cEH) is directed, we have analyzed the carboxy terminal region of rat liver cEH by means of cDNA cloning to define the structure of its possible peroxisomal targeting sequence (PTS). Purified cEH was subjected to peptide analysis following endoproteinase Glu-C digestion and HPLC-separation of the fragments. The obtained sequence information was used to perform PCR experiments resulting in the isolation of a 680 bp cDNA clone encoding the carboxy terminus of cEH. The deduced amino acid sequence displays a terminal tripeptide Ser-Lys-Ile which is highly homologous to the PTS (Ser-Lys-Leu) found in other peroxisomal enzymes. This slight difference appears to be sufficient to convert the signal sequence into an impaired and therefore ambivalent PTS, directing the enzyme partly to the peroxisomes and allowing part to reside in the cytosol.


http://www.sciencedirect.com/science/article/B6T36-449TJ2W-58/2/38ccb896beefb57237bb81c097fda5df

We have obtained and characterized 11 monoclonal antibodies (mAbs) specific for different domains of human tenascin (TN). Five of these mAbs reacted with epitopes contained in the TN area that undergoes alternative splicing and are thus able to recognize specific TN isoforms. These mAbs are a useful tool to study the expression and distribution of TN and its different isoforms in normal and pathological tissues.


http://www.sciencedirect.com/science/article/B6T36-3V5MRHV-13/2/afe9425316706f02f18c9c5946408a0b

Recently, we reported the purification of the novel enzyme limonene-1,2-epoxide hydrolase involved in limonene degradation by Rhodococcus erythropolis DCL14. The N-terminal amino acid sequence of the purified enzyme was used to design two degenerate primers at the beginning and the end of the 50 amino acids long stretch. Subsequently, the complete limonene-1,2-epoxide hydrolase gene (limA) was isolated from a genomic library of R. erythropolis DCL14 using a combination of PCR and colony hybridization. The limA gene encoded a 149-residue polypeptide with a deduced molecular mass of 16.5 kDa. It was functionally expressed in Escherichia coli. The amino acid sequence of limA contains neither any of the conserved regions of the [alpha],[beta]-hydrolase fold enzymes, to which most of the previously reported epoxide hydrolases belong, nor any of the conserved motifs present in leukotriene A4 hydrolase. The structural data presented in this paper confirm previous physical and biochemical findings [van der Werf et al. (1998) J. Bacteriol. 180, 5052-5057] that limonene-1,2-epoxide hydrolase is the first member of a new class of epoxide hydrolases.

http://www.sciencedirect.com/science/article/B6T36-3Y158RJ-H/2/cfd81a522b6a0c6d2a2075e475dd8f32

The chloride channel CIC-1 is required to maintain a normal excitability of mature muscle fibers; its blockade leads to hyperexcitability, the hallmark of the disease myotonia. In mouse and rat myotubes, representing the embryonic stage of muscle, CIC-1 mRNA is not detectable by Northern blotting. From neonatal to adult, CIC-1 expression increases at least fourfold. Using RT-PCR and hybridization on cultured myotubes were found CIC-1 mRNA at a level of 0.4-1.1% of that in mature mouse muscle, and <=0.01% in myoblasts, at stages when desmin mRNA levels are already high. The level of CIC-1 mRNA is thus a sensitive and specific indicator of the maturation of skeletal muscle cells.


http://www.sciencedirect.com/science/article/B6T36-3YGD3FC-W/2/29efc1e06f0ad666d451e1c8e2590661

For reconstruction or repair of damaged tissues, an artificially regulated switch from proliferation to differentiation would be of great advantage. To achieve conditional myogenesis, we expressed MyoD in mouse C3H 10T1/2 fibroblastic cells, using a gene regulation system based on the synthetic steroid RU 486. By stable co-transfection of a plasmid construct with the RU 486 dependent activator and an integrating inducible MyoD construct, a cell clone, designated 10T-RM, was obtained in which MyoD expression was stringently controlled by RU 486. 12 h after addition of 10 nM RU 486 to 10T-RM cells, saturation levels of MyoD mRNA were observed and >=2 days later, mRNA for embryonal myosin heavy chain (MyHCemb) was abundant and mononucleated cells fused into myotubes.


http://www.sciencedirect.com/science/article/B6T36-3R7B21X-1H/2/e855be2db90077b8b1efa1292f7b62000

Store-regulated Ca2+ entry represents a major mechanism for Ca2+ influx in non-excitable cells although many details remain to be evaluated including the identification of cation entry channels. Recently human homologues of the Drosophila proteins TRP and TRPL, have been described (TRPC1, TRPC1A, HTRP1) and suggested as candidate cation channels. In this study we sought to examine if the producers of blood platelets, megakaryocytic cells (using the cell lines MEG01, DAMI, HEL), expressed these genes. RNA was prepared from the cell lines and platelets and converted to cDNA. The cDNA was then subjected to 30-35 cycles of PCR using gene specific primers for TRPC1-3. PCR products of the expected sizes were observed for all three TRPC genes in the three cell lines. Direct sequencing confirmed their identity. Additionally for TRPC1, a larger species, and for TRPC2, a smaller species was detected in all three cell lines with sequencing revealing the fragments to contain TRPC sequence, suggesting that they were either products of alternative splicing events or from closely related genes. These results suggest that
TRPC genes are expressed in megakaryocytic cell lines and that the TRPC proteins may play a role in mediating cation influx in both megakaryocytes and platelets. (c) 1997 Federation of European Biochemical Societies.


http://www.sciencedirect.com/science/article/B6T36-3TMXY8S-G/2/9abcf3825afa2a95e652f9aa2cfcf902

Androgens are essential for normal prostate physiology and are intimately associated with the growth and progression of prostate cancer. However, few androgen regulated genes in the prostate have been identified. Using the mRNA differential display technique a 164-bp cDNA fragment was identified as being androgen regulated in the human prostate. Nucleotide sequence analysis of this fragment revealed 84% homology with the gene encoding the cytoskeletal protein talin. Confirmation of the androgen regulation of this gene was carried out using Northern analysis. Primary prostatic stromal cells treated with conditioned medium (CM) from androgen-treated primary prostatic epithelial cells showed an approximate 2-fold reduction in talin mRNA levels compared with stromal cells treated with CM from epithelial cells not exposed to androgens. Expression of talin mRNA in human prostatic tissue was confirmed by in situ hybridisation. The highest levels of expression were present in the epithelial cells, with lower levels of expression in the stroma. Thus, androgen regulation of talin expression may play a role in normal and/or aberrant growth and development of the prostate.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-4T/2/a9bd9c45fdefc6f93375d5b6b17282cece

Androgens are essential for normal prostate physiology and have a permissive role in the development and progression of prostate cancer. Using the mRNA differential display technique, ornithine decarboxylase (ODC) was identified to be up-regulated by androgens in human prostatic LNCaP cells. On Northern analysis, the induction of ODC expression by 10 nM androgen was rapid and continued up to 48 h exposure with a maximum 6.3-fold up-regulation. The anti-androgen Casodex inhibited the androgen-induced up-regulation of ODC, whereas the protein synthesis inhibitor cycloheximide did not. Together these data suggest that regulation is mediated through the androgen receptor protein and does require secondary protein synthesis, respectively. The kinetics of induction of ODC were almost identical to those of prostate specific antigen. Taken together these data suggest that ODC is directly regulated by androgens in LNCaP cells.


http://www.sciencedirect.com/science/article/B6T36-46P9TFM-2/2/75cde191a7eeef1bd3d35eddf8e82e70
The products of the human ARG gene and the human ABL gene characterize the Abelson family of non-receptor tyrosine protein kinases. Both genes are ubiquitously expressed. The interactions of these two similar protein kinases are still not well known, although it has been suggested that they could cooperate, with redundant actions, to provide intracellular signals in the cells. Lymphopenia occurs in mice with homozygous disruption of c-abl, indicating that in certain tissues Arg is unable to substitute c-abl functions. In B and T lymphoid cell lines at different stages of differentiation, we studied, by a reverse transcriptase-competitive polymerase chain reaction and Western blotting, Arg and c-abl in order to evaluate whether the expression pattern of the two genes could give insight as to why they do not exhibit overlapping roles in lymphocytes and whether the product levels of the two genes are related to lymphoid differentiation. The data showed that their expression is differently modified in lymphoid B cell lines. The highest Arg transcript and protein levels are in the mature B cells.


http://www.sciencedirect.com/science/article/B6T36-3WHK65P-D/2/0f079da7577bc990004867817b941790f

An enamine mechanism-based inactivator of mammalian [delta]-aminobutyric acid aminotransferase, 4-amino 5-fluoropentanoic acid is a potent inhibitor of cell growth and pigment formation in the cyanobacterium Synechococcus PCC 6301. It was demonstrated that 4-amino 5-fluoropentanoic acid inhibits the aminolaevulinate synthesis at glutamate 1-semialdehyde aminotransferase and that in the mutant obtained by exposing cells to 40 [mu]M 4-amino 5-fluoropentanoic acid, this enzyme was insensitive to the inhibitor. The specific activity of glutamate 1-semialdehyde aminotransferase in cell extracts was lower in the mutant, although the cell growth rate was unaffected. The decrease in sensitivity to 4-amino 5-fluoropentanoic acid in the mutant is due to a structural gene mutation, a single base change in the hemL gene resulting in a S162T substitution in the gene product.


http://www.sciencedirect.com/science/article/B6T36-3S4XR8S-11/2/3b699601d8a57859706d6d6aaedcf709

Human platelets express several sarco/endoplasmic reticulum Ca2+ATPase (SERCA) isoenzymes: SERCA2b of 100 kDa apparent molecular mass and two distinct enzymes of 97 kDa, one of them identified as being the SERCA3a isoform. The molecular identity of the third enzyme specifically recognized by the PL/IM430 monoclonal antibody has remained elusive. First, the study of the 3'-end part of platelet SERCA3 mRNA, by means of RT-PCR amplification using sets of primers covering the N-3 to N (ultimate) exons of the human SERCA3 sequence, revealed the presence of two distinct mRNA sequences, SERCA3a and a longer variant. Second, this additional sequence was identified as SERCA3b and found to refer to the insertion of a new exon of 73 bp, located at bp 349 from the beginning of the intronic sequence, linking the penultimate (N-1) exon to the last exon (N) of the human SERCA3 gene. Third, a relationship between the expression of this SERCA3b mRNA and the PL/IM430 recognizable SERCA protein was observed. SERCA3b mRNA was found to be absent in epithelial HeLa cells not recognized by the PL/IM430 antibody and the expression of this SERCA3b RNA species correlated with that of the SERCA protein recognized by PL/IM430 which was down-modulated in the platelet precursor megakaryocytic CHRF 288-11 cell line as well as upon in vitro lymphocyte activation.
Taken together, these results strongly support the notion of the presence of the SERCA3b protein in human cells by showing SERCA3b mRNA in platelets and the fact that the protein corresponding to this mRNA species is very likely the 97 kDa protein recognized by the PL/IM430 antibody.


http://www.sciencedirect.com/science/article/B6T36-44WKGD7-D/2/27261a0df74f99783a15c7f70bd08648

The multifunctional prohormone, proopiomelanocortin (POMC), is processed in the melanotrope cells of the pituitary pars intermedia at pairs of basic amino acid residues to give a number of peptides, including [alpha]-melanocyte-stimulating hormone ([alpha]-MSH). This hormone causes skin darkening in amphibians during background adaptation. Here we report the complete structure of Xenopus laevis prohormone convertase PC2, the enzyme thought to be responsible for processing of POMC to [alpha]-MSH. A comparative structural analysis revealed an overall amino acid sequence identity of 85-87% between Xenopus PC2 and its mammalian counterparts, with the lowest degree of identity in the signal peptide sequence (28-36%) and the region amino-terminal to the catalytic domain (59-60%). The occurrence of a second, structurally different PC2 protein reflects the expression of two Xenopus PC2 genes. The expression pattern of PC2 in the Xenopus pituitary gland of black- and white-adapted animals was found to be similar to that of POMC, namely high expression in active melanotrope cells of black animals. This observation is in line with a physiological role for PC2 in processing POMC to [alpha]-MSH.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-26/2/464688ac2a75e10079d3906dc4f31c08

P2X receptors are ion channels gated by extracellular ATP. We report here cloning of a P2X2 receptor splice variant (P2X2-2) carrying a 207 bp deletion in the intracellular C-terminus and the analysis of the corresponding genomic structure of the P2X2 gene. P2X2-2 is as highly expressed as the original P2X2 sequence in various tissues. ATP-activated currents mediated by heterologously expressed P2X2 or P2X2-2 receptors showed significant differences in desensitization time constants and steady-state currents in the continuous presence of ATP. These results imply functional differences between cells differentially expressing these P2X2 isoforms.(c) 1997 Federation of European Biochemical Societies.


http://www.sciencedirect.com/science/article/B6T36-4BX0GRB-2/2/655e12e7db1415a975c42d4156f5ba15

Damage to the central nervous system triggers rapid activation and specific migration of glial cells towards the lesion site. There, glial cells contribute heavily to secondary neuronal changes that take place after lesion. In an attempt to identify the molecular cues of glial activation following
brain trauma we performed differential display reverse transcription-polymerase chain reaction screenings from lesioned and control hippocampus. Here we report on the identification of the macrophage/microglia activation factor (MAF), a new membrane protein with seven putative transmembrane domains. Expression analysis revealed that MAF is predominantly expressed in microglial cells in the brain, and is upregulated following brain lesion. Overexpression of MAF in non-glial cells shows an intracellular codistribution with the lysosomal marker endosome/lysosome-associated membrane protein-1 (lamp-1). Furthermore, MAF-transfected cells show that MAF is primarily associated with late endosomes/lysosomes, and that this association can be disrupted by activation of protein kinase C-dependent pathways. In conclusion, these results imply that MAF is involved in the dynamics of lysosomal membranes associated with microglial activation following brain lesion.


http://www.sciencedirect.com/science/article/B6T36-3YNY44-CC/2/1cb7bfe6fff21d80b805fc4b6316749

Rab proteins are small GTPases involved in the regulation of membrane traffic. Rab5a has been shown to regulate transport in the early endocytic pathway. Here we report the isolation of cDNA clones encoding two highly related isoforms, Rab5b and Rab5c. The two proteins share with Rab5a all the structural features required for regulation of endocytosis. Rab5b and Rab5c colocalize with the both transferrin receptor and Rab5a, stimulate the homotypic fusion between early endosomes in vitro and increase the rate of endocytosis when overexpressed in vivo. These data demonstrate that three Rab5 isoforms cooperate in the regulation of endocytosis in eukaryotic cells.


http://www.sciencedirect.com/science/article/B6T36-44DSMYD-G/2/8c943da3fb99724ce1e532f170b03d4e

Starting from total pancreatic mRNAs, the classical guinea pig pancreatic lipase was cloned using rapid amplification of 3' and 5' cDNA ends. Internal oligonucleotide primers were designed from a partial cDNA clone including the region coding for the lid domain. Using this strategy, we did not amplify the cDNA corresponding to the pancreatic lipase related protein 2 in which the lid domain is deleted. Amino acid sequences of the classical guinea pig pancreatic lipase and the related protein 2 were compared based on the primary and tertiary structures of the classical human pancreatic lipase. Their distinct physiological roles are discussed in the light of functional amino acid differences.


http://www.sciencedirect.com/science/article/B6T36-3R7B21X-5M/2/dbd4b55516dd718b3fd3739015fb49
Dynein heavy chains (DHCs) are the main components of multisubunit motor ATPase complexes called dyneins. Axonemal dyneins provide the driving force for ciliary and flagellar motility. Recent molecular studies demonstrated that multiple DHC isoforms are produced by separate genes. We describe the isolation of five human axonemal DHC genes. Analysis of the human genomic clones revealed the existence of intronic sequences that were used to demonstrate that human axonemal DHC genes are located on different chromosomes. The cloned human DHC sequences were integrated into an evolutionary approach based on phylogenetic analysis. Tissue expression studies showed that these human axonemal DHCs are expressed in testis and/or trachea, two tissues with axonemal structures that can be altered in primary ciliary dyskinesia, making DHC genes strong candidates in the genesis of these human diseases.


http://www.sciencedirect.com/science/article/B6T36-3V7JH3Y-5/2/95992c921456c775f2e80834cf2f4b38

We have isolated a 1785-bp complementary DNA (cDNA) encoding the murine P2X7 receptor subunit from NTW8 mouse microglial cells. The encoded protein has 80% and 85% homology to the human and rat P2X7 subunits, respectively. Functional properties of the heterologously expressed murine P2X7 homomeric receptor broadly resembled those of the P2X7 receptor in the native cell line. However, marked phenotypic differences were observed between the mouse receptor, and the other P2X7 receptor orthologues isolated with respect to agonist and antagonist potencies, and the kinetics of formation of the large aqueous pore.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-W/2/7b8fdbbc7a5883d32fe9fe040bc463e

Binding assays using 2-[125I]iodomelatonin revealed high-affinity, guanosine 5'-O-(3-thiotriphosphate) sensitive, melatonin binding sites (Bmax 1.1 fmol/mg protein) in the human embryonic kidney cell line HEK293. Competition studies using the selective melatonin receptor antagonist luzindole and RT-PCR techniques identified these sites as human Mel1a melatonin receptors. Challenge of HEK293 cells with 1 [mu]M melatonin had no effect on forskolin stimulated cyclic AMP levels, whereas in HEK293 cells engineered to stably over-express the human Mel1a melatonin receptor (Bmax>400 fmol/mg protein) melatonin dose-dependently inhibited stimulated cyclic AMP levels (IC50 7.7 pM). These data may indicate that certain tissues, expressing low levels of G protein-coupled melatonin receptors, do not display melatonin mediated inhibition of cAMP.


http://www.sciencedirect.com/science/article/B6T36-3RD0S7F-3/J/2/ae30d3a2d113ad81cbb46408da5931f3
Using two synthetic oligonucleotides, we have constructed a new gene containing three zinc finger motifs of the Cys2-His2 type. We named this artificial gene 'Mago'. The Mago nucleotide triplets encoding the amino acid positions, described to be crucial for DNA binding specificity, have been chosen on the basis of the proposed recognition 'code' that relates the zinc finger's primary structure to the DNA binding target. Here we demonstrate that Mago protein specifically binds the 'code' DNA target, with a dissociation constant (Kd) comparable to the Kd of the well known Zif268 protein with its binding site. Moreover, we show that the deduced Mago 'code' and the 'experimental' selected DNA binding sites are almost identical, differing only in two nucleotides at the side positions.

http://www.sciencedirect.com/science/article/B6T36-3S0FJ2M-1K/2/909c4b9b98eed00395a74f4850d91ecb

This work was undertaken to establish the forms of the calpain inhibitor, calpastatin, expressed in the brain tissue. Five cDNA clones were obtained and the corresponding amino acid sequences were deduced. Three of these proteins contain an N-terminal domain (domain L) and four inhibitory repeats typical of the calpastatin molecule. The other two are truncated forms, containing the domain L, free or associated with a single inhibitory repeat. Other differences, due to exon skipping, produce calpastatin forms with different susceptibility to posttranslational modifications. The more represented mRNA form corresponds to a calpastatin molecule containing the four inhibitory domains. These results may be useful to understand the involvement of calpain in the onset of acute and degenerative disorders of the central nervous system.

http://www.sciencedirect.com/science/article/B6T36-42G6KMT-X/2/82b7cd1f871713efcea650d41a3c71cb

We have identified several cDNAs for the human Kir5.1 subunit of inwardly rectifying K+ channels. Alternative splicing of exon 3 and the usage of two alternative polyadenylation sites contribute to cDNA diversity. The hKir5.1 gene KCNJ16 is assigned to chromosomal region 17q23.1-24.2, and is separated by only 34 kb from the hKir2.1 gene (KCNJ2). In the brain, Kir5.1 mRNA is restricted to the evolutionary older parts of the hindbrain, midbrain and diencephalon and overlaps with Kir2.1 in the superior/inferior colliculus and the pontine region. In the kidney Kir5.1 and Kir2.1 are colocalized in the proximal tubule. When expressed in Xenopus oocytes, Kir5.1 is efficiently targeted to the cell surface and forms electrically silent channels together with Kir2.1, thus negatively controlling Kir2.1 channel activity in native cells.

http://www.sciencedirect.com/science/article/B6T36-3YNY51-CP/2/b377eb55bc02de4b01a70b83edebf4a
The lytic transglycosylases of Escherichia coli are involved in peptidoglycan metabolism and resemble the lysozymes not only in activity, but in the case of the 70 kDa soluble lytic transglycosylase (Slt70), also structurally. Here we report the cloning of the gene that encodes the 35 kDa soluble lytic transglycosylase (Slt35) of E. coli. Based on the sequence of the full-length gene, Slt35 is very likely to be a proteolytically truncated form of a slightly large protein. The homology between Slt35 and Slt70, albeit poor, indicates that the active site architecture of both proteins may be alike. Using the T-7 promoter system, Slt35 was overproduced in large quantities and purified to homogeneity for crystallographic purposes.


http://www.sciencedirect.com/science/article/B6T36-3YS2BTR-BS/2/a82315e5067843ebc06baf4349853a03

The distribution of mRNA encoding the inwardly rectifying K+ channel, BIR1 [1] was investigated in rat tissues, and a comparison made with the expression of related genes rcKATP and GIRK1 using the reverse transcription-polymerase chain reaction (RT-PCR). This showed BIR1 to be expressed in all areas of the brain examined, in the eye but not in any other peripheral tissue. This pattern was distinct from rcKATP and GIRK1. Additional in situ hybridisation studies of the central expression of BIR1 demonstrated high levels of BIR1 mRNA in the hippocampus dentate gyrus, taenia tecta and cerebellum and at lower levels in the cortex, habenerular nucleus, olfactory bulb, primary olfactory cortex, thalamus, pontine nucleus and amygdaloid nucleus.


http://www.sciencedirect.com/science/article/B6T36-3SY8DJK-3/2/1f858d7c977fe47f636009a0c843c694

The 11-cis-retinol dehydrogenase (11-cis-RoDH) gene encodes the short-chain alcohol dehydrogenase responsible for 11-cis-retinol oxidation in the visual cycle. The structure of the murine 11-cis-RoDH gene was used to reinvestigate its transcription pattern. An 11-cis-RoDH gene transcript was detected in several non-ocular tissues. The question regarding the substrate specificity of the enzyme was therefore addressed. Recombinant 11-cis-RoDH was found capable of oxidizing and reducing 9-cis-, 11-cis- and 13-cis-isomers of retinol and retinaldehyde, respectively. Dodecyl-[beta]-1-maltoside used to solubilize the enzyme was found to affect the substrate specificity. This is the first report on a visual cycle enzyme also present in non-retinal ocular and non-ocular tissues. A possible role in addition to its role in the visual cycle is being discussed.


http://www.sciencedirect.com/science/article/B6T36-3T8F754-M/2/a1167a9d0e2a388b34502241365768a2

Ecto-ATPase activities of melanocytes and human melanoma cell lines differing in the stage of progression were compared. A dramatic increase in ecto-ATPase activity above the level of
normal melanocytes was demonstrated in the differentiated melanomas and was followed by a gradual decrease with tumor progression. The characteristics of this enzymatic activity were consistent with CD39/ecto-ATP diphosphohydrolase (ATPDase) which was found to be the major ecto-ATP-hydrolysing enzyme in melanomas. Indeed, the expression of CD39 and the level of CD39 mRNA followed a similar pattern. Since CD39 is known to regulate homotypic adhesion and, supposedly, affects the disaggregation step, we suggest that overexpression of CD39 may enable tumor cells to reduce contacts with T-lymphocytes and escape from immunological recognition.


http://www.sciencedirect.com/science/article/B6T36-3WSMG1S-D/2/96a7586d86c612041c66a157c47002f9

In our search for genes involved in oyster immunity we isolated a cDNA encoding a polypeptide closely related to the mammalian I[kappa]B kinase (IKK) family. IKK proteins play a central role in cell signaling by regulating nuclear factor-[kappa]B (NF-[kappa]B) activation. We report here the cloning of an oyster IKK-like protein (oIKK) which possesses the characteristic organization of the mammalian IKK proteins, namely an amino-terminal kinase domain followed by a leucine zipper region and a carboxyl-terminal helix-loop-helix motif. When transfected into human cell lines, oIKK activated the expression of NF-[kappa]B-controlled reporter gene, whereas transfections with mutants of oIKK deleted within the kinase domain or within the helix-loop-helix motif respectively abolished and greatly reduced reporter gene activation. These results indicate that oIKK can replace the hIKK-[alpha] in catalyzing NF-[kappa]B nuclear translocation, and in triggering gene expression. Our results sustain the concept of an evolutionarily conserved signaling machinery in which IKK plays a major role.


http://www.sciencedirect.com/science/article/B6T36-3YRNY98-H6/2/15424f52fde41f4eae0887842b933c8

PCR and primers derived from the telomeric repeat (CCCTAAA)n and from the tobacco subtelomeric tandemly repetitive sequence HRS60 (EMBL X12489) were used to amplify the region linking the two loci. A 131 bp PCR product was obtained both from total tobacco DNA and from the DNA fraction enriched for telomeres. Its sequence only consists of the telomeric primer and the attached region of the HRS60 repetitive unit up to the end of the sequence complementary to the HRS60 primer. The site of direct continuity between the two sequences is formed by a (dA)7 tract.


http://www.sciencedirect.com/science/article/B6T36-452YB6S-C/2/56ae20e9596f2d3fa35e85704e9462bd

A cDNA was cloned from a rabbit spleen cDNA library which encoded a G-protein [alpha] subunit
peptide of 374 amino acids, that at the peptide level exhibited 86% and 79% identity with human Gα16 and mouse Gα15, respectively. The rabbit Gα subunit cDNA was subcloned into a mammalian expression vector and transiently co-transfected into HEK-293 cells along with cDNAs encoding the human C3a, C5a, or nociceptin/orphanin FQ receptors. In all three cases the rabbit Gα subunit behaved similarly to Gα15 or Gα16 and effectively coupled the transfected receptors to intracellular calcium mobilization pathways. By nucleotide sequence homology and functional activity the rabbit Gα subunit appears to be the ortholog of human Gα16 and mouse Gα15.


http://www.sciencedirect.com/science/article/B6T36-44M430H-VJ/2/006a87e546cc920d3058628512f46f35

Multiple genes have been found to encode families of protein kinases in animals and yeasts. Little is known of the diversity of protein kinase families in plants. We have used the polymerase chain reaction to identify members of protein kinase gene family in rice. We have cloned eight partial cDNA sequences from which deduced amino acid sequences contained conserved sequences or amino acid residues characteristic of catalytic domains of eukaryotic protein serine/threonine kinases. Our results suggest that there is great complexity in the protein kinase gene family in plants and that protein phosphorylation may play an as important role in plants as in other eukaryotes.


http://www.sciencedirect.com/science/article/B6T36-3S9674S-6/2/50aa417df9c20af220e8bfc24f3d1257

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes S-methylation of aromatic and heterocyclic sulfhydryl compounds, including anticancer and immunosuppressive thiopurines. Here we report the isolation and functional characterization of the murine TPMT cDNA. The screening of expressed sequence tags database led to isolation of a murine cDNA clone containing an uninterrupted ORF encoding the protein with an amino acid sequence that is 82% similar and 78% identical to the human TPMT. The expression product of the murine cDNA in rabbit reticulocyte and wheat germ lysate coupled transcription-translation systems showed TPMT enzymatic activity. We conclude that the isolated cDNA clone represents the murine TPMT cDNA.


http://www.sciencedirect.com/science/article/B6T36-4B664KD-3/2/3f1913f535aaa3c55b1b83b2df32085bf

Peptide nucleic acids (PNAs) are effective antisense reagents that bind specific mRNAs preventing their translation. However, PNAs cannot cross cell membranes, hampering delivery to cells. To overcome this problem we made PNAs membrane-permeant by conjugation to the
lipophilic triphenylphosphonium (TPP) cation through a disulphide bond. The TPP cation led to efficient PNA uptake into the cytoplasm where the disulphide bond was reduced, releasing the antisense PNA to block expression of its target gene. This method of directing PNAs into cells is a significant improvement on current procedures and will facilitate in vitro and pharmacological applications of PNAs.


http://www.sciencedirect.com/science/article/B6T36-3SFWBN0-11/2/6839952eda9af06105a3c0282d2fbaa7a

The Serratia nuclease is a non-specific endonuclease which cleaves single- and double-stranded RNA and DNA. It is a member of a large family of related endonucleases, most of which are dimers of identical subunits, with the notable exception of the Anabaena nuclease which is a monomer. In order to find out whether the dimer state of the Serratia nuclease is essential for its function we have produced variants of this nuclease which based on the crystal structure (Miller, M.D. and Krause, K.L. (1996), Protein Science 5, 24-33) were expected to be unable to dimerise. We demonstrate here that these variants, H184A, H184N, H184T and H184R, are monomers and have the same secondary structure, stability towards chemical denaturation and activity as the wild-type enzyme. This allows to conclude that the dimeric state is not essential for the catalytic function of the Serratia nuclease. In contrast, the S179C variant which is also a monomer shows little activity, presumably because this amino acid substitution changes the structure of the enzyme.


http://www.sciencedirect.com/science/article/B6T36-41BV1R7-R/2/83c5f155f030628f1ebf4ed35b9f811

Genetic analysis of a Drosophila synaptotagmin (Syt) I mutant (AD3) has revealed that Tyr-334 within the C2B domain is essential for efficient Ca2+-dependent neurotransmitter release. However, little is known as to why a missense mutation (Tyr-334-Asn) disrupts the function of the C2B domain at the molecular level. Here, we present evidence that a Tyr-312 to Asn substitution in mouse Syt II, which corresponds to the Drosophila AD3 mutation, completely impairs Ca2+-dependent self-oligomerization activity mediated by the C2B domain but allows partial interaction with wild-type proteins in a Ca2+-dependent manner. This observation is consistent with the fact that the AD3 allele is homozygous lethal but complements another mutant phenotype. We also showed that the Ca2+-dependent C2B self-oligomerization is inhibited by inositol 1,3,4,5-tetrakisphosphate, a potent inhibitor of neurotransmitter release. All of these findings strongly support the idea that self-oligomerization of Syt I or II is essential for neurotransmitter release in vivo.


http://www.sciencedirect.com/science/article/B6T36-3Y158VW-
We had previously identified an estrogen responsive protein ULF-250, synthesized and secreted by the estrous rat uterus, which is immunologically distinct from complement C3 and [alpha]2-macroglobulin. The N-terminal microsequencing of ULF250 followed by sequence homology analysis showed that this protein is a new member of a class of estrogen responsive proteins in the uterus. Polymerase chain reaction with a ULF-250 specific primer yielded partial sequence information of its message. The observed pattern of ULF-250 message in the uterus during the various stages of the reproductive cycle in the rat suggested a possible regulation of ULF-250 message by 17[beta]-estradiol. Upstream sequencing of ULF-250 message and its promoter domains would provide insight into the mechanism of its regulation by estradiol.


We elucidated the intron-exon boundaries of the 15 coding exons of the human cystathionine [beta]-synthase (CBS) gene in order to establish an improved method based on PCR and direct sequencing for detection of CBS mutations. Using this method we identified the pathogenic mutations in two Danish siblings with CBS deficiency. Patients were compound heterozygotes: we detected the 833T->C mutation and a novel 22 bp deletion of exon 4 (493-514del) that introduces a frameshift and a stop codon immediately after the deletion. The deletion resulted in no detectable mRNA from this allele, as assessed by sequencing of cDNA. The established method represents an improvement of the existing method based on sequencing of cDNA because it permits the detection of mutations within the entire coding region of the CBS gene from a peripheral blood sample, including splice mutations and mutations resulting in the lack or a reduced amount of transcript.


Combining the patch-clamp method with single-cell reverse transcription polymerase chain reaction (scRT-PCR) a fusicoccin-induced current reflecting the activity of the plasma membrane H+ ATPase of lily pollen protoplasts was measured and subsequently, the ATPase-encoding mRNAs were collected and amplified. Southern blot signals were observed in all 'patch-catch' experiments and could be detected even in 2560-fold dilutions of the pollen contents. H+ ATPase mRNAs were detectable only in the vegetative but not in the generative cell of pollen as confirmed by immunolocalisation. In 15% of the scRT-PCR experiments, a random non-reproducibility of the PCR was observed, probably caused by varying amounts of ATPase mRNAs in the protoplasts.

Results from in vivo and from serum-free primary cultures of Ehrlich cells suggest that the expression of mitogen-regulated protein/proliferin (MRP/PLF) mRNAs is not essential for proliferation of this murine tumor. Two sizes for MRP/PRL-related open reading frames (ORFs) have been detected by reverse transcription/PCR amplification. They are almost identical to that reported for PLF-1; but 20% of the amplified cDNA included a shorter ORF, which lacks the entire sequence corresponding to that of the exon 3 of the mrp/plf genes. Ehrlich carcinoma may represent a good model to study regulation of expression and physiological roles of MRP/PLFs in vivo.


Rab11a is a member of the rab-branch of the ras-like small GTP-binding protein superfamily that is associated with both constitutive and regulated secretory pathways. Using a direct procedure for cDNA cloning of small ras-related GTPases, that is based on the screening of eukaryotic cDNA expression libraries using [[alpha]-32P]GTP as a probe, we have isolated two cDNA clones encoding rab11a. Both clones share identical coding sequences, but differ in the length and sequence of their 3' untranslated regions (3'-UTR). Northern blot hybridisation analysis of various human tissues revealed indeed two mRNA species with lengths of 1.0 and 2.3 kb, respectively. Sequence analysis of the cDNAs identified two different putative polyadenylation signals (AATAAA) at positions 927 and 2302 of the larger transcript. In addition, the 3'-UTR of the larger transcript exhibited several AU-rich elements (ARE) that are believed to control gene expression by regulating the rate of mRNA degradation. Southern blots of human DNA digested with several rare restriction enzymes, and separated by pulse-field gel electrophoresis, yielded the same macro-restriction fragment pattern when hybridised with probes that discriminate between the two transcripts. Taken together, these findings imply that the two mRNA species originate from a single gene, which we have mapped to 15q21.3-q22.31, by the use of different polyadenylation sites. As expected, both rab11a-cDNAs yielded the same protein product when transiently expressed in COS-1 cells, and surprisingly, upregulated the proteome expression profile (de novo synthesis or posttranslational modification of preexisting proteins) of a few other, yet unknown GTP-binding proteins.


Siroheme is a uroporphyrinogen III-derivative used by sulfite reductase as a prosthetic group. We investigated in Saccharomyces cerevisiae the possible involvement in siroheme biosynthesis of three genes, MET1, MET8 and MET20. The MET1 gene from S. cerevisiae was cloned and shown to be the same gene as MET20. Sequence similarities as well as complementation studies indicate that Met1p and Met8p are both involved in siroheme biosynthesis. In addition, we show formally that S. cerevisiae does not need vitamin B12 for growth.

http://www.sciencedirect.com/science/article/B6T36-44M4167-8K/2/4c4ba27ad781c2e20e2b385c0e3e5a8d

The neuropeptides substance P and neurokinin A are synthesised from a family of precursor polypeptides encoded by the preprotachykinin A (PPT) gene. In addition to mRNA ([beta]-PPT) containing all 7 exons of the gene, alternatively spliced mRNAs lacking either exon 4 ([gamma]-PPT) or exon 6 ([alpha]-PPT) have been identified. We have determined the sequences of cDNA clones encoding four variants of PPT mRNA from rat dorsal root ganglion (DRG), including a novel mRNA species ([delta]-PPT) in which both exons 4 and 6 are absent. The sequence of [delta]-PPT predicts the existence of a novel tachykinin precursor polypeptide.


http://www.sciencedirect.com/science/article/B6T36-3YS2BCS-1X/2/24b595362cf31f95db54522b38cdb3c

Growth-blocking peptide (GBP) is an insect biogenic peptide that prevents the onset of metamorphosis from larva to pupa. A cDNA coding for GBP is described. Mixed oligonucleotides derived from a GBP peptide sequence were used to generate amplified DNA by the polymerase chain reaction (PCR). Based on the sequence of the amplified DNA, a 41 bases oligonucleotide was designed for screening a cDNA library which was constructed from the armyworm Pseudalata separata larvae parasitized with the parasitic wasp Cotesia kariyai. The cloned cDNA for GBP was 809 base pairs in length. An open reading frame of 429 base pairs encodes a pre-pro-peptide of 143 amino acid residues in which GBP is localized at the C-terminal region, and other three peptides including a putative signal peptide and appropriate processing sites for endoproteolytic cleavage precede the GBP sequence. Northern blot analyses demonstrate the presence of a 800-base mRNA transcript in fat body and a 2.5-kilobase transcript in brain and nerve cord, suggesting the possibility that the transcription of GBP gene is regulated in a tissue-dependent manner. This interpretation was supported by isolating a GBP cDNA fragment from cDNA pool of brain-nerve cords. GBP mRNA is constantly expressed in both parasitized and non-parasitized last instar larvae and there is no difference in the levels of the mRNA between both larvae, thus indicating that parasitism may effect on translational or posttranslational level to elevate plasma GBP concentration.


http://www.sciencedirect.com/science/article/B6T36-4BYP29F-5/2/8df39ad81b624a14e73f7e251c7f50ae2

Inducible nitric oxide (NO) synthase (iNOS) appears to be a marker of tumor progression in colon carcinogenesis. Here we investigated effects of NO on selected chemokines that differentially regulate angiogenesis, namely pro-angiogenic interleukin (IL)-8 as well as tumor-suppressive interferon-inducible protein-10 (IP-10) and monokine induced by interferon-[gamma] (MIG). These chemokines are expressed by DLD-1 colon carcinoma cells after stimulation with IL-
Expression of IL-8 was markedly upregulated by NO. Moreover, NO enhanced expression of vascular endothelial growth factor (VEGF). In contrast, expression of IP-10 and MIG was suppressed by NO. The present data are consistent with previous observations that link NO to enhanced tumor angiogenesis and imply that NO-mediated upregulation of IL-8 and VEGF as well as downregulation of IP-10 and MIG may contribute to this phenomenon.


http://www.sciencedirect.com/science/article/B6T36-41CP99F-B/2/5e52bcb3ee2ea53bcde2745519991c992

NADPH:protochlorophyllide oxidoreductase (POR) catalyses the light-dependent reduction of protochlorophyllide to chlorophyllide, a key regulatory reaction in the chlorophyll biosynthetic pathway. POR from the cyanobacterium Synechocystis has been overproduced in Escherichia coli with a hexahistidine tag at the N-terminus. This enzyme (His6-POR) has been purified to homogeneity and a preliminary characterisation of its kinetic and substrate binding properties is presented. Chemical modification experiments have been used to demonstrate inhibition of POR activity by the thiol-specific reagent N-ethyl maleimide. Substrate protection experiments reveal that the modified Cys residues are involved in either substrate binding or catalysis.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-B6/2/29658101df0d2c27810564067f3fb297

The bchH gene of Rhodobacter capsulatus has been cloned into an expression strain of Escherichia coli. Following induction of expression of the BchH protein, it was found that the E. coli strain also accumulated porphyrins with the fluorescence properties of protoporphyrin and zinc protoporphyrin. It was also found that the soluble BchH protein increased the activity of S-adenosyl--methionine:magnesium protoporphyrin IX methyltransferase, when mixed with membranes of an expression strain of E. coli into which the bchM gene (which encodes the methyltransferase) had been cloned, as well as membranes of a bchH mutant of R. capsulatus.


http://www.sciencedirect.com/science/article/B6T36-464XYWD-H/2/72cd5cbe2f2229c88d7db7e2aa174e44

We have characterised a novel aldo-keto reductase (AKR7A5) from mouse liver that is 78% identical to rat aflatoxin dialdehyde reductase AKR7A1 and 89% identical to human succinic semialdehyde (SSA) reductase AKR7A2. AKR7A5 can reduce 2-carboxybenzaldehyde (2-CBA) and SSA as well as a range of aldehyde and diketone substrates. Western blots show that it is expressed in liver, kidney, testis and brain, and at lower levels in skeletal muscle, spleen heart and lung. The protein is not inducible in the liver by dietary ethoxyquin. Immunodepletion of
AKR7A5 from liver extracts shows that it is one of the major liver 2-CBA reductases but that it is not the main SSA reductase in this tissue.


http://www.sciencedirect.com/science/article/B6T36-3Y158TN-2F/2/bb91595d6c8e882513fe91fb0b3473b3

The cellular resistance to the potent anticancer agent cis-diamminedichloroplatinum(II) (cisplatin) is thought to be mediated by multiple mechanisms. The technique of differential display of mRNAs was applied to various cisplatin-resistant cell lines and the corresponding parental sensitive human bladder, prostatic, and head and neck cancer cells in order to identify genes that underlie cisplatin resistance. Twenty-four clones were confirmed by Northern blot analysis to be expressed differentially between resistant and the corresponding sensitive cells. Partial DNA sequences of the eight clones that showed a threefold or greater increase in expression in either the resistant cells (seven clones) or sensitive cells (one clone) revealed that two were derived from the T-plastin gene and one from the tissue factor gene. The abundance of T-plastin mRNA in cisplatin-resistant T24/DDP10 cell was ~12 times that in the parental T24 cells. Transfection of T24/DDP10 cells with a vector encoding full-length T-plastin antisense RNA demonstrated that reduced T-plastin expression was associated with increased sensitivity to cisplatin. These results are consistent with the hypothesis that several mechanisms participate cooperatively in the acquisition of cisplatin resistance in human cancer.


http://www.sciencedirect.com/science/article/B6T36-495NDGJ-R/2/8dabb3cf9dd28d0be3c60ef397c26f25

PK-120 is a substrate for plasma kallikrein (PK), recently purified from human plasma. Here we have established the cDNA sequence for human PK-120 mRNA. The deduced amino sequence of PK-120 revealed that it consists of 902 amino acid residues with a calculated mass of 116,423 Da. The putative cleavage sites by PK have been proposed, suggesting that PK-120 may be a precursor of a bioactive peptide. Most interestingly, PK-120 showed significant sequence identities to heavy chains (HCs) of the inter-[alpha]-trypsin inhibitor (ITI) superfamily.


http://www.sciencedirect.com/science/article/B6T36-3SY3H7M-M/2/3e1cb6af7893d9da1a8a514c8f3a7603

The cDNA of the peroxisomal membrane protein-1-like protein (PXMP1-L, synonyms: PMP69, P70R), a novel peroxisomal ATP binding cassette transporter of yet unknown function, has recently been cloned. The best known peroxisomal member of this protein family is the adrenoleukodystrophy protein, defects of which are the underlying cause of X-linked adrenoleukodystrophy (X-ALD). Here we describe the complete exon-intron structure (19 exons
and 18 introns covering 16.0 kb) of the human PXMP1-L gene, transcript variants, the localization on chromosome 14q24 by cytogenetic analysis and sequencing of the putative promoter region. PXMP1-L has been proposed to play a role as a modifier in determining the phenotypic variations observed in X-ALD. The data presented will enable sequence analysis of the PXMP1-L gene in X-ALD patients and facilitate the analysis of PXMP1-L function.


http://www.sciencedirect.com/science/article/B6T36-3YRNXMS-D/2/fed2117238298d424dtbd41c1dd625d

A class II type alcohol dehydrogenase from rat liver was characterized at the cDNA level after screening cDNA libraries in combination with PCR amplification of the 5'-part. The open reading frame translates into a polypeptide of 376 amino acid residues, which show 73% positional identity to the human class II enzyme. This suggests that the class II enzyme is the most variable form of the mammalian alcohol dehydrogenases. A deletion is apparent corresponding to position 294 of the human enzyme and amino acid residues unique to the rat protein of those interacting with the coenzyme NAD+ are found at positions 47, 51, 178, and 271. Position 47 is occupied by Pro instead of Arg or His found in most mammalian alcohol dehydrogenases. This exchanged residue will not hydrogen bond to the pyrophosphate of the coenzyme and will change the local environment around position 47 to strictly hydrophobic.


http://www.sciencedirect.com/science/article/B6T36-3S5BCS2-M/2/de3efd5f2e7c19e035bc3f7a1ec7ba7f

The collecting duct epithelium originates from the embryonic ureter by branching morphogenesis. Ontogeny-dependent changes of CFTR mRNA expression were assessed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in primary monolayer cultures of rat ureteric buds (UB) and cortical collecting ducts, microdissected at different embryonic and postnatal developmental stages. The amount of wild-type CFTR-specific PCR product in UB declined to 20% of the initial value between embryonic gestational day E15 and postnatal day P1. After birth the CFTR product increased transiently between P1 and P7 by a factor of 10 and decreased towards day P14. PCR products specific for TRN-CFTR, a truncated splice variant, however, were low in early embryonic cells, increased markedly between day E17 and P2, and reached a plateau postnatally. Therefore, mRNA encoding TRN-CFTR does not appear to have a specific embryonic-morphogenetic function. By contrast, such function is suggested for wild-type CFTR mRNA as its abundance was high in early embryonic nephrogenesis, as well as during a postnatal period shortly before branching morphogenesis is completed.


http://www.sciencedirect.com/science/article/B6T36-44F7M5N-50/2/eacb38d4b3d2329c2cc465fb45a7dedf
The cyclisation of lycopene to \([\beta]-\)carotene and the hydroxylation of \([\beta]-\)carotene to zeaxanthin are common enzymatic steps in the biosynthesis of carotenoids in a wide range of bacteria, fungi, and plants. We have individually expressed in E. coli the two genes coding for these enzymatic steps in Erwinia herbicola. The cyclase and hydroxylase enzymes have apparent molecular weights of 43 kDa and 22 kDa, respectively, as determined by SDS-PAGE. Hydroxylase in vitro activity was obtained only in the cytoplasmic fraction. Cyclase also demonstrated enzyme activity in a crude cell-free lysate, although to a lesser extent.


http://www.sciencedirect.com/science/article/B6T36-3V79DV9-N/2/fbb96321f2e781e2179ac062ba293637

Carica papaya produces four cysteine proteinases. Calculations show that the Cys25, His159 essential ion pair is fully ionised at pH 2.99, where activity cannot be detected, but apparently an additional ionisation with a pKa of 4 is essential for activity (an electrostatic switch). Caricain (EC 3.4.22.30) wt and D158E genetic backgrounds were used to study the contribution of E50A to activity. E50 or E135 are candidates for the switch, E50A would be expected to reduce activity. However, activity increased at pH 5.0 in both backgrounds and at the pH optimum in D158E E50A but decreased slightly in the wt background. This challenges the hypothesis of an electrostatic switch.


http://www.sciencedirect.com/science/article/B6T36-3V5MRHV-1B/2/f597d926a2968a5852eb71661f891156

We have identified a novel splice variant of chicken collagen XIV which contains an insert of three amino acids (Val-Arg-Thr) in the sixth fibronectin type III-like (FNIII) domain. The codons for these amino acids are inserted into the mRNA by skipping of a splice donor site and usage of another donor site 9 bp further downstream in the collagen XIV gene. The percentage of the new splice variant in the total collagen XIV mRNA varies between 22 and 46% in different embryonic tissues. After hatching, however, this percentage increases dramatically and reaches 86% in adult skeletal muscle and 58% in adult gizzard, indicating developmental regulation of this splicing event. Computer modeling suggests that the three extra amino acids cause an increase in the size of a flexible loop connecting two [beta]-strands in the sixth FNIII domain. This increase might affect the exact arrangement of the FNIII domain in the collagen XIV molecule, thereby modulating its interactions with other matrix molecules.


http://www.sciencedirect.com/science/article/B6T36-3Y0SKJ0-CJ/2/12c2fe30d3286f121270c70d81bdee07

We have used mRNA differential display to isolate genes that are induced by neural activity in rat
hippocampus. One of these encodes activin [beta]A subunit. Convulsive seizure caused by kainate significantly induced the expression of activin [beta]A mRNA. Furthermore high frequency stimulation (HFS) of perforant pathway, which produced a persistent long-term potentiation (LTP) (> 10 h), caused a marked increase at 3 h in the level of activin [beta]A mRNA at the dentate gyrus of urethane-anesthetized rat. The increase was NMDA receptor-dependent. By contrast the level of inhibin [alpha] mRNA was not changed following the induction of LTP. The results suggest a role for activin in maintenance of neural plasticity in the adult brain.


http://www.sciencedirect.com/science/article/B6T36-449TM3T-Y3/2/83318442aa9c70f1a18323d4b5554430

A new type of mitogenic factor, termed MF, has been found in the culture supernatant of Streptococcus pyogenes and its N-terminal amino acid sequence has been determined. On the basis of this sequence, an S. pyogenes gene encoding MF was cloned and its nucleotide sequence was determined. The MF gene includes a long, open reading frame with 813 nucleotides capable of encoding the MF precursor protein with 271 amino acids. Removal of the putative 43 residues as a signal peptide results in the mature MF protein with 228 amino acids. The molecular mass of the mature MF is calculated as 25,363 which is consistent with the previously determined value of 25,370 for MF secreted from S. pyogenes. Neither nucleotide nor amino acid sequence homology was found between the mature MF and other streptococcal pyrogenic exotoxins, such as SPE A, SPE B and SPE C. The mature MF was recombinantly overexpressed as a fusion protein with glutathione S-transferase in Escherichia coli. The recombinant protein showed mitogenic activity in rabbit peripheral blood lymphocytes and immunoreactivity with the rabbit antiserum raised against the secreted MF from S. pyogenes. These data indicate that a unique gene encoding MF was cloned from S. pyogenes.


http://www.sciencedirect.com/science/article/B6T36-3YS2BPP-87/2/ea2a941551ff9da0b66cc31d8e2d6d643

Utrophin is a large cytoskeletal protein which shows high homology to dystrophin. In contrast to the sarcolemmal distribution of dystrophin, utrophin accumulates at the postsynaptic membrane of the neuromuscular junction. Because of its localization within this compartment of muscle fibers, expression of utrophin may be significantly influenced by the presence of the motor nerve. We tested this hypothesis by denervating muscles of mdx mouse and monitoring levels of utrophin and its mRNA by immunofluorescence, immunoblotting and RT-PCR. A significant increase in the number of utrophin positive fibers was observed by immunofluorescence 3 to 21 days after sectioning of the sciatic nerve. Quantitative analyses of utrophin and its transcripts in hindlimb muscles denervated for two weeks showed only a moderate increase in the levels of both utrophin (~ 2-fold) and its transcript (~60 to 90%). The present data suggest that although utrophin is a component of the postsynaptic membrane, its neural regulation is distinct from that of the acetylcholine receptor.


http://www.sciencedirect.com/science/article/B6T36-450HHRN-S/2/9dc927abf408a22ded55db5459c8781b

Glycyrrhizin (GL), a triterpenoid saponin fraction of licorice, is reported to have anti-viral and anti-tumor activities and is metabolized to 18[beta]-glycyrrhetinic acid (GA) in the intestine by intestinal bacteria. However, the mechanism underlying its effects is poorly understood. To further elucidate the mechanism of GA, the aglycone of GL, we investigated the effects of GA on the release of nitric oxide (NO) and at the level of inducible NO synthase (iNOS) gene expression in mouse macrophages. We found that GA elicited a dose-dependent increase in NO production and in the level of iNOS mRNA. Since iNOS transcription has been shown to be under the control of the transcription factor nuclear factor [kappa]B (NF-[kappa]B), the effects of GA on NF-[kappa]B activation were examined. Transient expression assays with NF-[kappa]B binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA, induced by GA, was mediated by the NF-[kappa]B transcription factor complex. By using DNA fragments containing the NF-[kappa]B binding sequence, GA was shown to activate the protein/DNA binding of NF-[kappa]B to its cognate site, as measured by electrophoretic mobility shift assay. These results demonstrate that GA stimulates NO production and is able to up-regulate iNOS expression through NF-[kappa]B transactivation in macrophages.


http://www.sciencedirect.com/science/article/B6T36-44P8M4P-PH/2/7fd24e1bb24cdfcaacb6871d75bf9ef8

The full-length cDNA encoding the human calbindin-D9k (CaBP-9k) has been cloned using reverse transcription/PCR methodology with rat- and bovine-derived primers and intestinal RNA. A core product, and both a 5' and 3' product encompassing the full-length cDNA were obtained. The clones include coding region for 79 amino acids, 57 nucleotides 5'- and 159 nucleotides 3'-non-coding region, and a poly(A) tail. The deduced protein sequence is homologous to other mammalian CaBPs. Northern analysis revealed the mRNA in human duodenum to be about 600 nucleotides in length. Expression levels in adult human tissue were substantially lower than in child, rat or porcine intestine.


http://www.sciencedirect.com/science/article/B6T36-4281361-N/2/c9221e475c60ea9774480f7ba1e2f385d

Gastrin stimulates proliferation of progenitor cells in the neck zone of gastric fundic mucosa. However, whether it directly enhances this proliferation through its receptors remains unclear. We investigated the expression of gastrin receptors in neck zone proliferating cells in rat gastric fundic glands using a reverse transcription polymerase chain reaction (RT-PCR) coupled with laser capture microdissection and in situ RT-PCR. Gastrin receptor expression was identified in c-fos-expressing cells located in the neck zone, and results of the RT-PCR analysis argued against contamination by other cells, such as enterochromaffin-like, parietal or D cells. Supporting this finding, gastrin receptor gene expression was identified in the neck zone as well as base glands.
by in situ RT-PCR. Therefore, it is suggested that proliferating cells in the neck zone are stimulated directly by gastrin via their gastrin receptors.


Human MutT homologue (hMTH1) mRNA was overexpressed in SV-40-transformed non-tumorigenic human bronchial epithelial cells (BEAS-2B cells) and in 11 out of 12 human lung cancer cell lines relative to normal human bronchial epithelial cells. Expression levels of hMTH1 mRNA were inversely proportional to cellular levels of 8-oxo-deoxyguanosine. Together, these results suggest that hMTH1 gene expression may represent a molecular marker of oxidative stress that could ultimately be used to elucidate the temporal relationships between oxidative stress, genomic instability and the development of lung cancer.


The role of the 80-amino acid motif 1572-1651 in the C-terminal tail of [alpha]1C Ca2+ channel subunits was studied by comparing properties of the conventional [alpha]1C,77 channel expressed in HEK-tsA201 cells to three isoforms carrying alterations in this motif. Replacement of amino acids 1572-1651 in [alpha]1C,77 with 81 non-identical residues leading to [alpha]1C,86 impaired membrane targeting and cluster formation of the channel. Similar to [alpha]1C,86, substitution of its 1572-1598 ([alpha]1C,77L) or 1595-1652 ([alpha]1C,77K) segments into the [alpha]1C,77 channel yielded single-channel Ba2+ currents with increased inactivation, reduced open probability and unitary conductance, when compared to the [alpha]1C,77 channel. Thus, the C-terminal sequence 1572-1651 of the [alpha]1C subunit is important for membrane targeting, permeation and open probability of L-type Ca2+ channels.


A degenerate oligonucleotide corresponding to the K+ channel signature sequence (TMTTVGYGD) was used to isolate the genomic and cDNA forms of a new channel gene, AKT3, from Arabidopsis thaliana. The deduced protein sequence has a predicted membrane topography similar to Shaker-like K+ channels. Three distinct modules comprise the carboxyl-terminal half: a nucleotide-binding motif, an ankyrin repeat domain, and a polyglutamate track. Xenopus oocytes injected with cRNA exhibited an inward-rectifying K+ current, demonstrating that the AKT3 polypeptide is a functional transport protein. Two other Arabidopsis K+ transporters (AKT1 and
KAT1) share 60% homology with AKT3; together these proteins constitute a family of plant inward-rectifying K+ channels.


The relevance of MDR-1 gene expression to the multidrug resistance phenotype was investigated. Drug-resistant cells, KB-V1 and MCF7/ADR, constantly expressed mRNA of the MDR-1 gene and were more resistant to vinblastine and adriamycin than drug-sensitive cells, KB-3-1 and MCF7. The drug efflux rate of KB-V1 was the same as KB-3-1 although the MDR-1 gene was expressed in only the resistant cell. The higher intracellular drug concentration of KB-3-1 than KB-V1 was due to the large drug influx. In the case of MCF7 and MCF7/ADR, the influx and efflux of the drug had nearly the same pattern and drug efflux was not affected by verapamil. The amount of ATP, cofactor of drug pumping activity of P-glycoprotein, was not changed by the resistance. These observations suggested that drug efflux mediated by MDR-1 gene expression was not a major determining factor of drug resistance in the present cell systems, and that the drug resistance could be derived from the change in drug uptake and other mechanisms.


Fukuyama-type congenital muscular dystrophy (FCMD) is an autosomal recessive severe muscular dystrophy in combination with cerebral cortical dysplasia. Previously, we identified the gene responsible for FCMD, termed fukutin, through positional cloning. In this study, we have sequenced 131892 bp of genomic DNA in the region of the fukutin gene on chromosome 9q31 and obtained its complete genomic structure. The fukutin genomic sequence spans approximately 100 kb and is organized into 10 exons (41-6067 bp) and nine introns (1841-21460 bp). Using these sequence data, we have identified three novel fukutin mutations in FCMD patients. We have also located a putative TATA box in the flanking 5' region and identified numerous alternatively spliced fukutin mRNA transcripts. Analysis of expressed sequence tag clusters within the region revealed two novel genes upstream of the fukutin gene. These data provide fundamental information to support detailed genetic and functional analyses of the fukutin gene.


SecY is an integral membrane protein which participates in the translocation of proteins through
the bacterial cell membrane. We have cloned the secY gene of Lactococcus lactis, and found its
deduced protein sequence, 439 amino acids long, to be similar in length to the previously
determined SecY proteins of Escherichia coli, Bacillus subtilis and Mycoplasma capricolum.
Comparison of the L. lactis SecY to the 3 other SecY proteins revealed 90 conserved amino acid
residues (21%). Nearly half of the conserved residues are clustered in 2 of the 10 transmembrane
segments, and in 2 of the 6 cytoplasmic regions. Some of the conserved regions are apparently
responsible for the interactions of SecY with signal sequences, and the proteins SecE and SecA.

Korner, I., R. Weber-Nordt, et al. (1997). "Analysis of a regulatory element in the 5'-untranslated region of

http://www.sciencedirect.com/science/article/B6T36-3RC4V01-7/2/7908e46764f4ec9455912c8593fe3380

The bcl-2 gene is an important antagonist of apoptosis, the programmed cell death. Bcl-2 is
highly expressed in a variety of lymphomas. Lymphocytes of patients with chronic lymphocytic
leukemia (CLL) express high amounts of bcl-2 even in the absence of the t(14;18) translocation,
resulting in a strong resistance towards corticosteroid induced apoptosis. Within the 5'-
untranslated region of the bcl-2 gene a p53 dependent negative response element has been
described. Genetic alterations within this element could lead to uncontrolled overexpression of
bcl-2 and subsequent resistance towards apoptosis. We therefore analyzed the mRNA from the
5'-untranslated region of the bcl-2 gene by direct PCR sequencing from peripheral
blood derived lymphocytes from patients with CLL and normal healthy donors. Compared to
consistently found an exchange at position 1271 from A to G and at position 1284 from G to A in
all CLL as well as normal donor derived samples analyzed. Thus, CLL specific alterations
compared to normal cells could not be found and deregulated expression of bcl-2 in CLL cells
does not appear to be due to alterations in the p53 dependent negative response element of the
bcl-2 gene. However, our data add information to published sequence data of this region. (c)
1997 Federation of European Biochemical Societies.

Koshikawa, S., R. Cornette, et al. (2005). "Screening of genes expressed in developing mandibles during
soldier differentiation in the termite Hodotermopsis sjostedti." FEBS Letters 579(6): 1365.

http://www.sciencedirect.com/science/article/B6T36-4FBW3PM-4/2/7f75dc4cbe9eab9c296e216702f5d56d

We investigated the morphological changes accompanying soldier differentiation in the damp-
wood termite Hodotermopsis sjostedti. Genes expressed in the developing mandibles, which
undergo the most remarkable morphological changes during soldier differentiation, were
screened using fluorescent differential display. Database searches for sequence similarities were
conducted and the relative expression levels were then quantified by real-time polymerase chain
reaction. Among the identified candidate genes, 12 genes were upregulated during soldier
differentiation. These included genes for cuticle proteins, nucleic acid binding proteins, ribosomal
proteins and actin-binding protein, which were inferred to be involved in caste-specific
morphogenesis in termites.

Li, C. M., S. J. Campbell, et al. (2002). "Response heterogeneity of human macrophages to ATP is
associated with P2X7 receptor expression but not to polymorphisms in the P2RX7 promoter."
A region 2 kb upstream of exon 1 of the P2X7 gene was sequenced using DNA from nine healthy individuals who exhibited three different ATP response phenotypes (i.e. high, low and interferon gamma-inducible). Five single nucleotide polymorphisms were identified within the nine donor promoter sequences but none were associated with a specific ATP response phenotype. A P2X7 loss of function polymorphism (1513 in exon 13) was also screened for within donor DNA but no response associations were identified. ATP response phenotype was positively associated with P2X7 receptor expression, as assessed by flow cytometry, but not with any identified receptor or promoter gene polymorphisms.


1-Aminocyclopropane-1-carboxylate (ACC) synthase is a key enzyme in the biosynthesis of the plant hormone, ethylene. We have isolated, sequenced and expressed a functional tomato (cy Pik-Red) ACC synthase gene in Escherichia coli. ACC synthase expressed in E. coli was inactivated by incubation with S-adenosylmethionine (SAM), the half--time of which was concentration dependent. Mixing the tomato fruit protein extract with the cell-free extract from transformed E. coli did not affect SAM-dependent inactivation of ACC synthase activity. Thus, single isoforms of the ACC synthase enzyme, which demonstrate the biochemical features expected of the tomato fruit enzyme, can be expressed in E. coli and their structure--function relationships investigated.


Double-stranded (ds) RNA is a biologically active component of many viruses including rhinoviruses infecting the respiratory tract. Mucus production is a common symptom of such infections. Here, we show that mucin, the glycoprotein subunit of mucus gels, is transcriptionally upregulated in an NF--[kappa]B- and p38-dependent manner when homogeneous cultures of epithelial cells are exposed to dsRNA. Furthermore, upstream of p38 in this system, dsRNA stimulates the extracellular release of ATP and activation of cell surface ATP receptors, which are G protein-coupled. This results in the stimulation of phospholipase C and protein kinase C. These findings suggest that ATP receptor antagonists could be used to modulate mucus production induced by virus.

Up to 7% of Caucasians may demonstrate ultrarapid metabolism of debrisoquine due to inheritance of alleles with duplicated functional CYP2D6 genes. Here we describe the genomic organization of the duplicated CYP2D6 genes in the 42 kb XbaI allele. We postulate that this duplication originates from a homologous, unequal cross-over event which involved two 29 kb XbaI wild-type alleles, and had break points within a 2.8 kb direct repeat (CYP-REP) flanking the CYP2D6 gene. Moreover, we have designed two different PCR assays for detection of alleles with duplicated CYP2D6 genes. Both assays correctly identified 29 out of 29 subjects positive for the 42 kb XbaI allele. No false negative or false positive reactions were observed.


The protein RPE65 has an important role in retinoid processing and/or retinoid transport in the eye. Retinoids are involved in cell differentiation, embryogenesis and carcinogenesis. Since the kidney is known as an important site for retinoid metabolism, the expression of RPE65 in normal kidney and transformed kidney cells has been examined. The RPE65 mRNA was detected in transformed kidney cell lines including the human embryonic kidney cell line HEK293 and the African green monkey kidney cell lines COS-1 and COS-7 by reverse transcription PCR. In contrast, it was not detected in human primary kidney cells or monkey kidney tissues under the same PCR conditions. The RPE65 protein was also identified in COS-7 and HEK293 cells by Western blot analysis using a monoclonal antibody to RPE65, but not in the primary kidney cells or kidney tissues. The RPE65 cDNA containing the full-length encoding region was amplified from HEK293 and COS-7 cells. DNA sequencing showed that the RPE65 cDNA from HEK293 cells is identical to the RPE65 cDNA from the human retinal pigment epithelium. The RPE65 from COS-7 cells shares 98 and 99% sequence identity with human RPE65 at the nucleotide and amino acid levels, respectively. Moreover, the RPE65 mRNA was detected in three out of four renal tumor cultures analyzed including congenital mesoblastic nephroma and clear cell sarcoma of the kidney. These results demonstrated that transformed kidney cells express this retinoid processing protein, suggesting that these transformed cells may have an alternative retinoid metabolism not present in normal kidney cells.


A full-length cDNA encoding a novel cytosolic protein-tyrosine phosphatase (PTP), PTP-BAS, was cloned from human basophils. Due to in-frame deletions in the coding region, PTP-BAS exists in three isoforms: 7,455 bp (2,485 aa) for type 1, 7,398 bp (2,466 aa) for type 2 and 6,882 bp (2,294 aa) for type 3. All three isoforms contain a single PTP catalytic domain at the carboxyl termini as well as two distinct structural sequences. Amino terminal sequences of 300 amino acids are homologous to membrane-binding domains of cytoskeleton-associated proteins. Three 90 amino acid internal repetitive sequences are homologous to the GLGF repeats found in guanylate kinase proteins. PTP-BAS was expressed in various human tissues, especially highly
in the kidney and lung. Interestingly, the BAS mRNA level in the fetal brain was remarkably high.


http://www.sciencedirect.com/science/article/B6T36-44XN071-86/2/f3b78e958a54319fe6257b06f676e2da

The cDNA encoding the [beta] subunit of the human high-affinity IgE receptor was cloned by a combination of various polymerase chain reactions (PCR). A major portion of the [beta] cDNA was amplified using primers homologous within the sequences of rat and mouse. The 3' unknown sequence was preferentially amplified using the RNA template-specific PCR and the improved two-step PCR. The 5' unknown sequence was specifically amplified by our newly developed PCR walking. Random heptanucleotides tagged with a unique sequence at the 5' end were used as the walking primer. Finally, the entire coding region was amplified and sequenced. The two extracellular loops of the human [beta] subunit were the least homologous to those of rat and mouse.


http://www.sciencedirect.com/science/article/B6T36-3PNRX2B-29/2/2f42e70a21a74b4f96a974dcbf7ea5b

A cDNA, BCA1, encoding a calmodulin-stimulated Ca2+-ATPase in the vacuolar membrane of cauliflower (Brassica oleracea) was isolated based on the sequence of tryptic peptides derived from the purified protein. The BCA1 cDNA shares sequence identity with animal plasma membrane Ca2+-ATPases and Arabidopsis thaliana ACA1, that encodes a putative Ca2+ pump in the chloroplast envelope. In contrast to the plasma membrane Ca2+-ATPases of animal cells, which have a calmodulin-binding domain situated in the carboxy-terminal end of the molecule, the calmodulin-binding domain of BCA1 is situated at the amino terminus of the enzyme.


http://www.sciencedirect.com/science/article/B6T36-44XN30T-S9/2/6d0ec5aaffe3e019b2df9baa5f04ff79

A 5 kilobase deletion in mitochondrial DNA (mtDNA) has been reported to be responsible for the specific complex I deficiency in the substantia nigra (SN) of the Parkinson's disease (PD) brain. We have studied mitochondrial respiratory chain function in the SN from control and PD subjects, and analysed mtDNA, extracted from the same tissues, by Southern blot and the polymerase chain reaction (PCR). Quantitation of the levels of the deletion indicate that it does not contribute to the pathogenesis of PD nor to a complex I deficiency but seems likely to be an age-related observation.

The potent anti-hypertensive peptide, RPLKPW, has been designed based on the structure of ovokinin(2-7). The sequence encoding this peptide was introduced into three homologous sites in the gene for soybean [beta]-conglycinin [alpha]' subunit. The native [alpha]' subunit as well as the modified, RPLKPW-containing [alpha]' subunit were expressed in Escherichia coli, recovered from the soluble fraction and then purified by ion-exchange chromatography. The RPLKPW peptide was released from recombinant RPLKPW-containing [alpha]' subunit after in vitro digestion by trypsin and chymotrypsin. Moreover, the undigested RPLKPW-containing [alpha]' subunit given orally at a dose of 10 mg/kg exerted an anti-hypertensive effect in spontaneously hypertensive rats, unlike the native [alpha]' subunit. These results provide evidence for the first time that a physiologically active peptide introduced into a food protein by site-directed mutagenesis could practically function in vivo even at a low dose.


Fatty acid hydroperoxide lyase (HPL) is a novel P-450 enzyme that cleaves fatty acid hydroperoxides to form short-chain aldehydes and oxo-acids. In cucumber seedlings, the activities of both fatty acid 9HPL and 13HPL could be detected. High 9HPL activity was especially evident in hypocotyls. Using a polymerase chain reaction-based cloning strategy, we isolated two HPL-related cDNAs from cucumber hypocotyls. One of them, C17, had a frameshift and it was apparently expressed from a pseudogene. After repairing the frameshift, the cDNA was successfully expressed in Escherichia coli as an active HPL with specificity for 13-hydroperoxides. The other clone, C15, showed higher sequence similarity to allene oxide synthase (AOS). This cDNA was also expressed in E. coli, and the recombinant enzyme was shown to act both on 9- and 13-hydroperoxides, with a preference for the former. By extensive product analyses, it was determined that the recombinant C15 enzyme has only HPL activity and no AOS activity, in spite of its higher sequence similarity to AOS.


Latrophilin is a brain-specific Ca2+-independent receptor of [alpha]-latrotoxin, a potent presynaptic neurotoxin. We now report the finding of two novel latrophilin homologues. All three latrophilins are unusual G protein-coupled receptors. They exhibit strong similarities within their lectin, olfactomedin and transmembrane domains but possess variable C-termini. Latrophilins have up to seven sites of alternative splicing; some splice variants contain an altered third cytoplasmic loop or a truncated cytoplasmic tail. Only latrophilin-1 binds [alpha]-latrotoxin; it is abundant in brain and is present in endocrine cells. Latrophilin-3 is also brain-specific, whereas latrophilin-2 is ubiquitous. Together, latrophilins form a novel family of heterogeneous G protein-
coupled receptors with distinct tissue distribution and functions.


http://www.sciencedirect.com/science/article/B6T36-4772S3F-8/2/e504c547d278102a2a83d1f2f49d97fc

Placentas of mice lacking p57Kip2 expression have trophoblastic hyperplasia. To elucidate the mechanism underlying this phenomenon, we studied expression of two angiogenic factors, vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF). Immunohistochemical analysis with anti-VEGF antibodies indicated that VEGF expression was stronger and more clearly detectable in placentas of p57Kip2 null embryos compared to wild-type placentas. PIGF showed no significant differences between placentas of p57Kip2 null and wild-type embryos. In quantitative analysis, placentas of p57Kip2 null embryos showed higher VEGF messenger (m)RNA and protein levels than did wild-type placentas. PIGF mRNA and protein levels were not significantly different. These findings suggest that VEGF is involved in the hyperplasia that occurs in placentas of p57Kip2 null embryos.


http://www.sciencedirect.com/science/article/B6T36-447G3F5-B6/2/3dd6349de20b2af92b176c0f9d8b4d1a

A synthetic version of the human D4 (hD4) dopamine receptor was prepared. The G/C content of the natural gene was reduced by 14% without altering the amino acid composition of the corresponding protein sequence. HEK293 cells were transfected with the synthetic hD4 gene and stable clones resistant to G418 selected. The hD4 receptor expressed from the synthetic gene had identical pharmacological characteristics to the native hD4 receptor [(1991) Nature 350, 610-619; (1992) Nature 358, 149-152]. Functional studies with cells expressing the synthetic hD4 gene indicated negative coupling of this receptor to adenylate cyclase.


http://www.sciencedirect.com/science/article/B6T36-3S0FJ2M-Y/2/29637f4e27aaaf9d5f0e592aac00a1f71

The dystrophin-glycoprotein complex (DGC) is critical for muscle membrane stability. The sarcoglycans are transmembrane proteins within the DGC, and the function of the sarcoglycans is unknown. Mutations in sarcoglycan genes cause autosomal recessive muscular dystrophy. We have identified a new sarcoglycan gene with high homology to [alpha]-sarcoglycan highlighting the redundancy of the DGC. This gene, named [epsil]-sarcoglycan, has an identical intron-exon structure to [alpha]-sarcoglycan, and is more broadly expressed. The characterization of [epsil]-sarcoglycan should make it possible to determine if it, like the other sarcoglycan genes, is mutated in muscular dystrophy.

http://www.sciencedirect.com/science/article/B6T36-3YWWV7G-1/2/a59f6c251de8d16da2544596975e5be1

We have used the yeast two-hybrid system to search for cytoplasmic proteins that might assist in the intracellular trafficking of the soluble [beta]-galactoside-binding protein, galectin-3. We utilised as bait murine full-length galectin-3 to screen a murine 3T3 cDNA library. Several interacting clones were found to encode a partial open reading frame and a full-length clone was obtained by rapid amplification of cDNA ends methodology. In various assays in vitro the novel protein was shown to bind galectin-3 in a carbohydrate-independent manner. The novel protein contains an unusually high content of cysteine and histidine residues and shows significant sequence homologies with several metal ion-binding motifs present in known proteins. Confocal immunofluorescence microscopy of permeabilised 3T3 cells shows a prominent perinuclear, as well as cytoplasmic, localisation of the novel protein.


http://www.sciencedirect.com/science/article/B6T36-3W4GJS0-B/2/bfc860bf5e381f2ba1342ac5ff1cf620

A gene encoding an antifreeze protein (AFP) was isolated from carrot (Daucus carota) using sequence information derived from the purified protein. The carrot AFP is highly similar to the polygalacturonase inhibitor protein (PGIP) family of apoplastic plant leucine-rich repeat (LRR) proteins. Expression of the AFP gene is rapidly induced by low temperatures. Furthermore, expression of the AFP gene in transgenic Arabidopsis thaliana plants leads to an accumulation of antifreeze activity. Our findings suggest that a new type of plant antifreeze protein has recently evolved from PGIPs.


http://www.sciencedirect.com/science/article/B6T36-3Y15908-69/2/a0a17e9005e149ba9b16bcdfb3693ec1

To analyze the possible involvement of c-ski and c-sno during the course of in vitro myogenesis, expression of their transcripts during differentiation of a murine muscle cell line (C2C12) was monitored by competitive reverse transcription-polymerase chain reaction (RT-PCR). The transcripts of c-snoN were temporarily increased 25-fold above basal level at 12 h prior to the onset of transcription of muscle-specific gene, e.g. myogenin and muscle creatine kinase, whereas c-ski was expressed invariably. The transient increase of c-snoN was blocked when myogenesis was interrupted by the presence of fetal calf serum in culture medium, probably due to growth factors being included; basic fibroblast growth factor (b-FGF) blocked the transient increase whereas epidermal growth factor (EGF) did not, consistent with the inhibitory effect of b-FGF and no effect of EGF on myotube formation of C2C12. In fibroblastic C3H10T1/2 cells, snoN exhibited a similar transient increase of transcript when growth arrested under the same conditions as for in vitro myogenesis, indicating that the expression of snoN is not sufficient to
induce the onset of muscle differentiation and an unknown factor involved in myogenic cells is necessary. The transient increase of snoN transcript may represent a common entrance step of cells into the GO phase where muscle differentiation is substantiated, considering that it was observed upon growth arrest of fibroblastic C3H10T1/2 cells and prior to the elevation of MCK in C2C12 but undetected when entry into G0 was blocked by b-FGF.


http://www.sciencedirect.com/science/article/B6T36-497C8B7-2V/2/fa1ff088f384738eb681bb894a742c0d

We detected alternative splicing of the mouse brain type ryanodine receptor (RyR3) mRNA. The splicing variant was located in the transmembrane segment. The non-splicing type (RyR3-II) included a stretch of 341 bp, and that of the 13th codon was stop codon TAA. Reverse transcription-polymerase chain reaction (RT-PCR) analysis shows that RyR3-II mRNA was expressed in various peripheral tissues and brain at all developmental stages. However, interestingly, the splicing type (RyR3-I) mRNA was detected only in the cerebrum. These findings suggest that the splicing variants RyR3-I and RyR3-II may generate functional differences of RyR3 in a tissue-specific manner.


http://www.sciencedirect.com/science/article/B6T36-4CRXYTW-4/2/08c3ad60ca127659ff2091d2b81447a6

The psbD blue light-responsive promoter (BLRP), whose activation has been considered to require strong blue light, is recognized only by SIG5 among six [sigma] factors of plastid RNA polymerase in Arabidopsis. We found SIG5 transcript accumulation was rapidly induced after a 30-min induction time by blue light (470 nm) with an intensity threshold of 5 [mu]mol m-2 s-1 through cryptochromes. Besides this weak blue light, the psbD BLRP activation required the stronger light such as 50 [mu]mol m-2 s-1 irrespective of blue or red light (660 nm). Thus, the two independent light signalings, the cryptochrome-mediated signaling to induce SIG5 transcription and the stronger light-dependent signaling, cooperate to activate the psbD BLRP.


http://www.sciencedirect.com/science/article/B6T36-449TM3T-X5/2/d526cad6f56cc1069919498bca82a0e5

Using an improved 3' RACE (PCR) amplification system containing oligonucleotide primer with an inosine at ambiguous codon positions and inverse PCR to amplify the 5' ends, we have isolated and characterized cDNA clones which encode cionin, a protochordan homologue of the mammalian hormones, cholecystokinin (CCK) and gastrin. The full-length cloned cDNA of 510 bp encoded a 128 amino acid preprocionin. Reverse transcription-PCR and subsequent cDNA cloning revealed that cionin mRNA is expressed in both the neuronal ganglion and the gut of the protochordate Ciona intestinalis. The primary structure of procionin resembles that of proCCK.
more than that of progastrin. Sequence-specific immunochemical analysis showed that the cionin gene is expressed also at peptide level in both the gut and the neural ganglion. The neuronal processing of procionin is, however, more complete both with respect to carboxyamidation and tyrosine O-sulfation. Hence, the tissue-specific expression of the cionin gene in Ciona intestinalis resembles that of the CCK gene in mammals.


http://www.sciencedirect.com/science/article/B6T36-3V7JRTN-10/2/003053f85d567679c6ed0586007e5062

Neurotransmitter transport systems are major targets for therapeutic alterations in synaptic function. We have cloned and sequenced a cDNA encoding the human type 2 glycine transporter GlyT2 from human brain and spinal cord. An open reading frame of 2391 nucleotides encodes a 797 amino acid protein that transports glycine in a Na+/Cl--dependent manner. When stably expressed in CHO cells, human GlyT2 displays a dose-dependent uptake of glycine with an apparent Km of 108 [mu]M. This uptake is not affected by sarcosine at concentrations up to 1 mM. Radiation hybrid analysis mapped the GlyT2 gene to D11S1308 (LOD=8.988) on human chromosome 11p15.1-15.2.


http://www.sciencedirect.com/science/article/B6T36-44F7MXT-FB/2/465875585af5f296362c97173507964b

We have cloned and sequenced a novel cDNA (RPR7) encoding a receptor for pituitary adenylate cyclase activating polypeptide (PACAP). RPR7 was identified by PCR of rat pituitary cDNA, and full-length clones were isolated from a rat olfactory bulb cDNA library. When expressed in COS cells, RPR7 was functionally coupled to increases in intracellular cyclic adenosine monophosphate (cAMP) in response to stimulation by PACAP-38, PACAP-27, vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI). The order of potency of these ligands was PACAP-38 ~ PACAP-27 > VIP > PHI, suggesting that the receptor corresponds to the pharmacologically characterised PACAP Type I receptor.


http://www.sciencedirect.com/science/article/B6T36-3Y0SM18-SJ/2/ae12915301071173c5d65dc97b6621

A novel estrogen receptor (hereinafter referred to as ER[beta]) was cloned using degenerate PCR primers. A comparison of the amino acid sequence of ER[beta] with the 'classical' ER (ER[alpha]) shows a high degree of conservation of the DNA-binding domain (96%), and of the ligand-binding domain (58%). In contrast, the A/B domain, the hinge region and the F-domain are not conserved. Northern blot analysis revealed that ER[beta] is expressed in human thymus, spleen, ovary and testis. Transient transfections of an ER[beta] expression construct together
with an ERE-based reporter construct in CHO cells clearly demonstrated transactivation of ER[\beta] by 17[\beta]-estradiol. In addition, the ER[\alpha] antagonist ICI-164384 is a potent antagonist for ER[\beta] as well. Interestingly, the level of transactivation by 17[\beta]-estradiol is higher for ER[\alpha] than for ER[\beta], which may reflect suboptimal conditions for ER[\beta] at the level of the ligand, responsive element or cellular context.


http://www.sciencedirect.com/science/article/B6T36-4FH0T2R-6/2/bd1b705f4972eb363f0348bf40c0859f

The potential use of [\alpha]-cyclodextrin and its hydrophilic [\alpha]-cyclodextrin derivatives ([\alpha]-CyDs) as antagonists against lipopolysaccharide (LPS), which stimulates the nitric oxide (NO) and tumor necrosis factor-[\alpha] (TNF-[\alpha]) production as well as nuclear factor-\kappaB (NF-\kappaB) activation in macrophages was examined. Of three [\alpha]-CyDs used in the present study, 2,6-di-O-methyl-[\alpha]-CyD (DM-[\alpha]-CyD) had greater inhibitory activity than did the other CyDs against NO and TNF-[\alpha] production through an impairment of gene expression in macrophage cell lines and primary macrophages stimulated with LPS and lipid A in a concentration-dependent manner. Concomitantly, DM-[\alpha]-CyD inhibited NF-\kappaB translocation into nucleus. These inhibitory effects of DM-[\alpha]-CyD could be attributed to the release of CD14 from lipid rafts caused by an efflux of phospholipids, but not cholesterol. These results suggest that DM-[\alpha]-CyD may have promise as a potent and unique antagonist for excess activation of macrophages stimulated with LPS.


http://www.sciencedirect.com/science/article/B6T36-3R85JMS-C/2/8df5aae47b87c5e53f6eecd5ab4630db

The 5'-untranslated region (5'-UTR) sequences of 33 GB virus C/hepatitis G virus (GBV-C/HGV) obtained from different geographic areas were determined through reverse-transcription polymerase chain reaction and dideoxy chain termination sequencing, the alignment of sequences, the estimation of the number of nucleotide substitution per site, and construction of phylogenetic trees. The 5'-UTR of GBV-HGV was found to be heterogeneous, with 70.9-99.5% homology. Three distinct phylogenetic branches were observed consistently in all phylogenetic trees. GBV-C is the prototype for one, HGV for another, and there is a new branch which consisted of GBV-C/HGV isolates from Asia. Genotype-specific restriction sites for the restriction enzymes, ScrFI and BsmFI, were identified, and a simple restriction fragment polymorphism analysis was developed for genotyping. These data provide evidence that GBV-C/HGV consists of three different genotypes. Our simple genotyping assay will also provide a tool for epidemiological studies of GBV-C/HGV infection.


http://www.sciencedirect.com/science/article/B6T36-44G8D5G-
The human platelet-activating factor (PAF) receptor gene exists as a single copy on chromosome 1. We identified two 5'-noncoding exons, each of which has distinct transcriptional initiation sites. These exons are alternatively spliced to a common splice acceptor site on a third exon that contains the total open reading frame to yield two different species of functional mRNA (Transcript 1 and 2). Transcript 1 has consensus sequences for transcription factor NF-κB and Sp-1, and the Initiator (Inr) sequence homologous to the murine terminal deoxynucleotidyltransferase gene. Transcript 2 also contains consensus sequences for transcription factor AP-1, AP-2, and Sp-1. Transcripts 1 and 2 were both detected in heart, lung, spleen, and kidney, whereas only Transcript 1 was found in peripheral leukocytes, a differentiated human eosinophilic cell line (EoL-1 cells), and brain. Existence of distinct promoters was thus suggested to play a role in the regulatory control of PAF receptor gene expression in different human tissues and cells.


Catecholamine-induced and [beta]-adrenergic receptor ([beta]-AR)-mediated thermogenesis in skeletal muscle is a significant component of whole-body energy expenditure. Skeletal muscle expresses uncoupling protein (UCP) 2 and UCP3, which can dissipate the transmembrane electrochemical gradient and thereby may be involved in regulation of energy metabolism. We investigated the effects of [beta]-AR stimulation on UCP2 and UCP3 expression in L6 myotubes. Stimulation of the cells with epinephrine increased the UCP3 mRNA level transiently at 6 h, and also the UCP2 mRNA level at 6-24 h. The stimulatory effects of epinephrine were also observed in the presence of carbacyclin and 9-cis retinoic acid, and mimicked by isoproterenol and salbutamol ([beta]2-AR agonists), but abolished by propranolol and ICI-118,551 ([beta]2-AR antagonists). Pharmacological and mRNA analyses revealed the existence of [beta]2-AR, but not [beta]1- and [beta]3-ARs, in L6 myotubes. These results suggested that catecholamines up-regulate UCP2 and UCP3 expression through direct action on the [beta]2-AR in skeletal muscle.


Astrocytes are generated from neuroepithelial cells after neurons during brain development. However, the mechanism of this sequential generation is not fully understood. Here, we show that a particular cytosine residue in the promoter of the gene encoding the immature astrocyte marker, S100[beta], becomes demethylated, correlating with the time when the S100[beta] expression commences at embryonic day (E) 14. In addition, astrocyte-inducing cytokine, BMP2, increased histone acetylation around the CpG site in neuroepithelial cells at E14 but not E11 when S100[beta] expressing astrocytes are absent. Furthermore, binding of a methyl DNA binding protein, MeCP2, to the S100[beta] gene promoter in neuroepithelial cells was reduced at E14 compared to E11. Thus, demethylation of specific CpG site is suggested to be a critical determinant in regulating astrocyte differentiation in the developing brain.

http://www.sciencedirect.com/science/article/B6T36-4BFXHKS-3/2/e7e343e7f96ed9679ba791c736f504c4

The small GstI protein (63 amino acids) of Rhizobium leguminosarum inhibits the expression of the glnII (glutamine synthetase II) gene, thus reducing the bacterial ability to assimilate ammonium. In order to identify the residues essential for its inhibitory activity, all the 53 non-alanine amino acid residues of GstI were individually mutated into alanine. Based on their capacity to inhibit glnII expression (in two genetic backgrounds) three groups of mutants were identified. The first group displayed an inhibitory activity similar to the wild-type; the second and the third ones showed partial and total loss of inhibitory activity, respectively. Several mutations of the latter group concerned residues conserved in two related sequences from Sinorhizobium meliloti and Agrobacterium tumefaciens. Additionally, we performed experiments to exclude a GstI-mediated mechanism of glutamine synthetase II inhibition/degradation. Finally, the protein was over expressed in Escherichia coli, purified and characterised.


http://www.sciencedirect.com/science/article/B6T36-3YN9FDJ-23/2/60d01de668ea24b97d454aca6f725b78

Tissue inhibitors of metalloproteinase (TIMPs) are inhibitory counterparts of collagenases, containing 12 cysteine residues paired to six internal disulphide bridges. TIMP-2, an inhibitory protein of 72 kDa gelatinase/type IV collagenase (MMP-2), was expressed in Escherichia coli as a fusion protein with a 34 amino acid NH2-linked tail containing six consecutive histidine residues. The protein was purified in a single-step using an ion metal affinity column (IMAC) in denaturing conditions. The immobilized fusion TIMP-2 protein was refolded at a high concentration in the column, producing about 5 mg of protein per litre of bacterial cells. It shows specific binding and inhibitory activity against MMP-2, but has no effect against 92 and 45 kDa gelatinases.


http://www.sciencedirect.com/science/article/B6T36-4638RRR-1R/2/aec63a590d673ca7fa1f9cee2735748a

Adequate means for genotype assignment to phenotype is essential in evolutionary molecular engineering. In this study, construction of ‘in vitro virus’ was carried out in which a genotype molecule (mRNA) covalently binds to the phenotype molecule (protein) through puromycin on the ribosome in a cell-free translation system. Bonding efficiency was ~10%, thus indicating a population of the in vitro virus to have ~1012 protein variants, this number being 104 that in the phage display. The in vitro virus is useful for examining protein evolution in a test tube and the results may possibly serve as basis for a general method for selecting proteins possessing the most desirable functions.

http://www.sciencedirect.com/science/article/B6T36-3XY1H1K-8/2/bfa2ba542534a256231e99ffdb2d7125

We have developed a new method for the C-terminus-specific fluorescence labeling of proteins. This method is based on the experimental finding that a fluorescent puromycin analogue at lower concentrations bonds efficiently to the C-terminus of mature proteins in cell-free translation systems using mRNA without a stop codon. This labeling is performed under moderate conditions and its labeling efficiency is in the range of 50-95%. Here we demonstrate a protein-protein interaction assay using fluorescence polarization measurement. This labeling method should also be useful for other rapid molecular interaction assays without purification of the labeled proteins, such as fluorescence correlation spectroscopy.


http://www.sciencedirect.com/science/article/B6T36-4F4H7NX-4/2/b240bc99ee004e05bb07c583be1eb6ad

Chronic ethanol exposure increases the density of N-type calcium channels in brain. We report that ethanol increases levels of mRNA for a splice variant of the N channel specific subunit [alpha]12.2 that lacks exon 31a. Whole cell recordings demonstrated an increase in N-type current with a faster activation rate and a shift in activation to more negative potentials after chronic alcohol exposure, consistent with increased abundance of channels containing this variant. These results identify a novel mechanism whereby chronic ethanol exposure can increase neuronal excitability by altering levels of channel splice variants.


http://www.sciencedirect.com/science/article/B6T36-4BDW15Y-5/2/941f6d82cbbcc59e493b754a0d550db5

Differential mRNA display revealed that a cDNA encoding the major urinary protein 2 (MUP2) that belongs to the lipocalin superfamily was absent in livers of mice treated with 3-methylcholanthrene (MC). The expression of MUP2 is known to be stimulated by growth hormone (GH), through the GH receptor (GHR), Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (STAT5) signal transduction pathway. Since MC is an aryl hydrocarbon receptor (AhR) ligand, the effects of MC treatment on the expression of GHR, JAK2 or STAT5 in the livers of wild-type or AhR-null mice were examined. The result indicated that the expression of GHR and JAK2 mRNA was greatly decreased by MC in wild-type mice but not in AhR-null mice. In addition, the binding activity of STAT5 bound to STAT5-binding element was reduced after MC treatment in wild-type mice but not in AhR-null mice. Based on these results, we conclude that the suppression of MUP2 mRNA expression by MC is caused by the AhR-mediated disruption of the GH signaling pathway. Possible mechanism(s) by which exposure to aromatic hydrocarbons causes a decrease in the body weight of mice, which has been referred to as wasting syndrome, will also be discussed.

http://www.sciencedirect.com/science/article/B6T36-3RTXJV-JV-C/2/f88f7bf23a57e238f165f0b9f5f8efefe

We describe here (1) the heterogeneous expression of Ca2+-independent transient (A-type) K+ channel [alpha]-subunits (Kv1.4, Kv3.3, Kv3.4, Kv4.2 and Kv4.3) in rat smooth muscle, heart and brain, (2) the molecular cloning and tissue distribution of a novel alternatively spliced variant of an A-type K+ channel [alpha]-subunit, Kv4.3, and (3) the functional expression of A-type K+ channels in HEK293 cells by the transfection with the novel splice variant of Kv4.3. A cDNA encoding this splice variant was identified from rat vas deferens by RT-PCR cloning. This cDNA clone contains a 1965 bp open reading frame that encodes for a protein of 655 amino acids. It has a 19 amino acid insertion in comparison with Kv4.3 previously reported in rat brain. RT-PCR analyses showed that the mRNAs of this longer variant are abundantly expressed in a number of smooth muscles of the rat, and that the mRNAs of the previously reported clones are absent. The longer splice variant is very weakly expressed in brain, but is the major product in heart.


http://www.sciencedirect.com/science/article/B6T36-3Y0SKX0-NJ/2/74594989c69bd5f090d94b28133466b15

We isolated a 1.7 kb gene (UbcPl) for a ubiquitin-conjugating enzyme from a P. tetraurelia cDNA library and sequenced it. Its deduced polypeptide sequence consists of 425 amino acid residues (48 kDa). The UbcP1 protein contains novel N- and C-terminal extensions in addition to a UBC domain, and within the UBC domain it shares low identity with sequences of other known E2s. A constructed phylogenetic tree suggests that the UbcP1 protein may represent a member of a distinct subfamily of E2s. Southern blot analysis showed that the N-terminal extension of the UbcP1 is conserved in P. multimicronucleatum.


http://www.sciencedirect.com/science/article/B6T36-455691V-6/2/aa12c15b4f5445ce5d8c34a062607584

The Lewis X (Lex) bearing glycolipids were noticeably increased in amounts during the course of neural differentiation of P19 EC cells induced by retinoic acid (RA, all-trans form). Applying neoglycolipid technology and in situ TLC-LSIMS, the oligosaccharide chains of these scarce Lex bearing glycolipids were partially characterized after released by endoglycoceramidase and subsequent conversion into neoglycolipids. In order to understand the enzymatic basis for the expression of Lex bearing glycolipids, we measured glycolipid, glycoprotein and oligosaccharide fucosyltransferase (Fuc-T) activities using appropriate substrates in P19 EC cells with or without RA treatment. All three Fuc-Ts were increased after RA treatment and the highest activity was in the differentiated neural cells. We then investigated the two possible Fuc-T genes that might be responsible for these changes using RT-PCR analysis. Mouse Fuc-TIX (mFuc-TIX) transcript was detected in all cell types but it was only strongly expressed in RA-induced aggregates and neural
cells. In the case of mouse Fuc-TIV (mFuc-TIV) gene, its transcript was only detectable in RA-induced aggregates and not found in either undifferentiated or RA-induced neural cells. These results strongly support that RA induces only a transient expression of the mFuc-TIV gene in cell aggregates but a more persistent expression of the mFuc-TIX gene at the transcription level throughout neural cell differentiation. The mFuc-TIX gene is probably the main cause for the increased expression of Lex glycoconjugates during neural differentiation of P19 EC cells.


http://www.sciencedirect.com/science/article/B6T36-3SXDXVF-8/2/56a7258709787a19202e1d3824c6afaf

The sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA) type 1 and 2 genes are alternatively spliced at their 3' end. We hypothesized that similar mechanism may occur for SERCA 3. Two spliced variants were identified by RNase protection analysis. We then isolated and sequenced the 3' end portion of the mouse SERCA 3 gene, and confirmed the presence of an alternative mRNA transcript by sequencing a cDNA fragment obtained by RT-PCR. Tissue distribution of the alternatively spliced mRNAs was studied by RT-PCR: SERCA 3b was the only isoform expressed in endothelial cells from aorta and heart and also was the major isoform in lung and kidney whereas SERCA 3a and 3b were coexpressed in trachea, intestine, thymus, spleen, and fetal liver.


http://www.sciencedirect.com/science/article/B6T36-44KP5R9-4H/2/83c129f0c1dd400f36bf1ed6007446d0

The gene of ecotin, an E. coli proteinase inhibitor, was cloned, and by site-directed mutagenesis the active site residue of the protein, Met84, was mutated to Lys, Arg and Leu. The recombinant wild-type and mutant inhibitors were overexpressed in E. coli, purified to homogeneity and their inhibitory effects on trypsin, chymotrypsin and elastase were compared. Of these serine proteinases trypsin is the most strongly inhibited by wild type ecotin and its mutants. According to our results the character of residue 84 of ecotin significantly but not dramatically modifies the specificity of the inhibitor.


http://www.sciencedirect.com/science/article/B6T36-3VXNBMH-1B/2/a1b1319497f92fca52102e2bdcd366

Ecotin, a homodimer protein of E. coli, is a unique member of canonical serine proteinase inhibitors, since it is a potent agent against a variety of serine proteinases having different substrate specificity. Monomers of ecotin are held together mostly by their long C-terminal strands that are arranged as a two-stranded antiparallel [beta]-sheet in the functional dimer. One ecotin dimer can chelate two proteinase molecules, each of them bound to both subunits of ecotin at two different sites, namely the specific primary and the non-specific secondary binding sites. In
this study the genes of wild type ecotin and its Met84 Arg P1 site mutant were truncated resulting in new forms of ecotin that lack 10 amino acid residues at their C-terminus. These mutants do not dimerize spontaneously, though in combination with trypsin they assemble into the familiar heterotetramer. Our data suggest that this heterotetramer exists even in extremely diluted solutions, and the interaction, which is responsible for the dimerization of ecotin, contributes to the stability of the heterotetrameric complex.


Fibulin-1 is a 90 kDa calcium-binding protein present in the extracellular matrix and in the blood. Two major variants, C and D, differ in their C-termini as well as the ability to bind the basement membrane protein nidogen. Here we characterized genomic clones encoding the mouse fibulin-1 gene, which contains 18 exons spanning at least 75 kb of DNA. The two variants are generated by alternative splicing of exons in the 3' end. By searching the database we identified most of the exons encoding the human fibulin-1 gene and showed that its exon-intron organization is similar to that of the mouse gene.


We developed a rapid method to determine DNA-binding sites for putative DNA-binding proteins. This procedure has been successfully used to define a specific consensus site for the human ZNF35 zinc finger gene. ZNF35 encodes a 56-kDa polypeptide containing 11 consecutive finger motifs located at the amino terminus, and an acidic domain located at the carboxy terminus. These features suggest that ZNF35 is a site-specific DNA-binding protein involved in the regulation of gene expression. We have expressed the ZNF35 protein from E. coli and have employed a Southwestern-polymerase chain reaction method using random oligonucleotides to identify its high-affinity binding site. The core sequence for the ZNF35 protein-binding site is 5'-C/GC/GAAG/TA-3'.


The CC chemokines RANTES and MIP-1[alpha] are known to activate certain leucocytes and leucocytic cell lines. We have produced and fully characterised the recombinant proteins expressed in E. coli. They induce chemotaxis of the pro-monocytic cell line, THP-1 and T cells. THP-1 cells express three of the known CC chemokine receptors. In order to study the activation of a single receptor, we have expressed the shared receptor (CC CKR-1) for RANTES and MIP-
1[alpha] stably in the HEK 293 cell line. We have examined the effects of RANTES and MIP-1[alpha] on the CC CKR-1 transfectants by equilibrium binding studies and in a chemotaxis assay. RANTES competes for [125I]RANTES with an IC50 of 0.6 +/- 0.23 nM, whereas MIP-1[alpha] competes for its radiolabelled counterpart with an IC50 of 10 +/- 1.6 nM in the transfectants. These affinities are the same as those measured on the THP-1 cell line. The stably transfected HEK 293 cells respond to both these chemokines in the chemotaxis assay with the same EC50 values as those measured for THP-1 cells. This indicates that this cellular response can be mediated through the CC CKR-1 receptor.


Cellophane wrapping of the hamster pancreas induces islet neogenesis. We have used the mRNA differential display technique to select for genes expressed during islet neogenesis but not in control pancreata. Ten candidate clones have been identified. Upon sequencing, 6 clones showed a high degree of homology to known genes, 1 showed some, and 3 showed no homology to genes of known sequence. Thus, mRNA differential display is a useful technique to identify genes induced during islet neogenesis, and in combination with screening hamster pancreatic cDNA libraries for full length clones, will enhance the likelihood of capturing the participants in this process.


The serine protease [alpha]-thrombin (thrombin) potently stimulates G-protein-coupled signaling pathways and DNA synthesis in CCL39 hamster lung fibroblasts. To clone a thrombin receptor cDNA, selective amplification of mRNA sequences displaying homology to the transmembrane domains of G-protein-coupled receptor genes was performed by polymerase chain reaction. Using reverse transcribed poly(A)+ RNA from CCL39 cells and degenerate primers corresponding to conserved regions of several phospholipase C-coupled receptors, three novel putative receptor sequences were identified. One corresponds to an mRNA transcript of 3.4 kb in CCL39 cells and a relatively abundant cDNA. Microinjection of RNA transcribed in vitro from this cDNA in Xenopus oocytes leads to the expression of a functional thrombin receptor. The hamster thrombin receptor consists of 427 amino acid residues with 8 hydrophobic domains, including one at the extreme N-terminus that is likely to represent a signal peptide. A thrombin consensus cleavage site is present in the N-terminal extracellular region of the receptor sequence followed by a negatively charged cluster of residues present in a number of proteins that interact with the anion-binding exosite of thrombin.

A chickpea cDNA encoding a cell wall copper amine oxidase (CuAO) was cloned and characterised. The 2010 bp open reading frame encodes a protein of 76.5 kDa which shares significant primary structure homology with other known CuAOs. Southern blot analysis indicates that in chickpea CuAO is encoded by a single gene or a small gene family. This cDNA was essential for studying the role of CuAO during seedling development and wound healing in chickpea seedlings. CuAO transcript level and activity were modulated during seedling development in parallel with cell maturation. Moreover, mechanical wounding induced a rapid increase of CuAO mRNA accumulation and enzyme activity which remained high during the wound-healing process. Aminoguanidine, a specific CuAO inhibitor, decreased the deposition of lignin-suberin barrier along the lesion. CuAO may be a limiting factor in H2O2 production in the cell wall of chickpea seedlings and its expression seems to integrate with the remodelling of plant cell wall occurring during ontogenesis and wound healing.


We have cloned an orphan G protein-coupled receptor from a human pituitary cDNA library using a probe generated by PCR. The cDNA, designated H9, encodes a protein of 613 amino acids that is 45% identical at the amino acid level to the recently cloned human Mel1a and Mel1b melatonin receptors. Structural analyses of the encoded protein and its gene, along with phylogenetic analysis, further show that H9 is closely related to the G protein-coupled melatonin receptor family. Unusual features of the protein encoded by H9 include a lack of N-linked glycosylation sites and a carboxyl tail >300 amino acids long. H9 transiently expressed in COS-1 cells did not bind [125I]melatonin or [3H]melatonin. H9 mRNA is expressed in hypothalamus and pituitary, suggesting that the encoded receptor and its natural ligand are involved in neuroendocrine function.


The ATP-sensitive K-channel plays a central role in insulin release from pancreatic [beta]-cells. We report here the cloning of the gene (KCNJ6) encoding a putative subunit of a human ATP-sensitive K-channel expressed in brain and [beta]-cells, and characterisation of its exon-intron structure. Screening of a somatic cell mapping panel and fluorescent in situ hybridization place the gene on chromosome 21 (21q22.1-22.2). Analysis of single-stranded conformational polymorphisms revealed the presence of two silent polymorphisms (Pro-149: CC-CC and Asp328: GA-GA) with similar frequencies in normal and non-insulin-dependent diabetic patients.

We isolated an INF1 elicitin-inducible cDNA encoding a pleiotropic drug resistance (PDR)-type ATP-binding cassette (ABC) transporter homolog (NtPDR1) in suspension-cultured tobacco Bright Yellow-2 (BY-2) cells by application of differential display PCR. The NtPDR1 (Nicotiana tabacum PDR protein 1) gene also encodes a 162 kDa protein that includes two putative hydrophilic domains containing the ABC signature motif and two putative hydrophobic domains. Expression of the NtPDR1 gene was rapidly and strongly activated by treatment of BY-2 cells with INF1 elicitin. Further, treatment of BY-2 cells with flagellin, a bacterial proteinaceous hypersensitive reaction elicitor, or yeast extract, a general elicitor, also induced NtPDR1 gene expression. These results indicate that NtPDR1 may be involved in the general defense response in tobacco. This is the first report that microbial elicitors induce the expression of a plant ABC transporter gene.


We have investigated the C-terminal tail of the rat substance P receptor (SPR) as a domain essential for agonist-induced desensitization. Four progressively shorter mutants, using premature termination in the C-terminus, were constructed and compared with the unaltered SPR using ectopic expression of wild-type and mutant receptors in Xenopus oocytes. These mutants were designated D16, D47, D70 and D96 with 16, 47, 70 and 96 amino acids residues deleted from the tail, respectively. Wild type SPR, D16 and D47 exhibited normal current responses when challenged with substance P, but D70 and D96 had reduced maximal current responses (70% and 5% of wild type SPR, respectively). D70, however, exhibited substantial resistance to substance P-induced desensitization in that 55%, versus 8% for wild type SPR, of the peak current of the first response was preserved on second challenge with substance P. Therefore, a domain from residues 338 to 360 of the rat SPR, though not necessary for the functional activity of the receptor, plays an essential role in agonist-induced desensitization.


Diacylglycerol (DG) kinase attenuates the level of the second messenger DG in signal transduction, and therefore possibly modulates protein kinase C (PKC). DG kinase was purified to homogeneity from human white blood cells, showing an M1 of 86 kDa as determined by SDS-PAGE and gel filtration. Two amino acid sequences of tryptic peptides from DG kinase were determined and degenerate oligonucleotides were prepared and used in the polymerase chain reaction. An amplified DNA fragment was subsequently used to clone the full-length human DG kinase cDNA. This sequence is the human homolog of porcine DG kinase cDNA sequence reported recently [1]. The sequence contains a double EF-hand structure typical for Ca2+ binding proteins. DG kinase further contains a double cysteine repeat that is present in all PKC isoforms, where it constitutes the phorbol ester (and most likely diacylglycerol) binding site. Therefore we speculate that the double cysteine repeat in DG kinase is involved in DG binding.
DG kinase is transcribed as a single mRNA of 3.2 kb, that is highly expressed in T-lymphocytes. The human DG kinase cDNA when transfected in mammalian cells (COS-7) results in a 6-7-fold increase of DG kinase activity.


http://www.sciencedirect.com/science/article/B6T36-3Y0SK4Y-2J/2/469306a405ab854a98dd1f4424dda1eb

We report the identification of a mouse cDNA, SIG41, encoding a protein of 288 amino acids that is 45% identical (58% similar) to the Drosophila splicing regulator Tra2. SIG41 cDNA contains four polyadenylation signals whose alternative use gives rise to four types of transcripts (2.1, 2.0, 1.5, and 1.4 kb) in mouse cells. Northern analysis and RT-PCR assays showed that SIG41 mRNA is present in virtually all the cell lines and tissues studied, with remarkable levels of expression in uterus and brain tissues. Differential stability of the SIG41 mRNAs was detected in mouse macrophage cells.


http://www.sciencedirect.com/science/article/B6T36-449T3JV-4R/2/644251b9b2574e4b320389e5aa468c93

A 569 bp probe against the [beta]-chain of hepatotropin was used to examine expression of RNA for this growth factor in human adult and foetal liver, foetal kidney and pancreas, and rat liver after partial hepatectomy. Low level expression of a 6kb RNA occurred in human adult and normal rat liver. 70% hepatectomy increased expression, peaking at 10 h and returning to near normal levels 24 h after resection. The 6 kb band was strongly expressed in human foetal liver, as compared with adult, but not in foetal kidney or pancreas, suggesting a major role for hepatotropin in both foetal development and regeneration of the liver.


http://www.sciencedirect.com/science/article/B6T36-3YS2BMK-74/2/8067f165a5c0e05d50efba9b8aa882e4

The role of a conserved arginine (R104) in the putative phosphoenol pyruvate binding region of 5-enolpyruvyl shikimate-3-phosphate synthase of Bacillus subtilis has been investigated. Employing site directed mutagenesis arginine was substituted by lysine or glutamine. Native and mutant proteins were expressed and purified to near homogeneity. Estimation of Michaelis and inhibitor constants of the native and mutant proteins exhibited altered substrate--inhibitor binding mode and constants. Mutation R104K hypersensitized the enzyme reaction to inhibition by glyphosate. The role of R104 in discriminating between glyphosate and phosphoenol pyruvate is discussed.
An important component of the extracellular matrix is the group of non-collagenous proteins belonging to the small leucine-rich repeat (SLR) protein family. A new SLR protein, podocan, with structural characteristics different from the known classes of the SLR protein family has been identified recently from the kidney. In this study, we examined the functional characteristics of this SLR protein expressed in cultured cells. Podocan was clearly observed intracellularly and was also detectable in the supernatant. Treatment of the expressed protein with various glycoenzymes suggested that podocan is a glycoprotein containing N-linked oligosaccharides but not a classical proteoglycan. Moreover, podocan was found to bind type 1 collagen. Cells transfected with podocan showed reductions in cell growth and migration, concomitant with increased p21 expression. Podocan mRNA was detected by reverse transcription polymerase chain reaction not only in the kidney, but also in other tissues including the heart and vascular smooth muscle cells, suggesting that podocan may have a potential role in growth regulation in cardiovascular tissues.

Flowering plant male gametic cell-specific gene expression has been reported recently but the regulatory elements controlling specificity of such genes expressed in generative cell and sperm cells have not been identified and studied. Here, we report the 0.8 kb promoter sequence upstream of the start of the transcription site of the generative cell-specific gene, LGC1, sufficient to regulate the expression of reporter genes in a cell-specific manner. In addition, the diphtheria toxin A-chain- (DT-A)-coding region under the control of the LGC1 promoter sequence confirmed unequivocally the lack of LGC1 expression in vegetative tissues. Transgenic tobacco plants carrying the LGC1-DT/A construct showed normal phenotype except for anthers of these plants that contained sterile and aborted pollen. Truncation and internal deletion analysis of the LGC1 promoter identified -242 bp as the minimal sequence necessary for male gametic cell-specific expression. In addition, a regulatory sequence required for determining generative cell-specific expression of LGC1 was identified. Deletion of this regulatory sequence led to loss of the generative cell specificity resulting in activation of this promoter in other tissues where it is normally repressed. Therefore, male gametic cell specificity of the LGC1 gene seems to be regulated by factors that suppress its activation in other plant cells. This is the first report of a male gametic cell-specific promoter, hence can be used as a novel tool in molecular analyses and experimental manipulation of flowering plant spermatogenesis and fertilization.

Polymorphisms of G-protein coupled receptor (GPCR) genes are associated with disease risk.
and modification, and the response to receptor-directed therapy. Genomic sequencing (~1700 automated runs) from as many as 120 chromosomes from 60 multiethnic individuals was performed to confirm non-synonymous coding polymorphisms reported in the dbSNP database from 25 randomly selected GPCR genes. These polymorphisms were in regions of the receptors responsible for structural integrity, ligand binding, G-protein coupling and phosphoregulation. However, most of these putative polymorphisms could not be confirmed (false positive rate of 68%). Based on these results, we suggest that the variability of the superfamily is not well defined, and we caution against exclusive reliance on databases for selection of candidate GPCR polymorphisms for disease association and pharmacogenetic studies.


http://www.sciencedirect.com/science/article/B6T36-44BMWXC-W8/2/847804d2cc740f274ceb81e30d9566f8

We have determined the sequence of a venom allergen phospholipase A1 from white-faced hornet (Dolichovespula maculata) by cDNA and protein sequencings. This protein of 300 amino acid residues (Dol m I) has no sequence similarity with other known phospholipases. But it has sequence similarity with mammalian lipases; about 40% identity in overlaps of 123 residues. Tests suggest that hornet phospholipase has weak lipase activity. Hornet venom has 3 major allergens, and another hornet allergen antigen 5 (Dot m V) was previously found to have sequence similarity with a mammalian testis protein and a plant leaf protein.


http://www.sciencedirect.com/science/article/B6T36-3XM3KN9-2/2/9d7cda3183ab0002a60f91183f8240

Murine erythroleukemia (MEL) cells, in addition to an mRNA coding for a 30 kDa high mobility group (HMG)-1 protein, contain an mRNA coding for a 6 kDa HMG1 protein having the following structural properties: (1) its primary structure has 90% homology with the N-terminal sequence of the 30 kDa HMG1 protein; (2) it contains a consensus region of the HMG1 protein family; (3) it is deprived of the cluster of acidic amino acids that characterizes the C-terminal region of the 30 kDa HMG1 protein. This novel small Mr HMG1 protein has been expressed in prokaryotic cells and tested to establish similarities and differences in activity compared to the homologous higher Mr HMG1 protein. It has been found that the low Mr HMG1 form is not released from MEL cells following induction to erythroid differentiation, but is still effective, although with much less efficiency, when added to the external medium, in promoting acceleration in the rate of MEL cell differentiation as well as in activation of [alpha]-protein kinase C. Altogether these results provide evidence for the presence in MEL cells of a multigene family that encodes at least two different HMG1-type sequences most presumably involved, at distinct cellular sites, in different functions although commonly related to the promotion of cell differentiation. Additional information can be considered concerning the relationship between the characteristic N-terminal sequence of HMG1 protein and the extracellular activity on MEL cell differentiation.

upregulate the expression of vascular endothelial growth factor in vascular smooth muscle cells."

http://www.sciencedirect.com/science/article/B6T36-3YMWT7S-5J/2/0993dcf2076c822739e6d54c5b1dddb42

Vascular endothelial growth factor (VEGF) mRNA expression was analysed in rabbit vascular smooth muscle cells following exposure to hypoxia and platelet-derived growth factor-BB (PDGF-BB). Hypoxia potently upregulated VEGF mRNA steady-state levels in a time- and concentration-dependent manner reaching a maximum level (~30-fold increase) after 12-24 h at 0% O2. In contrast, PDGF-BB caused a modest increase in VEGF expression. However, the combination of PDGF-BB and a threshold hypoxic stimulus (2.5% O2 for 4 h) had a marked synergistic effect. Synergy between hypoxia and PDGF-BB was selective for VEGF expression as hypoxia had no effect on the PDGF-induced upregulation of the proto-oncogene c-myc. These results raise the possibility that hypoxia and PDGF-BB may act in concert to induce VEGF expression in the arterial wall during the development of atherosclerosis.


http://www.sciencedirect.com/science/article/B6T36-3YMWT11-1P/2/adb569c19ef6c6e93cb82aba5246a32c

We report the molecular analysis of the transthyretin gene in a large Italian pedigree with familial amyloidotic polyneuropathy and demonstrate the presence of a Met30 mutation. The usefulness of the genetic analysis in the identification of presymptomatic persons and the diagnosis of individuals with partial symptoms is discussed.


http://www.sciencedirect.com/science/article/B6T36-3RB8YT6-4Y/2/c8ada68d62065bd6ce3a02b2a240f38

We analysed the expression of members of the hh gene family in adult ocular tissues of newt, frog and mouse by RT-PCR method. Shh displayed restricted expression in the neural retina that was conserved in each species analyzed. X-bhh, X-chh and mouse Ihh were detected in the iris and in the retinal pigment epithelium, while mouse Dhh was detected additionally in the neural retina and faintly in the cornea. We also found that two types of ptc genes, potential hh targets and receptors, were expressed in these tissues, suggesting the presence of active hh signalling there.


http://www.sciencedirect.com/science/article/B6T36-44G8CWW-45/2/5b70f1f09d78d74388c074a72ef9b4ca

The nucleotide sequence of a 25.7 kilobase Drosophila melanogaster genomic DNA segment
containing a gene for a ryanodine receptor/calcium release channel homologue has been determined. Computer analysis and partial cDNA cloning revealed 26 exons comprising the protein-coding sequence in this gene. The predicted protein is homologous in amino acid sequence and shares characteristic structural features with the mammalian ryanodine receptors. In blot hybridization analysis, a ~16 kilobase RNA species was identified abundantly in a 6-12 h embryo as the transcript from this gene. In situ hybridization to polytene chromosomes indicated that this gene locates at band position 44F on the second chromosome.


http://www.sciencedirect.com/science/article/B6T36-3VS2KSG-10/2/7c4211853740c53f824df131015dc1ce

The family of the RACK molecules (receptors for activated C kinases) are present in all the species studied so far. In the genus Leishmania, these molecules also induce a strong immune reaction against the infection. We have cloned and characterised the gene that encodes the RACK analogue from the parasite trypanosomatid Crithidia fasciculata (CACK). The molecule seems to be encoded by two genes. The sequence analysis of the cloned open reading frame indicates the existence of a high degree of conservation not only with other members of the Trypanosomatidae but also with mammals. The study of the protein kinase C phosphorylation sites shows the presence of three of them, shared with the mammalian species, additional to those present in the other protozoa suggesting a certain phylogenetic distance between the protozoon Crithidia fasciculata and the rest of the Trypanosomatidae. The CACK-encoded polypeptide shows an additional sequence of four amino acids at the carboxy-terminal end, which produces a different folding of the fragment with the presence of an [alpha]-helix instead of the [beta]-sheet usual in all the other species studied. A similar result is elicited at the amino-terminal end by the change of three amino acid residues. The immunolocalisation experiments show that the CACK displays a pattern with a distribution mainly at the plasma membrane, different from that of the related Leishmania species used as control, that displays a distribution close to the nucleus. Altogether, the data suggest that the existence of the structural differences found may have functional consequences.


http://www.sciencedirect.com/science/article/B6T36-3V4CRFD-8/2/489193c88161748d07a606a31af5cfc6

A phylogenetic analysis, using the open reading frame 1 sequence of 93 TT viruses (TTV) obtained from various geographical areas, indicated that the virus could be classified into six different genotypes including three hitherto unreported genotypes. The high reliability of the six clusters was confirmed by bootstrap analysis. On the basis of these sequence data, a new simple genotyping assay based on a restriction fragment length polymorphism of TTV was developed. Using the enzymes Ndel and Pstl, followed by cleavage with NlaIII or MseI, it was possible to distinguish between the six TTV genotypes. This system will provide the framework for future detailed epidemiological and clinical investigations.
S100A4 (Mts1) is a Ca2+-binding protein of the S100 family. This protein plays an important role in promoting tumor metastasis. In order to identify S100A4 interacting proteins, we have applied the yeast two-hybrid system as an in vivo approach. By screening a mouse mammary adenocarcinoma library, we have demonstrated that S100A4 forms a heterocomplex with S100A1, another member of the S100 family. The non-covalent heterodimerization was confirmed by fluorescence spectroscopy and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mutational analysis revealed that replacement of Cys76 and/or Cys81 of S100A4 by Ser abolishes the S100A4/S100A1 heterodimerization, but does not affect the S100A4 homodimerization in vivo.

The cDNA encoding murine procathepsin E was isolated and sequenced and recombinant enzyme was produced in Escherichia coli. The activity of the purified recombinant mouse cathepsin E was characterised quantitatively using two synthetic peptide substrates and naturally occurring inhibitors. The majority of the recombinant enzyme was present as a homodimer (Mr ~80) in which the two monomers were linked by an intermolecular disulfide bond. By analogy to previous studies with human cathepsin E, this is most likely a consequence of the presence of a unique cysteine residue near the N-terminus of the mature proteinase. The availability of (i) recombinant murine enzyme in reasonable quantities and (ii) a full-length cDNA now enables structural investigations and attempts to generate 'knock-out' mice deficient in this important aspartic proteinase to be undertaken.

Pheromone receptors are expressed in the accessory olfactory system, which is vital for non-specific chemical communication and for sexual behavior. Under the hypothesis that some of the pheromone molecules released from female reproductive organs might regulate sperm chemotaxis or chemokinesis, we examined whether the V1R type pheromone receptor mRNAs are expressed in developing germ cells. By a reverse transcription-PCR method, we obtained nine kinds of cDNA fragments belonging to the receptor family. In situ hybridization analysis in testicular sections using probes of testicular pheromone receptors (TVRs) revealed that TVR mRNAs were expressed by spermatids. TVRs were also expressed in the accessory olfactory organ. In the testis, hybridization signals were localized in subsets of the seminiferous tubules, suggesting that TVRs were expressed by selective subsets of the spermatids. In situ hybridization study suggests also that each sperm expresses multiple pheromone receptors. The testicular
pheromone receptors might have an important role in the maturation and/or migration of sperm.


http://www.sciencedirect.com/science/article/B6T36-3SHT1NY-F/2/0891fa5218ed5320b7aa76fd6326d851

The first complete amino acid sequence of a flavin-containing polyamine oxidase was solved by a combined approach of nucleotide and peptide sequence analysis. A cDNA of 1737 bp, isolated from maize seedlings by reverse transcription-polymerase chain reaction and rapid amplification of cDNA ends strategies, was cloned and its sequence determined. This cDNA contains information for a polypeptide chain of 500 amino acids. Its amino-terminal sequence shows the typical features of secretion signal peptides. The primary structure of the mature protein was independently confirmed by extensive amino acid sequencing. Structural relationships with flavin-containing monoamine oxidases are also discussed.


http://www.sciencedirect.com/science/article/B6T36-3RSNBYV-P/2/6ade010c18d51d50516670418256d03d

In this paper we describe isolation and molecular characterization of human dihydroxyacetonephosphate acyltransferase (DAP-AT). The enzyme was extracted from rabbit Harderian gland peroxisomes and isolated as a trimeric complex by sucrose density gradient centrifugation. From peptide sequences matching EST-clones were obtained which allowed cloning and sequencing of the cDNA from a human cDNA library. The nucleotide-derived amino acid sequence revealed a protein consisting of 680 amino acid residues of molecular mass 77187 containing a C-terminal type 1 peroxisomal targeting signal. Monospecific antibodies raised against this polypeptide efficiently immunoprecipitated DAP-AT activity from solubilized peroxisomal preparations, thus demonstrating that the cloned cDNA codes for DAP-AT.


http://www.sciencedirect.com/science/article/B6T36-44M4167-9H/2/a475fa032649338aab4919b71434f25d

The polymerase chain reaction was carried out with primers hybridizing to conserved regions of the phytochrome genes. With DNA from the moss Ceratodon purpureus 5 overlapping fragments were obtained resulting in a continuous nucleotide sequence of 1474 bp. The deduced amino acid sequence showed homology of around 60% with all known phytochrome sequences. The sequences contained a conserved chromophore attachment site. In light-grown Ceratodon protonemata the phytochrome mRNA with the size of about 4.5 kb was detected.
Kynureninase [E.C.3.7.1.3.] is one of the enzymes involved in the biosynthesis of NAD cofactors from tryptophan through the kynurenine pathway. By tryptic and CNBr digestion of purified rat liver kynureninase, we obtained about 28% of the amino acid sequence of the enzyme. The rat kynureninase cDNA, isolated by means of reverse-transcribed polymerase chain reaction and hybridization screening, codes for a polypeptide of 464 amino acids. Northern blot analysis revealed the synthesis of a 2.0 kb rat kynureninase mRNA. A cDNA encoding human liver kynureninase was also isolated. The deduced amino acid sequence is 85% identical to that of the rat protein. COS-1 cells were transfected with both cDNAs. The $K_m$ values of the rat enzyme, for -kynurenine and -3-hydroxykynurenine, were 440+/−20 [μM] and 32+/−5 [μM] and of the human enzyme 440+/−20 [μM] and 49+/−6 [μM], respectively. Interestingly, COS-1 cells transfected with the cDNA coding for rat kynureninase also display cysteine-conjugate [beta]-lyase activity.

The effect of ultraviolet B (UVB) irradiation on endothelin-1 (ET-1) and ET receptor expression was examined using cultured normal human keratinocytes. Keratinocytes secreted ET-1 in the medium at a level of 2.1 pg/day/105 cells. UVB irradiation up to 10 mJ/cm2 increased ET-1 secretion 3-fold, and potentiated expression of mRNA for ET-1. Both ETA and ETB receptor mRNAs were detected in keratinocytes, and their expression was up-regulated by 5 mJ/cm2 UVB irradiation.

Transport of karyophilic proteins into the nucleus is mediated by nuclear localization signals (NLSs) via a multistep process. The karyophiles are recognized by the importin [alpha] subunit in the cytoplasm to form a stable complex, termed the nuclear pore-targeting complex (PTAC). To date, three different mammalian [alpha] subunits (mSRP1/NPI-1, PTAC58/mPendulin/Rch1 and Qip1) have been identified. In this study, we report the identification of three additional mouse genes homologous to the known [alpha] subunits using RT-PCR methodology and show that the mouse [alpha] subunits can be classified into at least three subfamilies, [alpha]-P, [alpha]-Q and [alpha]-S families, each composed of closely related members (more than 80% amino acid sequence identity). These three subfamilies, however, have ~50% amino acid identity to one another. Northern blot analysis showed that all were differentially expressed in various mouse tissues. These results suggest that the function of these proteins may be controlled in a tissue-specific manner and that their combinatorial expression may play a role in differentiation and
organogenesis.


http://www.sciencedirect.com/science/article/B6T36-3YRNYDD-MT/2/ceb889ac1b29983c4f26e6af7f62272d

Chimeric receptor subunits of the AMPA receptor subunit GluR2 and the kainate receptor subunit GluR6 were constructed and stably expressed in baby hamster kidney cells. By using cal imaging and radioligand binding, we demonstrated that substitution of a specific domain showing homology to a bacterial leucine-isoleucine-valine binding protein (LIVBP) had no effect on the affinities of the tested agonists, but decreased the affinities of the antagonists CNQX, DNQX, and NBQX. On the other hand, when the first of two domains showing homology to a bacterial glutamine binding protein (QBP) in GluR2 was substituted with the corresponding region from GluR6, the affinity of AMPA decreased sevenfold and the affinity of kainate increased fourfold, indicating the importance of this domain in binding of these agonists. In contrast to this, the affinities of quisqualate and domoate, two other agonists, were unchanged, indicating that a region located C-terminal to the QBP domain is also involved in agonist binding.


http://www.sciencedirect.com/science/article/B6T36-3XR2GT0-9/2/1ea22c1b102db257595a0744cf8cfdab

We show that in a series of eight breast cancer cell lines, a direct relationship exists between the overall DNA demethylation and the percentage of rearranged chromosomes, except for cell lines with a highly rearranged genome which can be weakly demethylated. A real time fluorescent detection method was used to quantify by reverse transcription-PCR the expression of the DNA methyltransferase 1 and of the newly discovered DNA demethylase. The overall DNA methylation status seems to result from a complex interplay between the expression of these two genes. Our results suggest that in these tumor cells, the overall DNA demethylation is implicated in one of the mechanisms at the origin of the genome instability and that besides the role of the DNA methyltransferase 1, that of the DNA demethylase may be essential in the control of DNA methylation.


http://www.sciencedirect.com/science/article/B6T36-44XN362-W5/2/a251806c35e0642bfc6e692d066b904c

The cloning, sequencing and overexpression of the gene coding for Bacillus stearothermophilus ribosomal protein L9 is described. The sequence corresponds directly to that presented for the protein itself by classical methods, differing at only a few amino acid positions. The purification and crystallisation of the corresponding L9 protein is presented. The crystals are isomorphous to those described for L9 obtained by conventional methods.

We report the detailed expression pattern of the voltage-dependent potassium channel KV3.4 (rat homologue, Raw3) in mouse skeletal muscle. Using semi-quantitative RT-PCR, we show that its expression is detectable at embryonic day 17 and rises to adult levels within 2 weeks after birth. Expression is fiber type-dependent, with mRNA levels being 5-6-fold lower in the mixed slow/fast soleus muscle than in the fast tibialis anterior and extensor digitorum longus muscles. Fast muscles from myotonic mice exhibit low KV3.4 mRNA levels similar to those of wild-type soleus. In denervated extensor digitorum longus, KV3.4 expression declines to perinatal levels. We conclude that KV3.4 expression in mouse skeletal muscle is regulated by the pattern of excitation.


We have recently cloned a cDNA for mouse matrilin-4. By sequence comparison we identified the 12 kb long human matrilin-4 gene as a part of a high-throughput genomic sequence (HS453C12) in the databases. Additionally we found a human matrilin-4 expressed sequence tag (H54037) in the database that had been mapped to chromosome 20q13.1-2. The gene contains 10 exons and, like the matrilin-1 gene, the human matrilin-4 gene contains an AT-AC intron between the two exons encoding the coiled-coil domain. The cDNA sequence of human matrilin-4 was determined by sequencing of RT-PCR products obtained from mRNA of the human embryonic kidney cell line HEK 293. At the amino acid level it showed an overall sequence identity to the mature mouse matrilin-4 of 91% with a maximum of 97% in the second vWFA-like module. Alternative splicing leads to three different mRNAs. They all encode the putative signal peptide, the two vWFA-like domains and the potential coiled-coil [alpha]-helical oligomerisation domain but differ in that either one, two or three EGF-like domains are retained in the mature mRNA. Due to a G to A mutation at the splice donor site of intron C, the third exon encodes an untranslated pseudo-exon specifying the first EGF-like domain when compared to mouse matrilin-4.


Mouse cDNA encoding for matrilin-4 was cloned and the primary structure of this fourth member of the matrilin family was deduced from the nucleotide sequence. The protein precursor of 624 amino acids consists of a putative signal peptide, two vWFA-like domains linked by four epidermal growth factor-like modules and a potential coiled-coil [alpha]-helical oligomerization domain at the C-terminus. The predicted Mr of the mature protein is 66442. Expression in lung,
brain, sternum, kidney and heart was detected by Northern blot analysis of mouse mRNA. Additionally an alternatively spliced mRNA lacking the sequence coding for the first vWFA domain was found in 7 weeks old mice leading to a protein precursor of 434 amino acids and a predicted Mr of the mature protein of 45468.


http://www.sciencedirect.com/science/article/B6T36-44XN0CY-9M/2/6f0e716298055555a78ad8c22f96cd1f

Complementary DNA encoding the isoform of protein phosphatase 2C, termed PP2C2, has been isolated. The cDNA predicts a protein of 390 amino acid residues with a molecular mass of 42,888 Da. The protein displays 76% identity to the PP2C1 isoform.


http://www.sciencedirect.com/science/article/B6T36-4F19SSK-4/2/e53d9426754224ccc5bcf11178e774a

It is clear that G1-S phase control is exerted after the mouse embryo implants into the uterus 4.5 days after fertilization (E4.5); null mutants of genes that control cell cycle commitment such as max, rb (retinoblastoma), and dp1 are embryonic lethal after implantation with proliferation phenotypes. But, a number of studies of genes mediating proliferation control in the embryo after fertilization-implantation have yielded confusing results. In order to understand when embryos might first exert G1-S phase regulatory control, we assayed preimplantation mouse embryos for the acquisition of expression of mRNA, protein, and phospho-protein for max, Rb, and DP-1, and for the proliferation-promoting phospho-protein forms of mycC (thr58/ser62) and Rb (ser795). The key findings are that: (1) DP-1 protein was present in the nucleus as early as the four-cell stage onwards, (2) max protein was in the nucleus, suggesting function from the four-cell stage onwards, (3) both mycC and Rb all form protein was present at increasing quantities in the cytoplasm from the 2 cell and 4/8 cell stage, respectively, (4) the phosphorylated form of mycC phospho was present in the nucleus at high levels from the two-cell stage through blastocyst-stage, and (5) the phosphorylated form of Rb was detected at low levels in the two-cell stage embryo and was highly expressed at the 4/8-cell stage through the blastocyst stage. Taken together, these data suggest that activation of mycC phospho/max dimer pairs, (E2F)/DP-1 dimer pairs, and repression of Rb inhibition of cell cycle progression via phosphorylation at ser795 occurs at the earliest stages of embryonic development. In addition, the presence of max, mycC phospho, DP-1, and Rb phospho in the nuclei of embryonic and placental lineage cells in the blastocyst and in trophoblast stem cells suggests that a similar type of cell cycle regulation is present throughout preimplantation development and in both embryonic and extra-embryonic cell lineages.


http://www.sciencedirect.com/science/article/B6T36-3WWDHMS-
The zebrafish is widely used as a model system for studying mammalian developmental genetics and more recently, as a model system for carcinogenesis. Since there is mounting evidence that selenium can prevent cancer in mammals, including humans, we characterized the selenocysteine tRNA[Ser]Sec gene and its product in zebrafish. Two genes for this tRNA were isolated and sequenced and were found to map at different loci within the zebrafish genome. The encoding sequences of both are identical and their flanking sequences are highly homologous for several hundred bases in both directions. The two genes likely arose from gene duplication which is a common phenomenon among many genes in this species. In addition, zebrafish tRNA[Ser]Sec was isolated from the total tRNA population and shown to decode UGA in a ribosomal binding assay.


Evidence suggests a physiological role of the GABAA receptor in the pancreas. Clinically, an autoimmune reaction involving the GABA biosynthesizing enzyme, glutamic acid decarboxylase has been implicated in the development of insulin-dependent diabetes mellitus. To determine the subtypes of GABAA receptor expressed in human pancreas, we analyzed, with the use of the reverse-transcription/polymerase chain reaction technique human pancreatic tissue for the presence of GABAA receptor subunits [alpha]1-6, [beta]1-3, and [gamma]1-2 transcripts. Unlike brain tissue, pancreatic tissue only expresses the [alpha]2, [beta]3 and [gamma]1 subunits. Our results provide evidence of a specific GABAA receptor subtype expressed in human pancreatic tissue.


The CX3C chemokine, fractalkine (FKN, CX3CL1), has multiple functions and exists as two distinct forms, a membrane-anchored protein and a soluble chemotactic peptide that cleaves from the cell surface FKN. In this study, we first demonstrated the expression of FKN in tumor necrosis factor (TNF)-[alpha]- and interleukin (IL)-4-stimulated human fibroblasts. The induction of FKN was observed for both forms. We also demonstrated monocyte chemotactic activity in the culture supernatant from the fibroblasts stimulated with these cytokines. These results suggest that TNF-[alpha]- and IL-4-stimulated fibroblasts may play an important role in accumulation of monocytes at inflammatory sites.

H/2/2ceb746a5e445cee172a9959f902efeb

We have isolated a cDNA clone encoding a deep brain photoreceptive molecule from the hypothalamic cDNA library of the toad, Bufo japonicus. The deduced amino acid sequence showed the highest similarity to that of pinopsin (75-76%) among vertebrate retinal opsins, indicating the expression of toad pinopsin in the deep brain. Antibodies raised against the C-terminal tail of toad pinopsin stained cell bodies and the knob-like structures of the cerebrospinal fluid-contacting neurons in the anterior preoptic nucleus. This region is known to play an important role in breeding behavior, suggesting that toad pinopsin acts as a photosensor for the photoperiodic gonadal response.


http://www.sciencedirect.com/science/article/B6T36-44W4WMV-59/2/27de7cf44722fac66f61a3f18bc9e74

The porcine leukocyte protegrins are a family of cysteine-rich antimicrobial peptides the primary structures of which combine features of defensins and tachyplesins. We cloned three protegrins from porcine bone marrow mRNA by PCR, including one (PG-4) that was previously unknown. The 691 bp protegrin cDNAs were > 98.8% identical, and each was surrounded by highly conserved 5' and (in some instances) 3' sequences present in structurally dissimilar antimicrobial and LPS-binding peptides of animal leukocytes.

FEMS Immunology and Medical Microbiology(28)


The inhibitory effect of human and porcine bile samples to detect Helicobacter DNA was studied by adding different concentrations of bile samples to PCR mixtures of six thermostable DNA polymerases containing cagA specific primers and Helicobacter pylori DNA. PCR products were amplified by using the Rotorgene system and SYBR Green I. Among the six DNA polymerases tested, rTth had the lowest sensitivity to bile inhibitors, whereas Taq and Tfl had the highest sensitivity. Bile proteins did not inhibit AmpliTaq DNA polymerase, whereas the fraction containing mainly bile acids and their salts inhibited the amplification capacity of AmpliTaq. Heating human bile at 98 [deg]C and adding casein and formamide to the reaction mixture reduced the PCR inhibitory effect of bile. Therefore, a pre-PCR treatment based on dilution and heating of bile, adding casein and formamide to the reaction mixture of rTth DNA polymerase was found efficient to amplify DNA directly in bile.

The promising arena of DNA-based vaccines has led us to investigate possible candidates for immunization against bacterial pathogens. One such target is the opportunistic pathogen Pseudomonas aeruginosa which produces exotoxin A (PE), a well-characterized virulence factor encoded by the toxA gene. In its native protein form, PE is highly cytotoxic for susceptible eukaryotic cells through ADP-ribosylation of elongation factor-2 following internalization and processing of the toxin. To study the biologic and immunological effects of PE following in situ expression, we have constructed eukaryotic plasmid expression vectors containing either the wild-type or a mutated, non-cytotoxic toxA gene. In vitro analysis by transfection of UM449 cells suggests that expression of the wild-type toxA gene is lethal for transfected cells whereas transfection with a mutated toxA gene results in the production of inactive PE which can be readily detected by immunoblot analysis of cell lysates. To investigate the effects resulting from the intracellular expression of potentially cytotoxic gene products in DNA vaccine constructs, we immunized mice with both the wild-type and mutant toxA plasmid constructs and analyzed the resulting humoral and cellular immune responses. Immunization with the mutated toxA gene results in production of neutralizing antibodies against native PE and potentiates a TH1-type response, whereas only a minimal humoral response can be detected in mice immunized with wild-type toxA. DNA-based vaccination with the non-cytotoxic toxAmut gene confers complete protection against challenge with the wild-type PE. Therefore, genetic immunization with genes encoding potentially cytotoxic gene products raises concern with regard to the selection of feasible gene targets for DNA vaccine development.


In order to estimate the rate of microsporidia, cryptosporidia and giardia contamination of swimming pools, sequential samples of water were collected during a one-year period in six different swimming pools in Paris, France. Forty-eight samples were submitted to filtrations. Eluates were examined for microsporidia using polymerase chain reaction (PCR) and for cryptosporidia and giardia using immunofluorescence staining. One of 48 specimens was positive for microsporidia. Using DNA sequence analysis, unknown microsporidia species were identified, which were close to an insect microsporidia Endoreticulatus schubergi. One sample was positive for cryptosporidia and none were positive for giardia. This study shows a low level of swimming pool water contamination by microsporidia, cryptosporidia or giardia, demonstrating the efficacy of cleaning filtration and disinfection procedures used in French swimming pools.


In order to estimate the rate and seasonal variation of Enterocytozoon bieneusi contamination of surface water, sequential samples of water from the River Seine in France were collected during a 1-year period. Each sample (300-600 l) was submitted to sequential filtrations, and the filters were then examined for microsporidia using light microscopy and nested polymerase chain
reaction (PCR) for E. bieneusi. Amplified products were hybridized with a E. bieneusi-specific probe. Twenty-five samples of water were analyzed during 1 year. Microscopic examination of stained filters proved unreliable for the identification of spores. Using nested PCR, 16 of 25 specimens were positive (64%). Unexpectedly, E. bieneusi was identified in only one sample by specific hybridization underlining the lack of specificity of ours primers. Nevertheless, using DNA sequence analysis, unknown microsporidia species were identified in eight cases, which had highest scores of homology with Vittaforma corneae or Pleistophora sp. This study shows a low rate of water contamination by E. bieneusi suggesting that the risk of waterborne transmission to humans is limited.


TnphoA mutagenesis was used to identify adhesins of Aeromonas veronii biovar sobria 3767, a strain isolated from a diarrhoeal stool specimen. Six mutants, from a library of 154, exhibited significantly reduced levels of adhesion to HEp-2 cells. Primers to the terminal regions of TnphoA were used for inverse PCR and the product from one mutant was cloned into pBluescript and partial sequence data obtained. Scanning GenBank and EMBL data bases revealed DNA sequence similarity to the copA gene of Pseudomonas syringae pv. tomato which confers resistance to copper and other heavy metals. The transposon was located within the copA gene and the mutant exhibited a reduced tolerance to copper. Primer walking, using the inverse PCR product as a template, revealed three open reading frames (ORFs) copA, B and C in A. veronii biovar sobria 3767. The predicted amino acid sequences of ORFs A and B had significant homology (55 and 34% respectively) to the copA and B proteins of P. syringae. No amino acid or DNA sequence homology existed between ORF C of strain 3767 and any other gene in the data bases scanned. Further analysis of the nucleotide sequence failed to reveal the presence of typical copper regulatory genes within the vicinity of the Aeromonas sequence. The association between copper tolerance and adhesion in A. veronii biovar sobria requires further study.


Besides group A (GAS), Lancefield group C [beta]-haemolytic streptococci (GCS) have been implicated as a causative agent in outbreaks of purulent pharyngitis. In this study we have investigated a class CI M protein of a Streptococcus dysgalactiae human wound isolate designated MC. MC shares similar properties with M proteins of GAS. It contributes to the virulence of the investigated GCS strain as revealed by in vivo phagocytosis in chicken embryos. Further, MC showed multiple binding to the human plasma proteins fibrinogen, albumin, plasminogen, IgA and all subclasses of IgG. Until now, an M protein, especially from a group C strain, with such a multiple binding behaviour has not been described. Immunoblot experiments with 150 patient sera, having a rheumatoid factor titre >1:256, revealed that 26% of these sera showed serological cross-reactivity between a 68-kDa cartilage protein and the N-terminal part of MC. Only 8% of the sera of healthy patients showed this property. In additional, MC also cross-reacted with antibodies recognising epidermal keratins. The cross-reacting 68-kDa protein from
cartilage was different from human serum albumin, but was recognised with anti-vimentin immune serum. The MC was cloned and the gene sequenced. By using PCR, recombinant gene fragments encoding characteristic peptide fragments of MC were expressed in Escherichia coli. The peptides were used to map the binding sites for plasma proteins and to locate the cross-reacting epitopes on the MC molecule. In consequence, sequence alignments revealed that MC shared homologous regions with vimentin and different keratins. Our data, obtained with MC, suggest that not only infections with GAS but also infections with GCS and possibly GGS (the latter species can also produce class CI M-like proteins) may be responsible for the formation of streptococcal-associated sequel diseases.


http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-5/2/31af0f8a6480395605681192a235a513

Twenty-five strains of Plesiomonas shigelloides isolated from aquatic environment, 10 strains from human cases of diarrhoea and five strains from animals were identified by the polymerase chain reaction technique based on 23S rRNA gene. For this purpose, two primers targeted against part of the 5′ half of the 23S rRNA gene of P. shigelloides (Escherichia coli number C-912, G-1195; Plesiomonas number C-906, G-1189) were designed. Results from our study indicated that this method might serve as a tool for a rapid and sensitive identification of P. shigelloides from different environmental and clinical sources.


http://www.sciencedirect.com/science/article/B6T2T-405KDGT-D/2/96d5ea12798ad7c921e9405037ce089c

In a period where the proportion of culture confirmed cases in the UK has been steadily declining, diagnosis by PCR has been used to increase the number of confirmed cases and provide additional epidemiological data. This report presents a comparative evaluation of the fluorogenic probe-based 5′ exonuclease assay (Taqman) using the Perkin-Elmer Applied Biosystems automated sequence detection system 7700 with previously reported polymerase chain reaction enzyme-linked immunosorbent (PCR ELISA) assays for the detection of meningococcal DNA in CSF, plasma and serum samples. Taqman assays developed were based on the detection of a meningococcal capsular transfer gene (ctrA), the insertion sequence IS1106 and the sialytransferase gene (siaD) for serogroup B and C determination and compared with similar assays in a PCR ELISA format. The Taqman ctrA assay was specific for Neisseria meningitidis, however the IS1106 assay gave false positive reactions with a number of non-meningococcal isolates. Sensitivity of the Taqman ctrA, IS1106 and siaD assays testing samples from culture-confirmed cases were 64, 69 and 50%, respectively, compared with 26, 67 and 43% for the corresponding PCR ELISA assays. Improvements to the DNA extraction procedure has increased the sensitivity to 93 and 91% for the TaqMan(TM) ctrA and siaD assays, respectively, compared to culture confirmed cases. Since the introduction of Taqman PCR a 56% increase in laboratory confirmed cases of meningococcal disease has been observed compared to culture only confirmed cases. The developed Taqman assays for the diagnosis of meningococcal disease enables a high throughput, rapid turnaround of samples with considerable reduced risk of contamination.

http://www.sciencedirect.com/science/article/B6T2T-3YKC6HH-8/2/76bac56992f4b4b8d5fcaa07ba0c046c

Fusobacterium nucleatum is known to adhere to human polymorphonuclear neutrophils (PMNs) and cause them to aggregate. In this study, we isolated a spontaneously occurring aggregation defective (AGG-) mutant and this mutant will be used for future study of the interactions between this bacterium and human PMN. Genomic DNA fingerprinting by random-primed polymerase chain reaction method revealed a difference between the parent strain and the AGG- mutant. This mutant also showed an altered phenotype in both microbicidal and phagocytic assays, suggesting that the bacterial factor involved in the aggregation may also be very important for the phagocytosis and, subsequently, the killing by human PMNs. Further study of this mutant may help to clarify the molecular mechanisms of the interaction between this pathogen and human PMNs.


http://www.sciencedirect.com/science/article/B6T2T-4BRSXJ6-1/2/4b6a36ff994e043d0cab8cf73ebfc586c

In many animal species different intestinal Helicobacter species have been described and a few species are associated with intestinal infection. In humans, the only member of the Helicobacter family which is well described in literature is Helicobacter pylori. No other Helicobacter-associated diseases have definitely been shown in humans. We developed a sensitive quantitative PCR to investigate whether Helicobacter species DNA can be detected in the human gastrointestinal tract. We tested gastric biopsies (including biopsies from H. pylori positive persons), intestinal mucosal biopsies and fecal samples from healthy persons, and intestinal mucosal biopsies from patients with inflammatory bowel disease (IBD) for the presence of Helicobacter species. All gastric biopsies, positive for H. pylori by culture, were also positive in our newly developed PCR. No Helicobacter species were found in the mucosal biopsies from patients with IBD (n=56) nor from healthy controls (n=25). All fecal samples were negative. Our study suggests that Helicobacter species, other than H. pylori, are not present in the normal human gastrointestinal flora and our results do not support a role of Helicobacter species in IBD.


http://www.sciencedirect.com/science/article/B6T2T-3Y6RF1B-6/2/accc6359e29aee3df0a13fe05bf9537

The polymerase chain reaction (PCR) method has been employed to amplify a chlamydial genome encoding four variable segments of the major outer membrane protein and genotyping of different Chlamydia trachomatis serovars was successfully achieved by means of restriction
fragment length polymorphism (RFLP) analysis and sequencing of amplified DNA. These methods were applied to identify the serotypes of C. trachomatis in endocervical specimens obtained from asymptomatic pregnant Japanese women at 28-30 weeks of gestation. Among the 218 specimens, 207 were serotyped 43 (19.3%) as serovar D, 53 (24.3%) as E, 24 (11.0%) as F, 39 (17.9%) as G, 15 (6.9%) as H, 15 (6.9%) as I, five (2.3%) as J, nine (4.1%) as K and four (1.8%) as mixed. Among the 11 unclassified strains by RFLP, six (2.8%) were identified as serovar B variants and five (2.3%) were identified as D/IC-Cal-8. It was suggested that variants of endemic trachoma serovars also have affinity for the urogenital tract of Japanese pregnant women.


http://www.sciencedirect.com/science/article/B6T2T-3S1PY2B-6/2/59f57d49a729dcb5d3cdd15c05c83d2

Using defined rfb mutants, defective in the biosynthesis of the O-antigen of the lipopolysaccharide (LPS), and monoclonal antibodies (MAbs) to the A, B and C LPS antigens, we have examined the distribution of the antigens and the effects of their loss. By immunogold electron microscopy, it has been possible to determine the relative amounts of the A, B and C antigens on Inaba and Ogawa cells, confirming previous studies based upon bacterial agglutination and hemagglutination inhibitions. These antigens are absent from rfb:Tn mutants selected as resistant to phages which have been shown to use the O-antigen as their receptor. These mutants were severely attenuated as measured by both LD50 and their ability to compete with the wild-type parents when analyzed in the infant mouse cholera model. These mutants were unchanged in the export of cholera toxin or other secreted proteins but revealed an altered outer membrane protein profile. The competition defect suggested an effect on TCP (toxin-coregulated pilus). An analysis of the rfb:Tn mutants revealed that they were unable to assemble TCP on their surface, but the major subunit, TcpA, could be found as an intracellular pool. These mutants could be complemented back to wild-type using the cloned rfb region, implying that functional TCP assembly is dependent upon an intact LPS.

Kawakami, K., M. H. Qureshi, et al. (2000). "Involvement of endogenously synthesized interleukin (IL)-18 in the protective effects of IL-12 against pulmonary infection with Cryptococcus neoformans in mice." FEMS Immunology and Medical Microbiology 27(3): 191.

http://www.sciencedirect.com/science/article/B6T2T-3YKC6HH-2/2/b1002971ec533e02d18630cc36da211

We previously demonstrated that interleukin (IL)-12 protected mice against fatal pulmonary infection with a highly virulent strain of Cryptococcus neoformans, which correlated well with the production of interferon (IFN)-[gamma] as well as IL-18 in the primary infected site. In the present study, we examined the role of endogenously synthesized IL-18 in IL-12-induced host resistance to this pathogen. There was little or no production of IFN-[gamma] and IL-18 both at mRNA and protein levels in lungs of mice infected with C. neoformans, while treatment with IL-12 induced a marked production of these cytokines. Caspase-1 mRNA was expressed in infected mice even without IL-12 treatment. Administration of neutralizing anti-IFN-[gamma] monoclonal antibody (mAb) clearly inhibited production of IFN-[gamma] and IL-18 induced by IL-12, while control IgG did not show such an effect. However, administration of IFN-[gamma] did not induce the production of both cytokines in infected mice, although tumor necrosis factor (TNF)-[alpha] and IFN-[gamma]-inducible protein (IP)-10 were synthesized by the same treatment. Finally,
neutralizing anti-IL-18 antibody (Ab) significantly interfered with the production of IFN-[gamma] and elimination of the microorganism from the lung induced by IL-12 treatment. Furthermore, both IFN-[gamma] synthesis and host protection caused by IL-12 were profoundly diminished in IL-18 gene-disrupted mice. Considered collectively, our results indicated that host protection against C. neoformans induced by IL-12 involved endogenously synthesized IL-18 and that the production of IL-18 was mediated at least in part by endogenous IFN-[gamma].


http://www.sciencedirect.com/science/article/B6T2T-3X942P1-9/2/d9929032264fb5bf75eab038831eef95

We examined the mechanisms involved in the development of lung lesions after infection with Cryptococcus neoformans by comparing the histopathological findings and chemokine responses in the lungs of mice infected with C. neoformans and assessed the effect of interleukin (IL) 12 which protects mice from lethal infection. In mice infected intratracheally with a highly virulent strain of C. neoformans, the yeast cells multiplied quickly in the alveolar spaces but only a poor cellular inflammatory response was observed throughout the course of infection. Very little or no production of chemokines, including MCP-1, RANTES, MIP-1[alpha], MIP-1[beta] and IP-10, was detected at the mRNA level using RT-PCR as well as at a protein level in MCP-1, RANTES and MIP-1[alpha]. In contrast, intraperitoneal administration of IL-12 induced the synthesis of these chemokines and a marked cellular inflammatory response involving histiocytes and lymphocytes in infected mice. Our findings were confirmed by flow cytometry of intraparenchymal leukocytes obtained from lung homogenates which showed IL-12-induced accumulation of inflammatory cells consisting mostly of macrophages and CD4+ [alpha][beta] T cells. On the other hand, C-X-C chemokines including MIP-2 and KC, which attract neutrophils, were produced in infected and PBS-treated mice but treatment with IL-12 showed a marginal effect on their level, and neutrophil accumulation was similar in PBS- and IL-12-treated mice infected with C. neoformans. Our results demonstrate a close correlation between chemokine levels and development of lung lesions, and suggest that the induction of chemokine synthesis may be one of the mechanisms of IL-12-induced protection against cryptococcal infection.


http://www.sciencedirect.com/science/article/B6T2T-3VWP3TW-B/2/6b070aa427b8f8be2c2cf0fa32025907

Two divergently transcribed open reading frames: cpsX and cpsY separated by a common regulatory region was identified upstream of the cpsA-D genes involved in polysaccharide capsule biosynthesis in group B streptococci (GBS). We suggest that these genes are involved in the regulation of capsule expression in GBS, since the CpsX protein shares sequence similarities with LytR of Bacillus subtilis, an attenuator of transcription while CpsY has similarity to a wide variety of members of the LysR family of transcriptional regulators. No deletions, insertions, DNA rearrangements, or apparent differences were discovered in the postulated regulatory genes when the gene region was compared in GBS with different capsule phenotypes. Thus, other yet unidentified gene loci may control capsule phase variation in GBS.

http://www.sciencedirect.com/science/article/B6T2T-3TMXY36-3/2/3e9a3f9571085280fe98567790736eae

S and F1C fimbrial adhesins often expressed by uropathogenic Escherichia coli are genetically homologous. A multiply primed polymerase chain reaction (PCR) was developed for discriminating the S (sfa) and F1C (foc) fimbrial operons. A total of 270 uropathogenic E. coli strains and 80 fecal isolates were examined. PCR specifically detected the sfa and foc alleles in 105 (93%) of 113 sfa/foc+ strains by DNA hybridization. Furthermore, 87% of sfa+ uropathogenic E. coli simultaneously possessed the genes encoding the class III P fimbrial adhesin (prsGJ96), [alpha]-hemolysin and cytotoxic necrotizing factor 1. Statistical analysis showed the class II P fimbrial adhesin (papGIA2) and F1C fimbria to be associated with high relative virulence in pyelonephritis and cystitis, respectively. The multiply primed PCR developed should be useful for assessing the contribution of the S and F1C fimbriae in the pathogenesis of urinary tract infections.


http://www.sciencedirect.com/science/article/B6T2T-3W32484-J/2/0196da240bd9b42c5e5ac58ddf42d8b6

We measured urinary endotoxin, IL-6 and IL-8 levels in 23 patients with gram-negative urosepsis. The endotoxin and cytokine levels showed a 100-1000 fold range. No correlation was found between levels of urinary endotoxin, and IL-6 or IL-8 levels. In all cases bacterial numbers were $\geq 10^5$ CFU ml$^{-1}$ urine. The endotoxin content of the isolated microorganisms neither correlated with the urinary cytokine levels, nor with IL-6 and IL-8 levels obtained in vitro when 103 log-phase CFU of each of the bacteria were incubated with heparinized whole blood of three healthy donors. Neither the haemolysin phenotype of the bacteria, nor the presence of the P-pili gene was correlated with the cytokine response in vivo or in vitro. Other factors than known bacterial virulence factors apparently contribute to the wide variation in urinary cytokine levels in urinary tract infection.


http://www.sciencedirect.com/science/article/B6T2T-4CYPVKP-1/2/f1c106edac1f2b8c3da53b7e016fd1e3

The aim of the study was to assess the quantitative and qualitative differences of the gut microbiota in infants. We evaluated gut microbiota at the age of 6 months in 32 infants who were either exclusively breast-fed, formula-fed, nursed by a formula supplemented with prebiotics (a mixture of fructo- and galacto-oligosaccharides) or breast-fed by mothers who had been given probiotics. The Bifidobacterium, Bacteroides, Clostridium and Lactobacillus/Enterococcus
microbiota were assessed by the fluorescence in situ hybridization, and Bifidobacterium species were further characterized by PCR. Total number of bifidobacteria was lower among the formula-fed group than in other groups (P = 0.044). Total amounts of the other bacteria were comparable between the groups. The specific Bifidobacterium microbiota composition of the breast-fed infants was achieved in infants receiving prebiotic supplemented formula. This would suggest that early gut Bifidobacterium microbiota can be modified by special diets up to the age of 6 months.


http://www.sciencedirect.com/science/article/B6T2T-3SCJ0T1-7/2/8cecc095b460538d33232f9ae0737c15a

Haemophilus influenzae and Streptococcus pneumoniae are often the cause of serious diseases such as meningitis. We designed a nested PCR assay to identify these pathogens from cerebrospinal fluid samples. The first-step PCR was able to detect eubacterial rRNA genes with a unified set of universal primers. In the second-step PCR, the identification primers, HI I and II and SP I and II, could detect H. influenzae and S. pneumoniae respectively through amplification of the rRNA spacer between the 16S and 23S RNA genes. We suggest that the two-step PCR assay can be used as a novel method for the immediate and retrospective diagnosis of bacterial meningitis caused by H. influenzae and S. pneumoniae.


http://www.sciencedirect.com/science/article/B6T2T-44JD8N7-1/2/2d1281ebfc2e1051fb0032ae19a6d4d9

A repetitive DNA motif was used as a marker to identify novel genes in the mucosal pathogen Moraxella catarrhalis. There is a high prevalence of such repetitive motifs in virulence genes that display phase variable expression. Two repeat containing loci were identified using a digoxigenin-labelled 5′-(CAAC)6-3′ oligonucleotide probe. The repeats are located in the methylase components of two distinct type III restriction-modification (R-M) systems. We suggest that the phase variable nature of these R-M systems indicates that they have an important role in the biology of M. catarrhalis.


http://www.sciencedirect.com/science/article/B6T2T-3S6TYBD-C/2/445f1dcf9fe5583544c58e13df1a224

Two Swedish isolates of Coxiella burnetii and the two prototype strains of the species, Nine Mile and Priscilla, were characterized with regard to their multiplication and cytopathic effect on BGM cells and by PCR-based amplification of repetitive element DNA and the C. burnetii-specific plasmids QpH1 and QpRS. Moreover, 1330 bp of each 16S rRNA gene were sequence-determined. All four strains multiplied at virtually the same rate and displayed the same type of
vacuoles in the BGM cells. Genetic homogeneity was observed inasmuch as the 16S rDNA sequences were identical and the strains showed identical PCR amplification patterns using primers specific to enterobacterial repetitive intragenic consensus DNA sequences. The two Swedish strains and the Priscilla strain also showed identical patterns after PCR amplification of repetitive extragenic palindromic DNA sequences, whereas the Nine Mile strain demonstrated a similar, but not identical pattern. Thus, the investigated strains demonstrated very similar phenotypic and genotypic characteristics. This finding is discussed in view of the very rare occurrence of domestic Q fever in Sweden.


http://www.sciencedirect.com/science/article/B6T2T-463NNRG-1D/2/00faa2e15bec91717ab0fdb533c16ae6

Genetic diversity of the streptokinase gene (sk) from 36 strains of S. equisimilis and 54 strains of group G streptococci was examined. The strains were isolated from patients with various streptococcal disease manifestations and healthy carriers. The region of the gene that corresponds to amino acid residues 174-244, was PCR amplified. The amplified product was subjected to MluI, PvuII, DraI and DdeI digestion. Based on the restriction enzyme digestion patterns nine sk alleles were recognized. There was no correlation between the various sk gene alleles and streptococcal disease manifestations. Three of the nine sk gene alleles, sk4, sk7, and sk8, were detected earlier among group A streptococci. The other six alleles were unique to S. equisimilis and group G streptococci. The most common allele were sk5, found in 21/90 (23%) and sk10 detected in 43/90 (47%) of the strains. Alleles sk1 and sk2, the most frequent among group A streptococci, were not found among the strains in the present investigation. Thus, it appears that the sk gene has been evolving in line with other species distinguishing features of the streptococci.


http://www.sciencedirect.com/science/article/B6T2T-3WG32M9-C/2/c941b3dcd90610501d00472670fbd0f3

A polymerase chain reaction assay (PCR) for the diagnosis of Helicobacter pylori in human gastric biopsies was developed. To prevent false-negative results while performing PCR on human tissues, an internal control is necessary. Primer set ACT1-ACT2 which specifically amplifies a 542-bp fragment of the 16S rRNA gene of H. pylori was used. dUTP and hot-start were used to prevent false-positives from carryover of previous products and avoid non-specific extension products. A competitive internal control DNA fragment was constructed to detect the presence of inhibitors. Biopsies from 101 unselected patients with gastric symptoms were tested. PCR results were compared with results from microscopy of histological sections and conventional culturing for H. pylori. Forty-two percent of the biopsies were found to contain compounds inhibiting the PCR. The addition of the internal control assures the performance of the PCR assay and is an important quality control parameter.
A primer-set was designed for specific detection of genes that encode for 16S rRNA of Helicobacter pylori, using direct polymerase chain reaction (PCR). The primers were selected in the hypervariable regions, derived from a complete small subunit 16S rRNA sequence of the reference strain H. pylori CCUG 17874. The primer-set amplified a 537 base pair (bp) sequence specifically from chromosomal H. pylori DNA. Amplification of purified chromosomal H. pylori DNA was achieved at concentrations as low as 1 femto gram (fg), equivalent to 5 bacteria. Furthermore, as few as 1 lysed H. pylori cell was detected by this PCR technique. The specificity of the primers was 100%, since purified chromosomal DNA was detected from all 32 various H. pylori isolates, whereas no other bacteria species were detected, whether related to Helicobacter or not. The 16S rDNA primers successfully detected H. pylori in antral biopsy specimens collected from infected patients.

Prevalence of Mycoplasma genitalium in humans is still not clear. We have developed a sensitive and specific serological assay for M. genitalium using lipid-associated membrane proteins (LAMPs) as antigens. Antibodies to LAMPs from M. genitalium showed little cross-reactivity to LAMPs from antigenically similar M. pneumoniae. For validity testing, urines from 104 patients were tested by PCR for M. genitalium. All 15 PCR+ patients had M. genitalium-LAMPs antibodies. Moreover, none of 64 antibody-negative patients were PCR+. Serological study of 1800 patients of various diseased groups and healthy blood donors showed M. genitalium was primarily a sexually transmitted microbe that infected patients with AIDS (44.0%), intravenous drugs users with or without HIV infection (42.5%), and also HIV- patients attending STD clinics (42.6%). Only 5.5% HIV- healthy blood donors and 1.3% HIV+ hemophiliacs tested positive. M. genitalium has been associated with acute non-gonococcal urethritis in male patients. However, many sexually active men and women appear to be chronically infected or colonized by the microbe without apparent clinical symptoms and may continue to transmit the organism through sexual contacts.

Campylobacter jejuni is a leading human food-borne pathogen. The rapid and sensitive detection of C. jejuni is necessary for the maintenance of a safe food/water supply. In this article, we present a real-time polymerase chain reaction (PCR) assay for quantitative detection of C. jejuni in naturally contaminated poultry, milk and environmental samples without an enrichment step.
The whole assay can be completed in 60 min with a detection limit of approximately 1 CFU. The standard curve correlation coefficient for the threshold cycle versus the copy number of initial C. jejuni cells was 0.988. To test the PCR system, a set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of C. jejuni. 30.6% (92/300) of chicken meat samples, 27.3% (82/300) of milk samples, and 13.6% (41/300) of water samples tested positive for C. jejuni. This result indicated that the real-time PCR assay provides a specific, sensitive and rapid method for quantitative detection of C. jejuni. Moreover, it is concluded that retail chicken meat, raw milk and environmental water are commonly contaminated with C. jejuni and could serve as a potential risk for consumers in eastern China, especially if proper hygienic and cooking conditions are not maintained.


http://www.sciencedirect.com/science/article/B6T2T-4BKDRSC-1/2/683fc1ee00e7478a93a8788c981e409

Phenolic glycolipid-I (PGL-I), a Mycobacterium leprae-specific antigen, has been widely used for the serodiagnosis of leprosy and has been implicated in the pathogenesis of leprosy. In an effort to produce an alternate antigen of PGL-I, the mimotope peptides of PGL-I, W(T/R)LGPY(V/M), were obtained using a monoclonal antibody, III603.8, specific to PGL-I by a phage library. The biotin-labeled predominant mimotope peptide of PGLP1, WTLGPYV, bound to III603.8 in a dose-dependent manner in an immunoassay. However, PGLP1 did not bind to anti-PGL-I antibodies in the serum samples from leprosy patients that were reactive to PGL-I. Although the mimotope peptide of WTLGPYV was not effective as an alternate antigen of PGL-I for the serodiagnosis of leprosy, but it would be of interest to know how the mimotope peptides mimic the role of PGL-I antigen in the pathogenesis of M. leprae infection.


http://www.sciencedirect.com/science/article/B6T2T-46VJGKR-1/2/89e30a15479a581872e32a6d617cc82b

Neisseria meningitidis shows great variation in expression of structurally different lipooligosaccharides (LOS) on its cell surface. To better understand the LOS diversity that may occur within an individual strain, a group C wild-type strain, BB305-Tr4, and two stable isogenic LOS variants, Tr5 and Tr7, were selected for this study. SDS-PAGE analysis showed a size reduction of Tr5 and Tr7 LOS compared to that of Tr4. Immunoblotting showed that parental Tr4 LOS reacted with L1, L2 and L3,7 antibodies, variant Tr5 LOS with L1 and L6 antibodies, while Tr7 LOS was non-typeable. Genetic analysis showed that the gene organization at the lgt-1 locus in the three strains was lgtZ,C,A,B,H4 in Tr4, lgtZ,C,A,H4 in Tr5 and lgtZ,C,A,H9 in Tr7. The genetic differences in the three strains were consistent with their phenotypic changes. Sequence comparison revealed two independent recombination events. The first was the recombination of repeated DNA fragments in the flanking regions to delete lgtB in Tr5. The second was the recombination of a fragment of two genes, lgtB and lgtH4, to create an inactive lgtH9 allele with a mosaic structure in Tr7. These findings suggest that besides phase variation, homologous recombination can contribute to the genetic diversity of the lgt locus and to the generation of LOS variation in N. meningitidis.

http://www.sciencedirect.com/science/article/B6T6W-433P7K2-F/2/539a2c9218681e8027a3aad5976a73c4

Nine Y-chromosome STRs were investigated in a male population sample from the Western Mediterranean region of Valencia (Eastern Spain). Complete nine Y-chromosomal STRs haplotypes were obtained in 140 individuals, among which 113 different haplotypes were observed. The most common haplotype was shared by 5% of the sample, while 99 haplotypes were unique. The gene diversity was 0.9892 and the discrimination capacity was 0.8071. Significant population differences were observed with respect to other Iberian populations, such as the Basques and Northern Portugueses.


http://www.sciencedirect.com/science/article/B6T6W-3VXJC1-7/2/173826338826962eece9411f3f5276ad

A polymerase chain reaction- (PCR) based short tandem repeat (STR) system has recently been developed for use in routine forensic identity testing [1]. The methodology involves the simultaneous amplification of alleles at four loci on different chromosomes, followed by the fluorescent detection of products using an automated DNA sequencer. The adoption of this technology into operational casework offers several advantages over systems currently in use, particularly the ability to obtain results from very old or small samples, reduced operator time when compared with conventional DNA (single locus probe) analysis and the potential for automation. Validation studies were incorporated into the development work on this system [2,11]. The scope of these studies has been extended by further investigation carried out in this laboratory to test the reliability of the system under normal operational procedures. It was demonstrated that the precision of size determination was sufficient for the discrimination of alleles and size windows for allelic designation were established. A collaborative exercise carried out in conjunction with two independent laboratories demonstrated the robustness of allelic designation. Having tested both the DNA quantification and amplification techniques against DNA samples from a wide range of animal and microbial species, it was confirmed that results are only obtained from higher primate DNA. The PCR methodology was tested with both simulated and real casework samples (over 250 in total). Reportable results were obtained from most items yielding extracted DNA. Approximately 20% of the casework items from which no grouping (ABO, PGM) nor SLP results were obtained, gave reportable STR results. A method for the routine purification of DNA extracts which failed to amplify was established and validated for use in forensic casework. The STR multiplex system developed by Kimpton et al. [1] proved robust and reliable when tested under the operational procedures in place in this laboratory.
Previously reported Y chromosome STR haplotype databases for three UK population groups, plus additionally analysed samples, have been scrutinised for the presence of non-standard (intermediate, null and duplicated) alleles. These alleles have been characterised by sequencing, some showing changes in the repeat structure, and the frequencies reported. Mutation rates for each of the 13 STRs have been calculated when analysis of father-son pairs has been possible. An example illustrating the use of non-standard alleles in a large family tree is outlined.

A systematic study was conducted to investigate whether DNA can be successfully extracted from latent fingerprints deposited on ordinary paper and analysed using short tandem repeat profiling and mitochondrial DNA sequencing. In order to evaluate the performance of latent fingerprint analysis in a criminal case, experiments with varying conditions were carried out to improve our understanding of low copy number (LCN) DNA typing. After optimising the extraction methods to achieve increased sensitivity, the examination of touched paper can routinely yield the STR profile of the individual who has touched it. A fingerprint can therefore be considered as a potential source of DNA for genetic identification. Nevertheless, the findings of our "after enhancement experiment" (using chemically or physically pre-treated fingerprints), and our "mixture experiment" (using fingerprints from three to four people on the same sheet of paper) help to define the limitations of the low copy number PCR technique in forensic casework.

PCR followed by SDS-PAGE in miniaturized non-denaturing gels permits in some cases the identification of single base pair substitutions in small DNA fragments and therefore, the study of human DNA polymorphisms. The usefulness of the system in forensic science is investigated by typing the HLA-DQA1 locus and the VNTR recognized with the probe pMCT118 (locus D1S80) and it shows to be advantageous over previously published methods for typing the MCT118 system, whereas in HLA-DQA1 typing for forensic casework, both dot-blot with ASO probes and this method could be complementary.

To permit quick identification of arthropods, random amplified polymorphic DNA typing (RAPD) was used to support classical morphological and medico-legal analysis of maggots on a human corpse. The method was employed to determine if maggots which were found on the inside of a body bag were identical (a) with maggots found on the outside of the bag, and (b) pupae found on the floor under the corpse. Pre-mixed RAPD reaction beads together with semiautomatic computer aided analysis of the PCR products are shown to discriminate between closely related necrophageous insect species (flies and beetles) found on corpses. From the 11 RAPD primers used, one alone was sufficient in resolving a practical forensic situation. This is the first report of a forensic application of RAPD DNA typing.


http://www.sciencedirect.com/science/article/B6T6W-49M0RB7-1/2/2944c505fd2699be5f92325aafe26423

DYS464 is a multi-copy STR system with four positions on the Y-chromosome (DYS464a, b, c, and d) which was recently identified and characterized [Forensic Sci. Int. 130 (2002) 97]. The aims of our study were to perform a population study, to estimate the mutation rate and an extensive sequence analysis in order to confirm the nomenclature. Fourteen different alleles were found in an Austrian population sample with an allele length varying from 9 to 19 repeats. All alleles were cloned and sequenced. Alleles 9-19 showed the general repeat structure (CCTT)n. (CCTT)2. (CCTT)3. (CCTT)4. (CCTT)2. (CCTT)2. The nomenclature is based on the number of repeated units of the variable (CCTT)n-stretch only. In 13% of the samples intermediate alleles, namely 14.3A, 14.3B and 15.3 were detected. In these alleles the variable repeat block is interrupted by a CTT motif (14.3A: (CCTT)3CTT(CCTT)11; 14.3B and 15.3: (CCTT)7CTT(CCTT)7/8). A comparison with GenBank entries revealed the existence of a length variant due to a deletion of one cytosine in the 5' flanking region of the first repeat block. We designed an alternative forward primer to circumvent possible ambiguities in the allele designation. A total of 54 different genotypes were identified in 135 men corresponding to a discrimination capacity (DC) of 40% and a gene diversity (GD) of 0.97. These values are much higher than those of other Y-chromosomal short tandem repeats (Y-STRs). DYS464 has the same haplotype diversity (HD) as the combination of the five Y-STR loci with the lowest gene diversities of the Y-STR core set. On the other hand, a combination of the three most diverse loci (DYS464, DYS385 and DYS390) has the same capacity to distinguish between paternal lineages than the complete minimal haplotype (minHT) consisting of eight Y-STR loci. In our population sample the addition of DYS464 to the minHT increases the number of different haplotypes from 110 to 122. The mutation-rate estimate based on the 70 meioses analyzed amounts to 2.86 x 10-2 (95% confidence interval 3.5 x 10-3 to 9.95 x 10-2). This value is approximately 10 times higher than the average mutation-rate estimate for Y-STRs.


http://www.sciencedirect.com/science/article/B6T6W-3TKKV2W-7/2/b20ab6944fcf140e81aa537cdfb2c211
Allele frequencies for six tetrameric short tandem repeat (STR) loci CSF1PO, TPOX, THO1, D3S1358, VWA, and FGA were determined in a Black African sample population from Zimbabwe. All loci are highly polymorphic and meet Hardy-Weinberg expectations. An inter-class correlation test analysis detected only one departure from independence out of 15 pair-wise comparisons of the six loci (i.e., CSF1PO/VWA loci, \(P=0.026\)). The allele frequency data at four of the six STR loci in the Black African sample population are similar to African American data.


A total of 263 U.S. Caucasians, 260 African Americans and 140 U.S. Hispanics or a subset of 31 Caucasians, 32 African Americans, and 32 Hispanics were typed for 27 Y-chromosome short tandem repeat (Y-STR) markers: DYS444, DYS446, DYS449, DYS463, DYS485, DYS490, DYS495, DYS504, DYS505, DYS508, DYS520, DYS522, DYS525, DYS532, DYS533, DYS534, DYS540, DYS556, DYS557, DYS570, DYS575, DYS576, DYS594, DYS632, DYS635, DYS641, and DYS643. Allele frequencies for each locus are reported along with nomenclature based on sequence analysis.


A multiplex polymerase chain reaction (PCR) assay capable of simultaneously amplifying 20 Y chromosome short tandem repeat (STR) markers has been developed to aid human identity testing and male population studies. These markers include all of the Y STRs that make up the "extended haplotype" used in Europe (DYS19, DYS385, DYS389I/III, DYS390, DYS391, DYS392, DYS393, and YCAII) plus additional polymorphic Y STRs (DYS437, DYS438, DYS439, DYS447, DYS448, DYS388, DYS426, GATA A7.1, and GATA H4). Primers for the markers DYS385, DYS389, and YCAII target duplicated regions of the Y chromosome and thus can provide two polymorphic peaks for each respective primer set. This Y STR 20plex, which utilizes 34 different PCR primers, is the first to include a simultaneous amplification of all the markers within the European "minimal" and "extended" haplotypes. Relative primer positions are compared between the newly developed primers described here and previously published ones. Efforts were made to avoid X chromosome homology in the primer design as well as close packing of PCR product size ranges in order to keep all alleles less than 350 bp through careful examination of known allele ranges. Haplotype comparisons between the 20plex and a commercially available kit found excellent agreement across the 76 samples in the Y chromosome consortium panel.


http://www.sciencedirect.com/science/article/B6T6W-4007G86-9/2/95e20cc62b13222cc68f6e4d545eaa8

The allele frequency distributions of four VNTR loci amplified by PCR have been studied in a
population of 205 individuals from Spain. The loci analysed are D1S80 and three STRs: HUMTH01, HUMFES/FPS and HUMACTBF2 (SE33). The former was visualized in Metaphor agarose gels, and the STRs in sequencing polyacrylamide gels under denaturing conditions which could separate alleles with differences of a single base. This is of particular importance in the HUMTH01 locus, a tetrameric STR in which two alleles (9.3 and 10) were detected differing in a single base. Furthermore, HUMACTBP2 has at least 30 alleles, some of which may vary by as little as one base. At this locus a variation in the allele mobility was observed, depending on the electrophoretic conditions. For this reason, there should be careful consideration before this marker is accepted and validated as a common interlaboratory system. This paper does not include any comparison of the frequencies obtained for this locus with other recent studies. For the rest of the loci, the frequencies found have been compared with other published population studies; they show a degree of difference, particularly in the D1S80 locus. Finally, the systems were tested for Hardy-Weinberg equilibrium, and some statistical parameters of forensic interest were calculated.


http://www.sciencedirect.com/science/article/B6T6W-3V5MP79-B/2/658ce23da9e6bc695d7e15123f10d6c9

The aim of this collaborative exercise was to determine whether uniformity of mtDNA sequencing results could be achieved among different EDNAP laboratories. Laboratories were asked to sequence mtDNAHV1 region (16024-16365) from three bloodstains, proceeding in accordance with the protocol and strategies currently used in each individual laboratory. Cycle sequencing was used by 11 laboratories and solid phase single stranded sequencing was used by one laboratory. Different PCR strategies and PCR conditions were used by the different laboratories. Three laboratories used semi-nested PCR, two nested PCR, three direct amplification of HV1 and four amplification of overlapping fragments covering the HV1 region. Despite the diversity of methodologies used, all the laboratories reported the same results. The successful result of this exercise shows that PCR based mtDNA typing by automated sequencing is a valid, robust and reliable means of forensic identification despite the different strategies and methodologies used by the different laboratories.


http://www.sciencedirect.com/science/article/B6T6W-42SX3V-7/2/1a7d7ddc5834d937aa3ef19dd4b62c71

In the field of molecular diagnosis, forensic casework analysis is one of the most demanding investigations, due to its social impact. Optimization of DNA typing multiplex reactions with identical cycling conditions as those required by autosomal short tandem repeats (STR) multiplex reduces errors, and saves time and reagents. Previously, we validated a five Y-STRs set, all of them generating single band patterns. This work reports the optimization of combined multiplexes, a triplex (DYS19, DYS390 and DYS391) and a duplex (DYS392 and DYS393), that can be amplified in identical cycling conditions as those required by commercially available multiplex autosomal STR kits. In addition both Y chromosome multiplexes can be combined for co-injection on a capillary electrophoresis based automated sequencer. Statistical attributes of the haplotypes of the five Y-STR investigated were evaluated in unrelated males from different metropolitan areas of Argentina. This system was successfully used for investigating more than 350 forensic routine cases in our country.

http://www.sciencedirect.com/science/article/B6T6W-3W4GJ8F-8/2/8ec1deeb8afa0fa7879943b64fc9db7b

Frequency data of the short tandem repeat (STR) loci HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS, D12S391 and HUMFIBRA/FGA were determined in blood stains obtained from a population of unrelated individuals from the Madeira Archipelago. The observed genotype distribution showed no significant deviation from the Hardy-Weinberg equilibrium and there was no evidence for association of alleles among the six loci. Population data showed a combined discrimination power of 0.9999998 and a chance of exclusion of 0.99597. The frequencies are similar to those of other compared caucasian populations but significant differences were found between the Madeira population and Japanese, Chinese, Greenland Eskimos and Quechua Amerindians. The six loci studied, together proved to be highly discriminating and valuable for forensic cases.


http://www.sciencedirect.com/science/article/B6T6W-40XNW8K-7/2/8ffdb40d8e75c5a5b8330e34e6040c3c

The AMPFISTR(R) SGM Plus(TM) system is a commercially available STR multiplex produced by Applied Biosystems, a division of Perkin Elmer, Foster City, California, USA that supersedes SGM. The multiplex contains the six SGM loci, amelogenin and four additional loci. These additional loci are D3S1358, D19S433, D16S539 and D2S1338. Consequently, the match probability is significantly improved (conservatively quoted as 1 in 109 for reporting a full profile match). The system was subjected to validation. For example, ageing and degradation studies demonstrated semen stains to be the most stable evidence type, whereas buccal scrapes were the least stable. An apparent rise in the sensitivity increases the chance of obtaining successful results from the more difficult samples submitted for analysis. Two of the new loci (D3S1358 and D19S433) are low molecular weight (between 100 and 150 base pairs); this improved the success rates of the degraded samples where high molecular weight loci may drop out. Of 26 non-primates tested, four gave results that appeared as single peaks and were unlikely to cause interpretation problems. None of the 19 micro-organisms tested gave discernible results. Extensive casework and simulated casework studies demonstrated that SGM and SGM plus results were comparable. There was one example of a null allele (primer binding site mutation) recorded at the HUMFIBRA locus (in both systems). However, a concordance study of 1000 samples using both SGM and SGM plus did not demonstrate any differences in typing.


http://www.sciencedirect.com/science/article/B6T6W-4575ST7-1/2/f175b9c09b7a6e29b6542e4c9638a6d9

A total of 280 persons were HLA-DQA1 typed by two different polymerase chain reaction (PCR) based methods: (i) a reverse dot-blot (RDB) method, which can differentiate between six alleles,
and (ii) a combined PCR-restriction fragment length polymorphism (PCR-RFLP) and allelespecific amplification (ASA) method, which together recognise eight alleles. In 146 unrelated Danish individuals, the HLA-DQA1 alleles were in Hardy-Weinberg equilibrium. For identity testing, the power of discrimination (PD) of HLA-DQA1 was 0.932 with the RDB method and 0.942 with the PCR-RFLP/ASA method. For paternity testing, the theoretical chance of exclusion in HLA-DQA1 of non-fathers was 0.634 with the RDB method and 0.660 with the PCR-RFLP/ASA method.


http://www.sciencedirect.com/science/article/B6T6W-44HXKBT-D/2/09c9e2584575efb397a75a2829fe0161

Allele frequencies for seven STRs loci were obtained from a sample of 215 unrelated healthy Italian individuals.


A single nucleotide polymorphism (SNP) multiplex has been developed to analyse highly degraded and low copy number (LCN) DNA template, i.e. Hb) and the drop-out threshold (Ht) defined as the maximum peak height of a surviving heterozygous allele, where its partner may have dropped out. The discrimination power of the system is estimated at 1 in 4.5 million, using a White Caucasian population database. Comparisons using artificially degraded samples profiled with both the SNP multiplex and AMPFISTR[trademark] SGM plus[trademark] (Applied Biosystems) demonstrated a greater likelihood of obtaining a profile using SNPs for certain sample types. Saliva stains degraded for 147 days generated an 81% complete SNP profile whilst short tandem repeats (STRs) were only 18% complete; similarly blood degraded for 243 days produced full SNP profiles but only 9% with STRs. Reproducibility studies showed concordance between SNP profiles for different sample types, such as blood, saliva, semen and hairs, for the same individual, both within and between different DNA extracts.


Dog DNA-profiling is becoming an important supplementary technology for the investigation of accident and crime, as dogs are intensely integrated in human social life. We investigated 15 highly polymorphic canine STR markers and two sex-related markers of 131 randomly selected dogs from the area around Innsbruck, Tyrol, Austria, which were co-amplified in three PCR multiplex reactions (ZUBECA6, FH2132, FH2087Ua, ZUBECA4, WILMSTF, PEZ15, PEZ6, FH2611, FH2087Ub, FH2054, PEZ12, PEZ2, FH2010, FH2079 and VWF.X). Linkage testing for our set of marker suggested no evidence for linkage between the loci. Heterozygosity (HET), polymorphism information content (PIC) and the probability of identity (P(ID)theoretical, P(ID)unbiased, P(ID)sib) were calculated for each marker. The HET(exp)-values of the 15
markers lie between 0.6 (VWF.X) and 0.9 (ZUBECA6), P(ID)sib-values were found to range between 0.49 (VWF.X) and 0.28 (ZUBECA6). Moreover, the P(ID)sib was computed for sets of loci by sequentially adding single loci to estimate the information content and the usefulness of the selected marker sets for the identification of dogs. The estimated P(ID)sib value of all 15 markers amounted to $8.5 \times 10^{-8}$. The presented estimations turned out to be a helpful approach for a reasonable choice of markers for the individualisation of dogs.


http://www.sciencedirect.com/science/article/B6T6W-4471K5D-7/2/8a71d833ae42ad553861b96d67973ded

We describe a minisequencing protocol for screening DNA samples for the presence of 12 mutations in the human melanocortin 1 receptor gene (MC1R), eight of which are associated with the red hair phenotype. A minisequencing profile which shows homozygosity for one of these mutations or the presence of two different mutations would strongly indicate that the sample donor is red haired. The absence of any red hair causing mutations would indicate that the sample donor does not have red hair. We report the frequencies of MC1R variants in the British red haired population.


http://www.sciencedirect.com/science/article/B6T6W-4FBM1R7-3/2/9ae4665f18b74d054e14b085a742137a

We report a recent case in which a wildlife warden had suspected that some people had killed and cooked a peacock. Cooked meat, intestine of bird and the wooden block used for chopping were seized from the site of crime and forwarded to our laboratory for DNA testing. Mitochondrial cytochrome b sequence analysis revealed that the cooked meat and remnants of the bird were of a chicken, but the DNA obtained from the wooden block was of an Indian Peafowl (Peacock) testifying that the wooden chopping block was used to chop the meat of an endangered bird, thus bringing to light a wildlife crime.


http://www.sciencedirect.com/science/article/B6T6W-4C35YFD-HT/2/17aa4f92e18100a009c00dc2faf543c23

Biological material in forensic casework frequently contains a mixture of genotypes, with a predominance of material from the victim and only trace amounts from the person committing the crime. Physical separation of the two genotypes or preferential lysis of different cell types may sometimes be possible. However, it is often difficult to achieve complete separation due to the lysis of cells or lack of material. We have developed an enzymatic amplification system for the HLA DQA1 locus, that will allow the presence of individual alleles in a sample with mixed genotypes to be determined, independent of their initial proportion in the sample. This system permits the identification of an allele representing less than 10-4 of the background genotype.
Use of polymerase chain reaction (PCR) with general primers allows only alleles representing more than about 1% to be detected, while the allele-specific amplification represents up to a 1000-fold increase in sensitivity. This method was applied to a rape case and a combined rape and murder case; in both cases the biological evidential materials contained a mixture of alleles from the victim and the rapist. Allele-specific PCR revealed the presence of alleles identical to those of the suspect using DNA from a vaginal swab taken after a rape incident, whereas by using general primers in the PCR only trace amounts of alleles other than those of the victim were found. Similarly, allele-specific amplification of DNA from vaginal swabs from the murder case revealed the presence of alleles identical to those of the suspect, while standard PCR only indicated the presence of genetic material from the victim.


http://www.sciencedirect.com/science/article/B6T6W-495680J-4/2/6334b6a80183df468bad52f350e821f2

For a variety of reasons, some victims of sexual assault provide vaginal samples more than 24-36 h after the incident. In these cases, the ability to obtain an autosomal STR profile of the semen donor from the living victim diminishes rapidly as the post-coital interval is extended. We have used a number of carefully selected Y-STR loci in a variety of multiplex or monoplex formats to extend the post-coital interval from which a genetic profile of the semen donor can be obtained. The proposed Y-STR typing strategies enable the routine detection of the male donor Y-STR haplotype in cervicovaginal samples recovered up to 4 days post-coitus. We attribute our success to a number of factors that significantly improve the sensitivity and specificity of the analysis. Firstly, we utilize a subset of Y-STR loci that have been carefully selected for their superior performance under stressed conditions in both multiplex and monoplex formats. Specifically these loci function with low copy number templates in the presence of a vast excess of potentially confounding female DNA. Secondly, sperm and non-sperm DNA is co-extracted without a differential extraction process to prevent the unnecessary loss of the small number of structurally fragile sperm remaining in the cervicovaginal tract several days after intercourse. Thirdly, low copy number detection is facilitated by increasing the cycle number to 34-35 cycles and by the ability to input up to 450 ng of co-extracted sperm/non-sperm DNA into the PCR reaction without the appearance of confounding female artifacts. Lastly, the proper collection of post-coital cervicovaginal samples, instead of the lower or mid-vaginal tract samples often taken, is required for optimal recovery of sperm for analysis. In this report we demonstrate that our previously described 19 Y-STR loci systems (MPI and MPII) permit a reliable high resolution haplotype determination of the semen donor in cervicovaginal samples taken up to 48 h after intercourse. However, as the post-coital interval is extended further, dramatic loss of signal is observed and haplotype determination of the male donor is no longer possible with MPI and MPII. Nonetheless, subsets of these 19 loci (MPA and MPB) have been developed specifically to detect the male haplotype in samples recovered 4 days after intercourse. Thus, it is possible to derive an 11-19 locus Y-STR profile of the semen donor in cervicovaginal samples recovered 2-4 days after intercourse.


http://www.sciencedirect.com/science/article/B6T6W-414N4VW-S/2/508645c54ca673448fa0bb9bb441a564
We experienced an autopsy case, small testes and tall stature, which suggested Klinefelter's syndrome. DNA analysis was performed to confirm the genetic abnormality. Case History: A 28-year-old man who was single and lived with his parents. He suddenly lost his consciousness in a sitting room and died. Autopsy findings: He was 176 cm in height and 57 kg in weight. The post-mortem hypostasis was red-purple on his back, and rigor mortis was strong in each joint of the whole body. The heart weighted 340 g, in which dark red fluidal blood (300 ml) without coagulation was contained. The testes were smaller than normal adult male (left and right testes with epididymides weighted 8.1 g and 6.0 g, respectively). As a results of pathological examination, clumped Leydig cells, sclerotic and hyalined tubules were observed. Some germ cells with spermatozoid were also present. DNA Analysis: Generally, Klinefelter's syndrome is determined by karyotype analysis and/or the detection of sex chromatin. However, in this case, karyotype analysis and the detection of sex chromatin could not be demonstrated, because the blood which was collected in the autopsy became too old. Therefore, we tried sex determination and STR analysis (HPRT, HUMARA and DXS 1470) using DNA extracted from stored blood materials. Consequently, in the sex determination, no different situation was found in the X- and Y-specific bands from normal male's and as results of STR analyses, we could not corroborate the Klinefelter's syndrome.

http://www.sciencedirect.com/science/article/B6T6W-4DS98WW-5/2/9407a44be7c975ae413a2dea390b71d5

Genetic profile of 15 short tandem repeats (STR) loci were determined in a Chinese Han population from the Min Nan mountainous area, Southeast China.

http://www.sciencedirect.com/science/article/B6T6W-4CJCWWD-2/2/26765b7bfb969b0e73b623b854ce0b

Chao Shao area is a littoral under the jurisdiction of Guangdong province, abutting on Fujian. Historically, the area was relatively isolated from other parts of China until 1990s, when it started to take a small number of immigrants from other regions. People residing in this area speak in unique dialect and have distinct lifestyle. Allele frequencies for the 15 short tandem repeats (STR) loci included in the AmpFLSTR(TM) Identifiler(TM) kit were obtained from a sample of 144 unrelated Chinese born and living in the Chao Shan area, South China.

http://www.sciencedirect.com/science/article/B6T6W-4CB676S-2/2/465e03547345e3086acf82727f5561cc

A novel 39-plex typing system for single nucleotide polymorphisms (SNPs) has been developed. This multiplex approach has the advantage of being able to type 38 autosomal SNPs and one sex-discriminating base exchange site on the X and Y chromosomes rapidly and simultaneously. The SNP loci on the autosomes, which we examined, contain 15 loci distributed on blood type
genes: three on RhCE, two each on Km and Gc, and one each on Duffy, AcP1, Tf, MN, GPT, EsD, Pi, and Kidd genes. Thirty-seven genomic DNA fragments containing a total of 38 SNPs and one sex-discriminating site were amplified in one multiplex PCR reaction. Following the reaction, single nucleotide primer extension reaction was performed by dividing these SNP loci into five groups. The SNP type of each of the 39 loci was determined at one time by capillary electrophoresis using the newly designed multi-injection method. The combined PD (power of discrimination) of this typing system was (1-1.1) x 10^{-14}, and the MEC (mean exclusion chance) was 0.9990. We applied this system to forensic cases, including 16 paternity testing cases (13 non-exclusion and three exclusion cases) and one personal identification case. For the paternity testing cases, the highest Essen-Moller's W-value was 0.9999995. The pM (matching probability) of the personal identification case was 2.22 x 10^{-17}. These data showed that this system was an excellent tool for use in forensic cases of paternity testing and personal identification.


To profile postmortem degradation of mRNA, total RNA was extracted, at given postmortem intervals, from the brain, lung, heart and liver of rats left at 20 \( \text{[deg]}\)C. In electrophoretic analysis, total RNA was most stable in the brain, moderately stable in the lung and heart, and most unstable in the liver. Northern blot analysis of total RNA extracts from the brain and liver of dead rats with a cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) showed that GAPDH mRNA degraded in a similar fashion to total RNA. Analysis of the postmortem degradation profile of GAPDH mRNA with real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) gave results consistent with those above, indicating that real-time RT-PCR is reliable for estimation of the mRNA level in specimens from dead bodies. Real-time RT-PCR analysis showed that degradation rates of three housekeeping genes, GAPDH, \([\beta]\)-actin and hypoxanthine guanine phosphoribosyltransferase, in the brains of dead rats were similar. The degradation rate of interleukin-1\([\beta]\) (IL-1\([\beta]\)) mRNA induced by intravenous injection of LPS to rats was higher than that of GAPDH mRNA in the lung. In real-time RT-PCR analysis using GAPDH mRNA as an internal standard, the detection level of IL-1\([\beta]\) mRNA decreased in the postmortem interval. However, enhanced expression of IL-1\([\beta]\) was detected for at least 3 days postmortem.


A novel approach to quantitative reverse transcription (RT)-PCR assay of mRNA component using fluorescent TaqMan methodology and a new instrument (ABI Prism 7700 sequence detection system) was developed for autopsy materials. Pulmonary surfactant apoprotein A (SP-A) mRNA from a cadaveric lung was quantitated in real-time. The target SP-A gene and the endogenous reference of glyceraldehyde-3-phosphate (GAPDH) were amplified in the same tube, and an amount of the target was normalized to the reference. This assay had a high reproducibility and discrimination even in forensic autopsy materials up to 96 h postmortem. Elevated SP-A expressions were determined in some cases. This system without post-PCR sample handling would be a very useful tool in pathological diagnosis and DNA analysis.

http://www.sciencedirect.com/science/article/B6T6W-490H2DF-D/2/75c34372623631608763b4a1de77aaf9

Conventional methods of body fluid identification use a variety of labor-intensive, technologically diverse techniques that are performed in a series, not parallel, manner and are costly in terms of time and sample. Theoretically, the identification of a body fluid may be made by determining a sufficient number of mRNAs that are expressed exclusively in cells that collectively comprise that body fluid. Advantages of an mRNA-based approach, compared to conventional biochemical methods of analysis, include greater specificity, simultaneous and semi-automatic analysis through a common assay format, improved timeliness, decreased sample consumption and compatibility with DNA extraction methodologies. In this report, we demonstrate that RNA is stable in biological stains and can be recovered in sufficient quantity and quality for analysis. Messenger RNA from the housekeeping genes S15, beta-actin and GAPDH was detected in blood, semen and saliva stains using a sensitive reverse transcriptase-polymerase chain reaction assay (RT-PCR). Additionally, we have identified a number of candidate tissue-specific genes, statherin, histatin 3, PRB1, PRB2 and PRB3 that may be useful for the positive identification of saliva. Messenger RNAs from these genes were detectable in saliva stains but not in blood or semen stains. Collectively these findings constitute the basis of a prototype RNA based assay system that may eventually supplant conventional methods for body fluid identification.


http://www.sciencedirect.com/science/article/B6T6W-4007G86-8/2/98573d921ca74aae07ac2b905cd89d25

The European DNA Profiling Group (EDNAP) has previously carried out collaborative exercises to determine which STR systems will produce results that can be reproduced by different laboratories. The first EDNAP exercise involving STR systems focused on different types of loci: a simple locus with six common alleles (HUMTH01) and a complex locus with >35 alleles (ACTBP2). Generally the simpler STR system was found to be readily amenable for use across a wide range of different technologies, whereas a more complex locus presented difficulties. The second EDNAP STR exercise was intended to take the process of investigation a stage further. Some laboratories are developing automation, coupled with fluorescent methods of detection and multiplex applications, whereas others use manual methods involving visual detection techniques such as silver staining. The purpose of this exercise was to determine whether loci amenable to multiplexing with automation (as a quadruplex reaction) could also be successfully used with manual methods, either by multiplexing in duplex reactions or alternatively by using just a single pair of PCR primers.


http://www.sciencedirect.com/science/article/B6T6W-490RG6H-3/2/76b79844ae546d13cc1caee470765c54
The aim of the study was to test the hypothesis that polymerase slippage correlates to the length of repeat stretches consisting of uniform repeats against the alternative hypothesis that the total number of repeats is most relevant. Two short tetrameric short tandem repeats (STRs) with different repeat structures were investigated: D3S1545 containing only homogeneous (GATA)n repeat stretches and D7S1517 with compound repeat arrays of GAAA and CAAA repeats. Additionally two different polymerases (Herculase and AmpliTaq Gold) were used which gave comparable results. No correlation was found for the hypothesis "total repeat number against percent of stutter"; in contrast, the other hypothesis that the number of uniform repeats is relevant for the degree of stutter gave a strong positive correlation (0.82 for selected D7S1517 alleles) which confirmed the hypothesis that polymerase slippage correlates to the length of repeat stretches consisting of uniform repeats.


http://www.sciencedirect.com/science/article/B6T6W-4D9R96Y-1/2/1feb186db95679e44bf07167cb29940d

Y chromosome-specific short tandem repeat (Y-STR) analysis has become another widely accepted tool for human identification. The PowerPlex[trademark] Y System is a fluorescent multiplex that includes the 12 loci: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439. This panel of markers incorporates the 9-locus European minimal haplotype (EMH) loci recommended by the International Y-STR User Group and the 11-locus set recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM). Described here are inter-laboratory results from 17 developmental validation studies of the PowerPlex[trademark] Y System and include the following results: (a) samples distributed between laboratories and commercial standards produced expected and reproducible haplotypes; (b) use of common amplification and detection instruments were successfully demonstrated; (c) full profiles were obtained with standard 30 and 32 cycle amplification protocols and cycle number (24-28 cycles) could be modified to match different substrates (such as direct amplification of FTA[trademark] paper); (d) complete profiles were observed with reaction volumes from 6.25 to 50 [mu]L; (e) minimal impact was observed with variation of enzyme concentration; (f) full haplotypes were observed with 0.5-2X primer concentrations; however, relative yield between loci varied with concentration; (g) reduction of magnesium to 1 mM (1.5 mM standard) resulted in minimal amplification, while only partial loss of yield was observed with 1.25 mM magnesium; (h) decreasing the annealing temperature by 2-4 [deg]C did not generate artifacts or locus dropout and most laboratories observed full amplification with the annealing temperature increased by 2 [deg]C and significant locus dropout with a 4 [deg]C increase in annealing temperature; (i) amplification of individual loci with primers used in the multiplex produced the same alleles as observed with the multiplex amplification; (j) all laboratories observed full amplification with [greater-than-or-equal]125 pg of male template with partial and/or complete profiles observed using 30-62.5 pg of DNA; (k) analysis of [less-than or equal to]500 ng of female DNA did not yield amplification products; (l) the minor male component of a male/female mixture was observed with [less-than or equal to]1200-fold excess female DNA with the majority of alleles still observed with 10,000-fold excess female; (m) male/male mixtures produced full profiles from the minor contributor with 10-20-fold excess of the major contributor; (n) average stutter for each locus; (o) precision of sizing were determined; (p) human-specificity studies displayed amplification products only with some primate samples; and (q) reanalysis of 102 non-probative casework samples from 65 cases produced results consistent with original findings and in some instances additional identification of a minor male contributor to a male/female mixture was obtained. In general, the PowerPlex[trademark] Y System was shown to have the sensitivity, specificity and reliability required for forensic DNA analysis.
A large number of alleles from the six different short tandem repeat (STR) loci FGA, D3S1358, vWA, CSF1PO, TPOX and TH01, used in human identity testing were sequenced to provide support for the robustness of fluorescent STR DNA typing by allele size. Sequence information for some of these loci (FGA, vWA, TH01) is an extension of published work, whereas no extensive sequence information is available with respect to the D3S1358, CSF1PO, and TPOX loci. Sequencing of alleles at each locus has provided quantitative data with respect to the true nucleotide length of common alleles, and of alleles that vary in length from the common alleles. All alleles that were identified as “off-ladder” alleles through fluorescent typing at these STR loci have proven to be true length variant alleles. Sequencing at the D3S1358 and CSF1PO loci allowed for the establishment of a common nomenclature for these loci. A correlation between percent stutter and the length of the core tandem repeat is demonstrated at the FGA locus. Alleles in which the core tandem repeat is interrupted by a repeat unit of different sequence have a reduced percent stutter. DNA samples from three non-human primates (chimpanzee, orangutan, and gorilla) were compared to the human sequences, and shown to differ markedly across loci with respect to their homology. The effects of primer binding site mutations on the amplification efficiency at a particular locus, and methods used to interpret amplification imbalance of heterozygous alleles at a locus is also addressed.

A number of DNA marker types suitable for human identification and parentage testing have been developed, of which single nucleotide polymorphisms (SNPs) merit attention as they are abundant, genetically stable, and amenable to high-throughput automated analysis. In this regard, 24 highly informative SNP markers representing each 22 autosome and both sex chromosomes were selected, and the allele and genotype frequencies of these SNPs were determined in a group composed of 30 unrelated Koreans. Based on frequency data from this group, the estimated probability of identity (PI) and probability of paternity exclusion (PE) with 22 autosomal SNP loci were 1.905 x 10-10 and 98.9%, respectively. The SNPs in this study offer a small but highly accurate database that will be an essential reference for SNP-based forensic application in the future.

Formalin-induced DNA degradation was studied at different fixation times (3, 7, 16 and 32 days) each on 10 formalin fixed paraffin embedded tissues (FFPET) stored for 15 years at room temperature. The four different extraction protocols used in this study showed that Chelex(R)100 extracts performed the best at 3 and 7 days of formalin fixation (DFF) (with regard to the quantity
and the quality of the DNA). However, Qiamp extracts showed better results for long sized alleles, as well for single polymerase chain reaction (PCR) amplifications after 16 and 32 DFF, as for multiplex PCR at shorter fixation times. DNA degradation is expressed by the size of the amplified alleles, only 100 bp templates surviving after 32 DFF (AMG locus). Single locus amplifications (CD4 and FES/FPS alleles) performed better than multiplex PCR (ProfilerPlus), with nearly 100% positive results at 7 DFF. In both types of amplifications, the success rate decreased proportionally with the time of formalin fixation and, consequently, with the size of the required DNA template.


http://www.sciencedirect.com/science/article/B6T6W-48KMJ252/2/d12c1f26e0d443658c1be592f8df46bd

A population study of Chamorros and Filipinos using short tandem repeat (STR) loci amplified with the AmpFlSTR(R) Profiler Plus(TM) PCR amplification kit demonstrated an excess of observed homozygosity at the D8S1179 locus. Use of a different set of D8S1179 primers to type the same samples did not demonstrate an excess of homozygosity and showed discordant genotypes at the D8S1179 locus. A single point mutation, G-to-A transition, 16 nucleotides from the 3’ end of the reverse primer, was identified to cause allele dropout when using the AmpFlSTR(R) Profiler Plus(TM) primer set. An additional D8S1179 reverse primer specific for the variant was constructed resulting in the recovery of the null allele. The primer was included in the newly developed AmpFlSTR(R) Identifier(TM) PCR amplification kit. No deleterious effects or non-specific peaks were observed in validation experiments evaluating primer concentration, Mg2+ concentration, annealing temperature and population samples.


http://www.sciencedirect.com/science/article/B6T6W-414N4VW-9/2/a1ab814a1d7edcb5f8abb71b096f7693

In order to apply a useful STR system we performed a population study in Western Saxony (Germany). The allele distribution was investigated in a sample of 431 unrelated adults. In addition, 170 families from routine paternity cases were examined for the presence of meiotic mutations, and two mutations were observed.


http://www.sciencedirect.com/science/article/B6T6W-42SXF3V-C/2/85c71972de3dd5e52a2fc14b421dd36

Y-chromosomal microsatellites (STRs) are potentially useful in forensic practice but, in contrast to autosomal systems, large and diverse population databases are required in order to facilitate the statistical evaluation of donor-stain matches. Since appropriate data from the Baltic region have so far been lacking, blood samples were obtained from 430 males originating from one of the
three Baltic states and these samples were genotyped using a previously described "extended core set" of nine Y-STR marker systems. Allele frequency distributions and discrimination indices were calculated, and the three populations were tested for genetic differences by means of analysis of molecular variance (AMOVA). A larger genetic difference became apparent between Estonian and both Lithuanian and Latvian males than between the latter two, non-Finno-Ugric speaking populations. The haplotype data reported here have been included into the Y-STR database maintained at the Institute of Legal Medicine, Humboldt University, Berlin.


http://www.sciencedirect.com/science/article/B6T6W-3S2BK/V5-8/2/46ff5449134702ff0235ef109ee34a80

The unambiguous identification of illicit substances, including Cannabis sativa, is a major concern of law enforcement agencies. Current methods of cannabis identification involve the use of techniques such as HPLC and GC to identify cannabinoids. A method for the identification of cannabis using DNA-specific primers has been developed and is described here. The nucleotide sequences between the trnL and trnF genes in the chloroplast of Cannabis sativa have been determined and Cannabis sativa-specific nucleotide sequences within the intergenic spacer between the trnL 3' exon and trnF gene identified. Primers, made to these sequences, have been tested on a range of different plant extracts but only give a PCR product in the presence of Cannabis sativa. The successful production of a PCR product using these primers identifies the presence of cannabis.


http://www.sciencedirect.com/science/article/B6T6W-4BFVP2F-1/2/3f8deeb5c306426cbcf39d381ff23c4f

Population frequencies for the eight Y-STR loci included in the "minimal haplotype" from Y-STR Haplotype Reference Database (YHRD) plus other eight Y-STRs (DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, GATA H4 and GATA A10) were obtained for a sample of 133 males from four main geographical areas in the Pyrenees (Spain): Vall D'Aran (Lerida), Cerdanya (Gerona), Alt Urgell (Lerida) and Jacetania (Huesca). Haplotype diversities were estimated in the four populations.


http://www.sciencedirect.com/science/article/B6T6W-3XH31K5-4/2/1afcc6d3e6ba60d6d20e6de7982edf1f

To determine precisely post mortem interval, larvae and puparium species found on a corpse have to be identified. Among more than 200 cases examined at the entomology department of the Institut de Recherche Criminelle de la Gendarmerie Nationale, two-thirds concerned corpses less than one month old. Therefore, insects from first and second screwworms are the most frequently found [1]. Some species commonly found in France, such as different Lucilia and
Calliphora vicina Robineau-Desvoidy, are easily identifiable at an adult stage, but are almost impossible to differentiate at immature stages when only fragments of puparium or necrosed first instar larvae are available. For this reason, an easy and objective method of identification was thus searched by genetic analysis of these insects. Sequencing of partial gene of sub unit I of cytochrome oxydase has been used to predict restriction sites. Restriction enzyme cleavage of PCR products with Dde I allowed us to differentiate these species.


http://www.sciencedirect.com/science/article/B6T6W-3SHBM46-G/2/dce9e1ddceb23b7b8e7b83be020

We report a sensitive and low-cost capillary electrophoretic typing method and its application to a short tandem repeat system widely used in forensic identification (HUMCD4). Separations are carried out with internally coated 100 μm fused-silica capillaries filled with a noncrosslinked gel sieving matrix based on 1.50-1.75 hydroxyethylcellulose. Detection was by laser-induced fluorescence detection. As running buffer, 100 mM Tris-borate with 0.1 mM EDTA, pH 8.7, was used. Samples were simply diluted (≥1:10 in water) prior to separation. Both electrokinetic injection and analytical separation were carried out at -180 V/cm. The method enabled separation of HUMCD4 alleles, with fair precision in terms of absolute and relative migration times (R.S.D.s of 0.22% and 0.04%, respectively, in intraday tests). The average accuracy of CD4 fragment sizing was 0.218 base pairs. These results confirmed the high suitability of capillary electrophoresis as a screening method for small-size DNA polymorphisms.


http://www.sciencedirect.com/science/article/B6T6W-414N4VW-3/2/3ecn5bac2569d7b3e6fd0d30176aed78a

The HUMVWA locus was examined in 160 samples from the Japanese population. A total of 142 fragments were sequenced, and the counterpart sequences were also determined in non-human primates. In humans, 10 different alleles were found; they could be grouped into seven allelic classes based on the total number of repeats. No variation was observed in the alleles 17, 18 and 19, which showed consensus sequence structures and in the allele 14, which showed a different structure. New variation was found in alleles 15, 16, and 20, which had differences occurred in a basic (TCTA)(TCTG)n repeat in the 5' side. The counterpart fragments were successfully amplified in three species (chimpanzees, gorilla, and orangutan) out of four kinds of anthropoids, three species (rhesus macaques, Japanese macaques, and green monkey) out of four kinds of old world monkeys, but not in one species of either new world monkey or prosimian. The sizes of the fragments distributed from 92 to 180 bp in non-human primates and showed allelic size differences in four species. The sequence of the 5' flanking region followed by primer sequences in humans and anthropoids, which consisted of 19 bp, was identical in all, but differed from that in old world monkeys. The basic repeat motifs of humans and anthropoids consisted of TCTA, TCTG, and TCCA but that of old world monkeys consisted of TCTG, TCCG and TCCA The structures of humans and anthropoids were essentially similar, but with characteristic difference in each species. Differences in the allelic structures of old world monkeys were complex. Seven different alleles were observed in two rhesus and two Japanese macaques and one type of allele was observed in two green monkeys. Duplication of more than two repeat units of 4 bp was found in an allele of an old world monkey. These data illuminate interesting features of mutational
changes in STRs during the long generations and also some insight into evolitional aspects of primates.


http://www.sciencedirect.com/science/article/B6T6W-47X1YJ0-5/2/547077a28619e5f5ace7aa67467f1854

The denaturant gradient gel electrophoresis (DGGE) method was used in order to simultaneously estimate the genotypes of different factors in a gel plate consisting of one sheet. A genotype analysis of the blood groups (MN, Duffy, Kidd type) and serotype (Gc system) was carried out. DNA samples were extracted from trace deposits which were transferred on adhesive film from a blood trace obtained from a car tire after a fatal car accident. The reference DNA was prepared from the victim’s blood. The PCR amplification fragments were amplified from the gene which controlled each blood group. The primers were designed in order to analyze the genotypes with one to three base substitutions in the amplification product. The denaturant concentration limit of the gel for the DGGE method to detect each genotype of the blood groups (MN, Duffy, Kidd type and Gc system) and other conditions of electrophoresis were performed according to previously methods. The each genotype of the blood groups and the Gc system were all simultaneously distinguished in one plate.


http://www.sciencedirect.com/science/article/B6T6W-3VXJ5D7-8/2/72d77128dc3b32e3cc03501acaa14a

DNA from the double short tandem repeat (STR) system MBP (locus 18q23-pter) was amplified by the polymerase chain reaction (PCR) and the two polymorphic repeat systems were separated by cutting with the restriction enzyme NlaIII. The lengths of the DNA fragments of the two MBP STR systems MBP-A and MBP-B were analyzed by vertical electrophoresis in polyacrylamide gels followed by silver staining. DNA samples from 112 unrelated Danes, 140 unrelated Greenland Eskimos, and 88 Danish mother/child pairs were analyzed. The distributions of MBP phenotypes were in Hardy-Weinberg equilibrium in both the Eskimo and Danish populations. Significant differences were observed between the distribution of fragments ('alleles') in Greenland Eskimos and in Danes. The allele MBP-A7 was considerably more frequent in Eskimos (0.2214) than in Danes (0.0775) and also the allele MBP-B9 was considerably more frequent in Eskimos (0.225) than in Danes (0.06). Strong gametic associations were found between fragments from the MBP-A and MBP-B series in both Danes and Eskimos. Some of the associations were different in Danes and Eskimos. In the 88 Danish mother/child pairs, the segregation of the MBP genotypes were in accordance with a genetic model of co-dominant inheritance and no mutation was found. Two MBP STR regions with irregular structures were sequenced. One fragment had a single base G to A transition at position 124 in the primer binding region between the MBP-A and MBP-B regions. In the other fragment, a deletion starting at position 117 and including the primer binding region between the MBP-A and MBP-B regions was found.
There are hundreds of millions of betel quid (BQ) lovers widely spreading around the world. Compositions in BQ may generate reactive oxygen species, which would induce DNA damage. However, oral epithelial cells as well as blood have often been used as reference samples in comparison with the mitochondrial DNA (mtDNA) sequence of hairs. The main purpose of this study was to investigate the extent of mtDNA sequence variation in regular BQ-chewers' oral epithelial cells, and thus to evaluate the forensic availability of the buccal cells from BQ-chewers using the mtDNA markers. The hypervariable segments I and II in the D-loop control region of mtDNA between paired samples of blood and buccal scrape cells from 75 non-BQ-chewers (to be a control group), 60 BQ-chewers, and 67 oral cancerous patients were DNA sequenced and compared. Among the three groups, the alteration rates of 1.3% (1 out of 75), 10% (6 out of 60), and 61% (41 out of 67) were identified from the control, BQ-chewers, and the cancerous group, respectively. In the cancerous group, as expected, high rate of DNA alteration between blood and buccal samples was found. In the BQ-chewers, one and five individuals had the length and point alterations, respectively. Interestingly, most of point alteration sites, e.g., mtDNA positions 153, 16189, 16093 identified from BQ-chewers, were also observed in previous literatures. As for the control subjects, one case with point alteration, and none with length alteration, was identified. For all the three groups, not only the oral cells but also the normal blood samples exhibited high frequency (>55%) of length heteroplasmy at poly-(C)n track. Statistical analyses revealed that significance was observed between the severity of mtDNA alteration in BQ-chewers' oral epithelial cells and the history of BQ-chewing (p = 0.02), with a tendency of positive association. Based on the guidelines by Carracedo et al., we suggest that the interpretation of mtDNA variations between criminal evidences and the oral epithelial cells (as a reference or known sample) from BQ-chewers should be performed with particular caution using the PCR-based mtDNA sequencing. Our findings would be valuable in mtDNA analysis of hair evidence, especially for those countries where the habit of BQ-chewing is popular.

Although cancerous specimens are usually not used in forensic DNA typing, they might be forcibly employed under certain instances. On the other hand, though the oral epithelial samples have been applied to forensic identification, the great popularity of betel quid (BQ)-chewing in Taiwan, which is known to be a risk factor leading to an oral cancer, makes this application questionable. The DNA stability of nine short tandem repeat (STR) markers (the AmpFlSTR(TM) kit) was first investigated and then used to evaluate the forensic appropriateness of the oral samples of both healthy BQ-chewers and the archived clinical specimens from oral cancer patients. The analyses were performed on buccal samples from 100 BQ-chewers and 100 oral cancer patients, as well as their paired peripheral blood samples, and a group of 100 non-BQ-chewers were used for the control. In the group of 100 oral cancer patients, two types of DNA instability were found. They were major allelic imbalance, and allelic alterations including the expansion, the contraction and the un-classified type (i.e. can not be confirmed as the expansion or the contraction). The overall percentage of the cancerous subjects demonstrating DNA instability was 33% (five patients possessing both types of DNA instability). Both types of DNA instability showed a tendency of increasing with the severity of the pathological stage of oral cancer. Forty-four occurrences of major allelic imbalance were found from 21 cancer patients.
The statistical result revealed that there was no significant difference in the allelic imbalanced occurrence among the nine STR loci. Allelic alterations were found in 17 patients, within which 12 individuals had the expansion, five had the contraction, and three were the un-classified type. Further, among these 17 patients, three were found to acquire multiple allelic alterations at multiple loci. In the group of 100 unrelated healthy BQ-chewers, two loci with major allelic imbalance were detected. However, the two imbalanced alleles were virtually half lost, and could still be recognized as heterozygous alleles. The statistical results of ANOVA, χ², and Scheffe tests indicated that the means of allelic imbalance at the nine STR loci of the oral cancerous group revealed a significant difference from those in the control group. Our results suggest that oral cancer tissues cannot be used as references for forensic purposes using the PCR-based STR systems, whereas the oral swabs from healthy BQ-chewers can be employed, but should be done with caution.


In this study, 19 Y-specific STR loci (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS388, DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, DYS446, DYS449, and DYS464) were analyzed in 301 unrelated Korean males by three multiplex PCR systems. The haplotype diversity using the classical set of Y-STRs (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS393, and DYS385; multiplex I) was 0.9963. For the same individuals, the haplotype diversity value using the new set of highly informative Y-STRs (DYS385, DYS446, DYS449, and DYS464; multiplex III) was 0.9989, while that using the combined set of Y-STRs by adding DYS388 to the previously studied DYS434, DYS435, DYS436, DYS437, and DYS439 (multiplex II) was 0.9509. A total of 297 different haplotypes were identified using the 19 Y-STR markers, of which 293 were unique and 4 were found twice. The overall haplotype diversity was 0.9999. The evaluation of the information of selected markers by combination of each marker with the minimal haplotype showed that DYS434, DYS435, DYS436, DYS437, and DYS438 do not significantly contribute to increment of haplotype diversity. However, respective conjunction of DYS464, DYS449, and DYS446 with the minimal haplotype considerably increased the haplotype diversity. Especially, DYS464 is expected to be the most useful marker that can be included in the expanded minimal haplotype. These results including the haplotype data at 19 Y-STR loci in the present study would provide useful information in forensic practice in a Korean population.


http://www.sciencedirect.com/science/article/B6T6W-4BCXKDN-1/2/25266d22fe4c3fe540c647c2b51e10d3

This paper presents an overview of the organisation and the results of the collaborative exercises (CE) of the European DNA Profiling (EDNAP) Group's mitochondrial DNA population database project (EMPOP). The aim of the collaborative exercises was to determine whether uniformity of mtDNA sequencing results could be achieved among different laboratories. These were asked to sequence either the complete mtDNA control region or the two hypervariable regions HVI (16024-16365) and HVII (73-340) from DNA extracts, buccal swabs or bloodstains, proceeding in accordance with the protocol and strategies used in each individual laboratory. The results of the collaborative exercises were employed to identify possible sources of errors that could arise during the analysis and interpretation of mtDNA profiles. These findings were taken as a basis to
tentatively make suitable arrangements for the construction of a high quality mtDNA database. One hundred fifty mtDNA profiles were submitted to the evaluating laboratory, and disaccord profiles were classified into four groups corresponding to the source of error: clerical errors, sample mix-ups, contaminations and discrepancies with respect to the mtDNA nomenclature. Overall, 14 disaccording haplotypes (16 individual errors) were observed. The errors included 10 clerical errors, 3 interpretation problems, 2 cases of sample mix-up and 1 case of point heteroplasmic mixture, where the 2 sequencing reactions brought inconsistent base calls. This corresponds to an error rate of 10.7% in a virtual mtDNA database consisting of the collaborative exercise results. However, this estimate is still conservative compared to conclusions drawn by authors of meanwhile numerous publications critically reviewing published mtDNA population databases. Our results and earlier published concerns strongly emphasize the need for appropriate safety regulations when mtDNA profiles are compiled for database purposes in order to accomplish the high standard required for mtDNA databases that are used in the forensic context.


DNA microsatellites play a major role in population genetics, linkage mapping, and parentage studies of mammals. In addition, they may be used for forensic purposes, if an individual identification of a specific animal is necessary. Therefore, we tested a variety of microsatellite polymorphism derived from reindeer (Rangifer tarandus) by PCR and sequencing analysis for use in red deer (Cervus elaphus), roe deer (Capreolus capreolus) and fallow deer (Dama dama). Twelve of these microsatellites were selected for further analysis. In all these microsatellite polymorphism short tandem repeats could be detected for one or all three species as shown by sequencing analysis. In red deer, more than two alleles were found in eight microsatellites, in roe deer more than two alleles could be demonstrated in seven microsatellites, whereas in fallow deer more than two alleles were found in only two microsatellite polymorphism. A comparison of
sequences of PCR products from the three deer species with the sequences of reindeer revealed several differences between the four species. In six microsatellites -- selected because of their reliability in PCR and because of their polymorphic character -- we established a sequenced allelic ladder and give population data of all three species from 82 deer of the Northeast region of Germany (Vorpommern). Our results show the possibility to use microsatellite polymorphism in the identification of deer in forensic applications like poaching.


http://www.sciencedirect.com/science/article/B6T6W-49M0RB7-4/2/7bc3338551d0a88f59c2f9a9ccd239bb

Mitochondrial DNA sequences of the hypervariable regions HV I and HV II were analyzed in 300 unrelated individuals born and living in the northeast corner of Germany (Western Pomerania) to generate a database for forensic identification purposes in this region. Sequence polymorphism were detected using PCR and direct sequencing analysis. A total of 242 different haplotypes were found as determined by 147 variable positions. The most frequent haplotype (263G, 315.1C) was found in 10 individuals and is also the most common sequence in Europe. Three other haplotypes were shared by 5 individuals, 2 sequences by 4, 8 haplotypes by 3, 15 sequences by 2 persons, and 213 sequences were unique. The genetic diversity was estimated to be 0.99 and the probability of two random individuals showing identical mitochondrial DNA (mtDNA) haplotypes is 0.6%. A comparison with other studies from Germany showed only little differences in the distribution of haplogroups. Nevertheless, one frequent haplotype in northeast Germany (five unrelated individuals) could only rarely be found in other German and European regions. Our results may indicate that despite a high admixture proportion in the German population some regions could demonstrate certain characteristic features.


http://www.sciencedirect.com/science/article/B6T6W-49XPTY2-1/2/1d5ec2ccc3e7c140b8b8b590d19847846

The present communication presents a new triplex PCR co-amplifying three loci (D3S1358, D8S1179 and D18S51) recommended for STR typing by the European Network of Forensic Science Institutes (ENFSI). Twenty-two different primers were tested to optimise the PCR. Four of the six primer sequences finally chosen were self selected, the fifth was a published one and the sixth derived from a commercially available multiplex kit. Using this PCR-setup, even minimum amounts of genomic DNA are sufficient to analyse the STR loci D3S1358, D8S1179 and D18S51 in parallel. Especially in forensic casework, where DNA is mostly limited and often contaminated with enzyme inhibitors, this new PCR proved to be very advantageous. To demonstrate the reliability, buccal swabs from 2874 persons were typed not only with the new triplex PCR but also with a commercially available multiplex kit.

A series of validation experiments was performed for a Y chromosome specific STR multiplex system following the suggestions made by the Technical Working Group DNA Analysis Methods (TWGDAM). The multiplex PCR products were detected on Perkin-Elmer 373 and 377 automated sequencers using two labeling colors. No problems regarding the stability, robustness and sensitivity of the Y STR multiplex were observed. Mixture studies revealed a cut off rate similar to autosomal STRs for mixtures of male DNAs and no interference of any female admixture. The comparison of the Y STR results to the autosomal typing results for 56 nonprobative semen stains and swabs, showed a slightly higher success rate in detecting the semen donor's alleles for the Y STR multiplex. Two examples are shown to illustrate the usefulness of Y STR typing for DNA mixtures. In one case the Y STR results confirmed an isolated exclusion; in the other case, the interpretation of a mixture was clarified since the Y STR results proved the presence of DNA from at least two semen donors. Y STR typing is a valuable addition to the forensic DNA testing panel.


We performed a population study on a Taiwan population using a set of nine short tandem repeat (STR) loci and the amelogenin locus. Allele and genotype frequencies of the STR systems D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820 were determined by polymerase chain reaction followed by automated sequencer analysis. A total of 80 alleles could be observed, using all systems, in a population of 500 individuals. No new intermediate fragments were found in these systems. Allele frequencies showed no deviation from Hardy-Weinberg equilibrium. The mean exclusion power (MEP) ranged from 0.294 for TPOX to 0.711 for FGA (the combined MEP was >0.999), the discriminating power (DP) ranged from 0.782 for TPOX to 0.964 for FGA, (the combined DP was >0.9999999949).

http://www.sciencedirect.com/science/article/B6T6W-4BRBK4G-C/2/1b41081564f6f10e7dd4185a81bc408d

The development of new methodologies for high-throughput SNP analysis is one of the most stimulating areas in genetic research. Here, we describe a rapid and robust assay to simultaneously genotype 17 mitochondrial DNA (mtDNA) coding region SNPs by minisequencing using SNapshot. SNapshot is a methodology based on a single base extension of an unlabeled oligonucleotide with labeled deoxy terminators. The set of SNPs implemented in this multiplexed SNapshot reaction allow us to allocate common mitochondrial West Eurasian haplotypes into their corresponding branch in the mtDNA skeleton, with special focus on those haplogroups lacking unambiguous diagnostic positions in the first and second hypervariable regions (HVS-I/II; by far, the most common segments analyzed by sequencing). Particularly interesting is the set of SNPs that subdivide haplogroup H; the most frequent haplogroup in Europe (40-50%) and one of the most poorly characterized phylogenetically in the HVS-I/II region. In addition, the polymorphic positions selected for this multiplex reaction increase considerably the discrimination power of current mitochondrial analysis in the forensic field and can also be used as a rapid screening tool prior to full sequencing analysis. The method has been validated in a sample of 266 individuals and shows high accuracy and robustness avoiding both the use of alternative time-consuming classical strategies (i.e. RFLP typing) and the need for high quantities of DNA template.


http://www.sciencedirect.com/science/article/B6T6W-4D490H0-D/2/73d906b63376bb8e4d5f5bf288280985

We extracted the DNA contained in samples of bones and teeth from 10 skeletons excavated from the Gravette site (400-1000 AD, south of France). Ancient DNA was analysed by autosomal short tandem repeats (STRs). The DNA present in these ancient remains appeared very degraded, but nevertheless, better conserved in tooth than in bone samples. Moreover, we showed that the DNA extracted from ancient dental pulp was not exempt from polymerase chain reaction (PCR) inhibitors, which could result from extreme DNA fragmentation. An adapted protocol with a supplementary step of purification removed this inhibition.


http://www.sciencedirect.com/science/article/B6T6W-3S2BKV5-9/2/1a8de0c1626090fbd9299fb83fa5bf60

The electrophoretic mobility of DNA fragments on denaturing polyacrylamide gel depends on various factors. One of these is the base composition of a single-stranded DNA (ssDNA). We confirmed that one strand and its complementary strand of polymerase chain reaction (PCR) products migrated with different mobilities in all alleles detected at 12 out of the 13 short tandem repeat (STR) loci studied. The mobility differences between complementary strands (MD) were also observed regardless of end-polishing with Pfu DNA polymerase. MD was therefore not influenced by additional nucleotides to each strand of the PCR products. We then analyzed the
relation between MD and the base composition using one representative allele at each of the 13 loci. The results indicated that MD was affected by the adenine plus cytosine (AC) content in the ssDNA and was proportional to the values of the AC content divided by the guanine plus thymine (GT) content in the AC-rich strand (the proportion AC/GT). When the proportion AC/GT was well-balanced, MD decreased. The same tendency was observed even in the end-polished strands. In this study, the electrophoretic mobility of an ssDNA on denaturing polyacrylamide gels was shown to depend on the proportion AC/GT. Unless the same side of the PCR products is labelled in the context of a PCR-based STR typing, distinct alleles may be mistaken for identical ones because of the different mobility of complementary strands. Accordingly, the labelled strand should be described if only one strand of the PCR products is detected. When using an allelic ladder marker as a size standard, the labelled side should be unified between STR alleles and the allelic ladder alleles.


http://www.sciencedirect.com/science/article/B6T6W-4C35YMH-MV/2/1392b36f0734a2c8efdc3275422a886

The application of DNA typing methods after amplification by the polymerase chain reaction (PCR) of DNA derived from body tissues from charred fire victims was investigated. A total of 26 different tissue specimens from ten extensively burned individuals were analyzed. The samples included femoral muscle, psoas muscle, bone marrow and blood. The post-mortem period varied from 38 to 183 h. After amplifying the DNA by PCR from the various tissues, the D1S80 locus was analyzed with a high resolution polyacrylamide gel electrophoresis technique followed by silver staining and the alleles of the HLA-DQ[alpha] locus were detected by using a reverse dot blot format. All samples could be typed for both loci and the genotypes were consistent in the various tissues from each individual. A parentage test was performed in two cases and Mendelian inheritance of the alleles for both loci was observed.


http://www.sciencedirect.com/science/article/B6T6W-49D6YWB-1/2/54c45c15778ca4940344bf2eb24a0f967

We have developed a robust single nucleotide polymorphism (SNPs) typing assay with co-amplification of 25 DNA-fragments and the detection of 35 human Y chromosome SNPs. The sizes of the PCR products ranged from 79 to 186 base pairs. PCR primers were designed to have a theoretical Tm of 60+/-5 [deg]C at a salt concentration of 180 mM. The sizes of the primers ranged from 19 to 34 nucleotides. The concentration of amplification primers was adjusted to obtain balanced amounts of PCR products in 8 mM MgCl2. For routine purposes, 1 ng of genomic DNA was amplified and the lower limit was approximately 100 pg DNA. The minisequencing reactions were performed simultaneously for all 35 SNPs with fluorescently labelled dideoxynucleotides. The size of the minisequencing primers ranged from 19 to 106 nucleotides. The minisequencing reactions were analysed by capillary electrophoresis and multicolour fluorescence detection. Female DNA did not influence the results of Y chromosome SNP typing when added in concentrations more than 300 times the concentrations of male DNA. The frequencies of the 35 SNPs were determined in 194 male Danes. The gene diversity of the SNPs ranged from 0.01 to 0.5.

http://www.sciencedirect.com/science/article/B6T6W-4BRBK4G-B/2/383e68af0d08b05f7b3a86c12500b6677

In the present study, we demonstrate that two commonly used Y-chromosome single nucleotide polymorphisms (SNPs), P25 and 92R7, are paralogous sequence variants (PSVs) originating from segmental duplications and that at least one of the sequence variants in each group of loci is polymorphic. Several methodologies were used in order to detect the SNP alleles and the PSVs of the loci. All results obtained with the various typing techniques supported the conclusion. The allele distributions of the binary markers were analysed in more than 600 males with seven different haplogroups. For P25, the ancestral allele C was found in several samples from different haplogroups. The derived allele A was always present with an additional C variant. Haplogroup P was defined by the derived allele A at the 92R7 locus. However, the ancestral allele G was always associated with an A variant due to the duplication.


http://www.sciencedirect.com/science/article/B6T6W-3RG55N8-10/2/3a061c80e5e170fe3f5726d4f1ae6fd81

Allele frequencies for six tetrameric short tandem repeat (STR) loci CSF1PO, TPOX, THO1, D3S1358, VWA and FGA were determined in a Caucasian population sample from Portugal. All loci are highly polymorphic and meet Hardy-Weinberg expectations. There is little evidence for association of alleles among the six loci. The three loci D3S1358, VWA and FGA are more polymorphic and, hence, are more informative than the loci CSF1PO, TPOX, and THO1. However, all six loci would be useful for human identification applications. The STR allelic frequency data are similar to other Caucasian data.


http://www.sciencedirect.com/science/article/B6T6W-4B4HKB1-2/2/51235e3cf3404bb7fdd0d2061d63407

Two Y-chromosome short tandem repeat (STR) multiplex polymerase chain reaction (PCR) assays were used to generate haplotypes for 19 single copy and 3 multi-copy Y-STRs. A total of 27 PCR products were examined in each sample using the following loci: DYS19, DYS385 a/b, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS426, DYS437, DYS438, DYS439, DYS447, DYS448, DYS450, DYS456, DYS458, DYS460, DYS464 a/b/c/d, H4, and YCAII a/b. The first multiplex is the Y-STR 20plex previously described by Butler et al. [Forensic Sci. Int. 129 (2002) 10]. The second multiplex is a novel Y-STR 11plex and includes DYS385 a/b, DYS447, DYS448 and the new markers DYS450, DYS456, DYS458, and DYS464 a/b/c/d. These two multiplexes were tested on 647 males from three United States population sample sets: 260 African Americans, 244 Caucasians, and 143 Hispanics. Haplotype comparisons between
common loci included in the 20plex and 11plex assays as well as commercially available kits found excellent agreement across a sampling of the population samples. The multi-copy loci DYS464, DYS385, and YCAII were the most polymorphic followed by the following single copy Y-STRs: DYS458, DYS390, DYS447, DYS389II, DYS448, and DYS456. Samples containing the most common type in the European database could be well resolved with additional markers beyond the minimal haplotype loci.


http://www.sciencedirect.com/science/article/B6T6W-4CG2KCF-1/2/ab50b93ec782b0fd39bc037227083c7b

Allele and haplotype frequencies were obtained for the six Y STR loci DYS19, DYS389II, DYS390, DYS391, DYS393 and DYS385 in the New Zealand population. Ninety-two different haplotypes were found. The Maori population had a specific haplotype that occurred in over 30% of the population. The Pacific Island population exhibited a triple repeat at the DYS385 locus in 26% of individuals, something rarely observed in other population groups.


http://www.sciencedirect.com/science/article/B6T6W-3WSMFJX-7/2/924b5addb152bd8d260f93a1664259d2

Using the polymerase chain reaction (PCR), we studied the short tandem repeat (STR) polymorphism observed at the D12S391 locus. In 350 Japanese examined, 14 different alleles ranging from 209 bp to 261 bp were detected. Allele 18 (221 bp) showed the highest frequency at 0.30. Observed and expected values of respective genotypes satisfied the Hardy-Weinberg equilibrium (\( \chi^2 = 24.08, P = 0.24, df = 20 \)). In addition, 18 additional sequence structures (suballeles), were detected in this study. Within the suballeles, sequence variants, in which the initial repeat of (AGAT) was replaced with (AGGT), was found in five samples. It was found that the analysis of single-strand conformation polymorphism (SSCP) before sequence analysis was useful for distinguishing these suballeles.


http://www.sciencedirect.com/science/article/B6T6W-4DVBJG-6/2/7c2d296501cccb6bd587000a0e0fd84fe

Cholecystokinin (CCK), a neurotransmitter in the central nervous system (CNS), co-exists in a large portion of A10 dopamine neurons to exert some effect on dopamine behavior. The aim of this study was to determine whether any association exists between the genotype of CCK gene promoter regions (−45C/T and −196G/A) and suicidal behavior. Genotypes and allele frequencies of CCK −45C/T and −196G/A were analyzed using polymerase chain reaction (PCR) followed by single-strand conformational polymorphism (SSCP) analysis on the genomic DNA from selected suicide victims (N = 154) and from control subjects (N = 328). Statistical analysis was performed using the Mantel-Haenszel \( \chi^2 \)-test and multiple logistic regression analysis with distinction of gender. An association between CCK −196G/A polymorphism and suicidal behavior in Japanese
males was confirmed by statistical analysis (Odds ratio: 3.462, 95% CI: 1.128-10.626, P = 0.038 by multiple logistic regression analysis). However, a significant association between CCK - 196G/A polymorphism and suicidal behavior was not discovered in females. The polymorphism of the CCK gene promoter region was found to represent a susceptibility factor for suicidal behavior in Japanese males.


http://www.sciencedirect.com/science/article/B6T6W-4DKKFD7-6/2/acc148e818469e40cccb1ae9848bd9a12

Forensic investigations involve several scientific branches among which biological analyses are much more frequently requested as a consequence of their importance and great versatility towards most of the traces found on the crime scene. Biological analyses are lead in subsequent steps: extraction, amplification and STR typing of the specimens collected on the crime scene. All of these techniques have been modified from original protocols according to the kind of sample to process. A critical point in our analysis is trying to amplify small amounts of DNA extracted from decomposed tissues or objects, small biological traces have been left on, with high fidelity and account. That's why we have decided to settle on an experimental procedure aimed to find the best DNA polymerase according to our purposes. We have tested different Taq polymerases on the same known DNA sample at several dilutions and have compared quality and amount of amplified DNA in order to appreciate the amplifying capability of each enzyme. These data have been analyzed as a function of the technical properties of each engineered Taq polymerase and results are shown in details.


http://www.sciencedirect.com/science/article/B6T6W-43J6SDP-B/2/db732b101c582180f48c63ef935898a8

The allele distribution of the systems DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385 and YCAII were investigated in a sample of 121 unrelated males from Slovenia.


http://www.sciencedirect.com/science/article/B6T6W-3VCVF84-7/2/a6a3f14dd78d5b1f50d8b1628312a50fd

Polymorphism of GPT was investigated in blood samples from 241 unrelated Japanese individuals by PCR-RFLP analysis. Three common types were identified which agreed with those obtained by starch gel electrophoresis. The allele frequencies were GPT*1=0.604 and GPT*2=0.396. The population data fitted the Hardy-Weinberg law. The PCR-RFLP patterns of GPT were also demonstrated in semen, and the types coincided with those in the corresponding blood. The present method permitted GPT genotyping in bloodstains and semen stains stored for 20 years. The GPT system determined by PCR-RFLP can still be a useful supplement in medicolegal individualization of biological stains.
This study details validation of two separate multiplex STR systems for use in paternity investigations. These are the Second Generation Multiplex (SGM) developed by the UK Forensic Science Service and the PowerPlex™1 multiplex commercially available from Promega Inc. (Madison, WI, USA). These multiplexes contain 12 different STR systems (two are duplicated in the two systems). Population databases from Caucasian, Asian and Afro-Caribbean populations have been compiled for all loci. In all but two of the 36 STR/ethnic group combinations, no evidence was obtained to indicate inconsistency with Hardy-Weinberg (HW) proportions. Empirical and theoretical approaches have been taken to validate these systems for paternity testing. Samples from 121 cases of disputed paternity were analysed using established Single Locus Probe (SLP) tests currently in use, and also using the two multiplex STR systems. Results of all three test systems were compared and no non-conformities in the conclusions were observed, although four examples of apparent germ line mutations in the STR systems were identified. The data was analysed to give information on expected paternity indices and exclusion rates for these STR systems. The 12 systems combined comprise a highly discriminating test suitable for paternity testing. 99.96% of non-fathers are excluded from paternity on two or more STR systems. Where no exclusion is found, Paternity Index (PI) values of >10000 are expected in >96% of cases.

DNA from the locus D1S80 was amplified by polymerase chain reaction (PCR) and analyzed by electrophoresis in vertical polyacrylamide gels followed by silverstaining. DNA samples from 119 unrelated Danes and 97 mother/child pairs were examined. The amplified fragment length polymorphism (AMP-FLP) analysis of the D1S80 locus demonstrated 21 alleles and a heterozygosity of 77%. Of the 231 possible phenotypes, 57 were observed. All mother/child pairs shared at least one D1S80 allele. The D1S80 typing results in 70 Danes were compared to the results obtained on the same samples in another laboratory and the results were concordant in all cases.

We examined the complex short tandem repeat (STR) locus at the 3'-flanking region of the neurotensin receptor (NTR) gene. The polymorphism of this locus was first reported as a simple
tetranucleotide repeat variation by Le et al. [1], but it also offers a surprisingly informative variation, that permits reliable individual identification by two complementary strategies: fluorescent-labelled polymerase chain reaction (PCR)/electrophoresis and direct sequencing of the PCR products. We determined the alleles in 203 Japanese by fluorescent-labelled PCR/electrophoresis. Determination was based on their length with a reliability of +/-1 bp, and the frequency of each allele was very low. Sequencing analysis further grouped these alleles in detail. Sequencing demonstrated that the locus varied by six repetitive units and three insertion/deletion positions of nucleotide fragments. We detected multiple alleles having different structures even in the same allele length. We found structural differences in homozygous alleles having the same base pair size. We also determined that apparently homozygous alleles were heterozygous from sequencing electropherograms showing an overlap of nucleotides or +/-1 bp difference. These results indicate that this locus is structurally hypervariable in addition to having allelic length variations, promising a great advance in individual identification in forensic practice.


http://www.sciencedirect.com/science/article/B6T6W-433P7K2-C/2/b7b74747c1a62d36a651677af06540cc

In order to demonstrate the sequence diversity of mitochondrial D-loop DNA in the Taiwanese Han population, we established a database of 155 unrelated individuals. For each individual, the complete 980 bp DNA region from the 5’ end of HVI to 3’ end of HVII segment was sequenced. In these 155 sequence data, 149 different haplotypes were observed, amongst these haplotypes, 144 were unique, 4 were found in 2 individuals and 1 was found in 3 individuals. When compare to the Anderson sequence, 144 transitions, 24 transversions, 5 insertions and 5 deletions were found. Eight positions exhibited more than one polymorphic sequence, six exhibited two variants while two exhibited three variants. Over the 1024 bp that was analysed, pairwise differences between the sequences were 11.35 +/- 3.53 bp. The sequence and nucleotide diversity were 0.9994 and 0.0116, respectively. The probability of two individuals randomly matching over the entire control region was 0.007. The diversity in the mitochondrial D-loop indicates the value of this locus for casework within Taiwan.


http://www.sciencedirect.com/science/article/B6T6W-43J6SDP-6/2/bd82097f2cd12ccc4703ac8d7c816823

Analysis of the polymorphic sequences in mitochondrial DNA (mtDNA) has been widely applied to forensic tests and anthropology studies. However, these polymorphic data in human have thus far been derived from the displacement-loop and intergenic regions only. Here, we report the identification of clustered polymorphic sites in the mitochondria coding region encompassing position 8389-8865. The DNA sequences of 119 unrelated Chinese were determined by PCR amplification and direct sequencing. The results showed that heteroplasmy was found in five individuals, 39 sites were noted in this 477 bp region, and 41 haplotypes were identified. The probability of identity and allelic diversity were estimated as 0.1265 and 0.8809, respectively. The results suggest that sequence polymorphism from position 8389-8865 in human mtDNA can be used as a marker for identity investigation.

http://www.sciencedirect.com/science/article/B6T6W-4DCWHF2-1/2/0afca869ac369cc6f8c7c8257a3e6f14

189 samples from 3 different U.S. sample groups Caucasian (74), African American (71) and Hispanic (44) were typed for 70 autosomal genetic markers. These 70 markers are bi-allelic (C/T) short nucleotide polymorphisms (SNPs). For each sample, the 70 SNP markers were typed in 11 unique 6-plexes and a single 4-plex PCR. A total of 10 of the 210 tests (70 loci X 3 populations) for Hardy-Weinberg equilibrium indicated a statistically significant result. In order to evaluate the minimum number of SNP loci needed to distinguish all 189 samples from one another, we ranked the loci according to their levels of observed heterozygosity and p-values obtained upon testing for Hardy-Weinberg equilibrium. The top 12 loci according to these ranking criteria were tabulated along with the number of unique genotypes observed when combining subsequent SNP markers. The 12 selected SNPs possessed an observed heterozygosity of >0.45 in all three populations examined and thus would be expected to exhibit more differences between samples. All of the 189 samples in this study were individualized with a subset of 12 SNP loci. However, it is likely that the addition of more than 12 SNP loci will be required to resolve larger sets of unrelated individuals from one another. By way of comparison, in these same 189 individuals all but one pair is resolved from one another with three of the traditional short tandem repeat (STR) loci possessing the highest heterozygosity values (D2S1338, D18S51, and FGA) run with the Identifier kit. The final pair of unrelated samples could be resolved with the combination of 4 STR loci: D2S1338, D18S51, FGA, and VWA.


http://www.sciencedirect.com/science/article/B6T6W-4B7YFXJ-2/2/d6b45079de49e01390a395e643b30f6e5

Malignant tissue samples may sometimes be the only source of biological material for forensic investigations, including identification of individuals or paternity testing. However, in use of such samples, uncertainties due to microsatellite instability (MSI) and loss of heterozygosity (LOH) often associated with neoplasias may be encountered. In this study, we have analysed the applicability of autosomal tetranucleotide short tandem repeat (STR) markers, which are routinely used in forensic analysis, to gain genetic information. MSI and LOH were analysed in 41 surgically removed gastrointestinal cancer specimens and the adjacent non-cancerous tissue marginals. The cancer specimens showed great variability in their genetic phenotypes due to MSI or LOH, with only 32% being microsatellite-stable. Of the 15 autosomal STR loci analysed, only TH01 had no MSI-type alteration in these samples. The loci most frequently affected by MSI were D8S1179, D21S11, D18S51 and D19S433 (MSI in 15-17% of cases). LOH-type alterations were observed at all of the loci, including the amelogenin locus used for sex determination. The highest LOH frequency was found at locus D18S51 (27%). The genetic alterations at the marker loci may indicate false homozygosity or heterozygosity, and false gender may result from erroneous deduction of DNA profiles. Therefore, typing of autosomal STRs from malignant tissues in forensic settings warrants careful interpretation of MSI and LOH results together with microscopic analysis of a tissue specimen. Results by two commercially available and widely used forensic DNA profiling kits used here were comparable.

http://www.sciencedirect.com/science/article/B6T6W-49FR99N-1/2/a9bbd4c3974f1a9059492e99f0846a49

In this study, we report the findings of a recent case in which the officials of an Indian zoo claimed that an animal, possibly a carnivore, is periodically visiting the zoo from a nearby vast forest area and causing panic in zoo and nearby villages. They collected some elusive faecal material from the vicinity of an herbivore enclosure. Looking to the pugmarks found in that area and faecal matter ceased, the officials could not decide whether it was a lioness, a tiger or a panther. We resolved this mystery by DNA-based analysis of the faecal material, using our recently developed novel universal primers to amplify and sequence a specific fragment of mitochondrial cytochrome b gene. The findings of the DNA-based analyses were confirmed after few days when the zoo officials trapped the animal of same species as suggested in our report. The potential of our procedure to investigate the cases related to wildlife offence is discussed.


http://www.sciencedirect.com/science/article/B6T6W-44KTHV6-3/2/c2ffcc382335c575151e8dd2f1b42cfa27

Cyanoacrylate ester (CA) is commonly used by criminalists to detect latent fingerprints on smooth surfaces. We investigated whether this treatment has an influence on a subsequent DNA typing of biological stains, and on the efficiency of three different forensic PCRs (mtDNA, Y-STR determination and the Profiler Plus kit). Using fluorescence labeled primers and an automated detection system, we could show that the presence of CA led to weaker PCR products. Depending on the DNA extraction method the amplification results were significantly weaker compared to untreated controls. To simulate forensic cases we prepared blood and saliva stains on glass slides, extracted the DNA using two different methods and compared the signal intensities of the amplified DNA fragments. Depending on the extraction methods, the presence of CA significantly hampered the amplification of DNA from small stains whereas there was virtually no difference comparing the amplification results of DNA extracted from bigger stains.


http://www.sciencedirect.com/science/article/B6T6W-43G30BC-C/2/8661b39c20f2ef5421f565668b955627

The applicability of mitochondrial DNA (mtDNA) sequencing was investigated for the identification of the following forensically important species of blowflies from southeastern Australia: Calliphora albifrontalis, C. augur, C. dubia, C. hilli hilli, C. maritima, C. stygia, C. vicina, Chrysomya rufifacies, Ch. varipes and Onesia tibialis. All breed in carrion except O. tibialis, which is an earthworm parasitoid. Emphasis was placed on Calliphora species because they predominate among the carrion-breeding blowfly fauna of southern Australia and their immatures are difficult to identify morphologically. A partial sequence of the mitochondrial COII gene was determined for all species and for COI for C. albifrontalis, C. augur, C. dubia and C. stygia only. Five other species of blowflies, Chrysomya albiceps, Ch. rufifacies, Protophormia terraenovae, Lucilia illustris and L.
sericata, for which sequence data were already available, were also included. Analysis of the COI and COII sequences revealed abundant phylogenetically informative nucleotide substitutions that could identify blowfly species to species group. In contrast, because of the low level of sequence divergence of sister species, the data could not distinguish among taxa from the same species group, i.e. the species within the C. augur and C. stygia groups. The molecular data support the existing species group separation of the taxa within Calliphora. Because of the speed and accuracy of current nucleotide sequencing technology and the abundant apomorphic substitutions available from mtDNA sequences, this approach, with the analysis of additional taxa and genes, is likely to enable the reliable identification of carrion-breeding blowflies in Australia.


http://www.sciencedirect.com/science/article/B6T6W-48S35HX-6/2/9e2841515bf5607a5c4e05db68041b08

Several variant alleles of the HumD21S11 locus have only been reported in Australasian population samples. Fifteen such alleles were observed in Caucasian and Australian Aborigine sub-population databases compiled from residents of the state of Western Australia. Each variant was sequenced to authenticate the allelic designation and determine the structural conformation. Nine novel structural variants are described. The structure of the repeat region of these rare alleles combined with the STR designation brings aspects of the HumD21S11 nomenclature guidelines into question, in particular the designation of common incomplete repeats (or "0.2's"). The conformation of the sequences provides evidence in support of a genetic relationship between the Australian Aborigine and the Papuan people.


http://www.sciencedirect.com/science/article/B6T6W-4C35Y8T-F7/2/4e9b173c6f2e35e19db09bd0db80b594c

The typing of the D1S8 (MS32) locus using the minisatellite variant repeat (MVR) polymerase chain reaction (PCR) method was performed by visualising amplified DNA stained with ethidium bromide. The results from rapid detection MVR-PCR were compared with those from the original MVR-PCR using Southern blot hybridisation with a 32P-labelled probe. With genomic DNA extracted from blood samples of 40 healthy unrelated Japanese individuals, the first 41 codes, on average, were correctly determined by rapid detection MVR-PCR without band intensity information, compared with at least 60 codes typed by the original MVR-PCR. The rapid detection MVR-PCR method was applied to bloodstains to simulate forensic samples. On average, 39 code positions could be determined from DNA extracted from 3-month-old bloodstains of six persons. Rapid detection MVR-PCR is more convenient than the original MVR-PCR, furnishes much information with regard to personal identification, and should be applicable to forensic fields.

Allele frequency data for 15 Short Tandem Repeat (STR) loci was studied for the three main ethnic groups residing in Singapore, namely Chinese, Malay and Indian. An in-house STR marker panel was employed, consisting all 13 tetranucleotide STR listed in CODIS (Combined DNA Index System, USA) and two pentanucleotide STR, Penta D and Penta E. This represents a comprehensive report for allele distribution in the Singapore population for these 15 microsatellite markers commonly used in forensic science.


A new method has been optimised to amplify five X chromosome short tandem repeat (STR) markers of interest in forensic medicine: human phosphoribosyl transferase (HPRTB), DXS101, androgen receptor (ARA), DXS7423 and DXS8377. Markers were conveniently amplified in a single PCR reaction with fluorochrome-labelled primers, which allowed the analysis of fragment sizes after injection into a capillary electrophoresis system. The most common alleles of each locus were sequenced and used in a control ladder to type unknown samples.


Eleven Y-chromosome STR loci (DYS19, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS385a,b, DYS438, DYS439) have been co-amplified in 96 healthy unrelated males of Chinese Mongol ethnic group, in order to investigate allele and haplotype frequencies of them, evaluate their usefulness in forensic casework.


We tested the long-term effects of sublethal oxidative stresses on replicative senescence. WI-38 human diploid fibroblasts (HDFs) at early cumulative population doublings (CPDs) were exposed to five stresses with 30 [mu]M tert-butylhydroperoxide (t-BHP). After at least 2 d of recovery, the cells developed biomarkers of replicative senescence: loss of replicative potential, increase in
senescence-associated [beta]-galactosidase activity, overexpression of p21Waf-1/SDI-1/Cip1, and inability to hyperphosphorylate pRb. The level of mRNAs overexpressed in senescent WI-38 or IMR-90 HDFs increased after five stresses with 30 [mu]M t-BHP or a single stress under 450 [mu]M H2O2. These corresponding genes include fibronectin, osteonectin, [alpha]1(I)-procollagen, apolipoprotein J, SM22, SS9, and GTP-[alpha] binding protein. The common 4977 bp mitochondrial DNA deletion was detected in WI-38 HDFs at late CPDs and at early CPDs after t-BHP stresses. In conclusion, sublethal oxidative stresses lead HDFs to a state close to replicative senescence.


http://www.sciencedirect.com/science/article/B6T38-4BRP0NV-1/2/f234d961cf588248a1e3df832438ba53

The detrimental role of oxidative stress has been widely described in tissue damage caused by ischemia-reperfusion. A nonenzymatic, reactive oxygen species-related pathway has been suggested to produce 8-iso-prostaglandin F2[alpha] (8-iso-PGF2[alpha]), an epimer of prostaglandin F2[alpha] (PGF2[alpha]), which has been proposed as an indicator of oxidative stress. Using an in vivo ischemia-reperfusion model in rat kidneys, we investigated intrarenal accumulation of 8-iso-PGF2[alpha] and PGF2[alpha]. Both prostanoids accumulated in the ischemic kidney and disappeared upon reperfusion. In addition, a nonselective (acetylsalicylic acid) or selective cyclooxygenase (COX) 1 inhibitor (SC-560) completely abrogated the 8-iso-PGF2[alpha] and PGF2[alpha] formation in kidneys subjected to ischemia. COX2 inhibition had no effect on the production of these prostanoids. Therefore the two metabolites of arachidonic acid seemed to be produced via an enzymatic COX1-dependent pathway. Neither COX overexpression nor COX activation was detected. We also investigated renal glutathione, which is considered to be the major thiol-disulfide redox buffer of the tissue. Total and oxidized glutathione was decreased during the ischemic period, whereas no further decrease was seen for up to 60 min of reperfusion. These data demonstrate that a dramatic decrease in antioxidant defense was initiated during warm renal ischemia, whereas the 8-iso-PGF2[alpha] was related only to arachidonate conversion by COX1.


http://www.sciencedirect.com/science/article/B6T38-3Y6GVRK-18/2/ddc9d8639fdcecd15962cbb8eadfa3ed

Deletions of mitochondrial DNA (mtDNA) are associated with aging and several chronic diseases. We have reported heterogeneous mutations between base pair 8468 and 13446 in mtDNA, the region known as the "common" deletion, in muscle of older humans with impaired glucose tolerance or diabetes mellitus. To further characterize potential effects of age and glycemia on mtDNA integrity, we studied corpulent JCR:LA-cp rats that are characterized by insulin resistance, hyperinsulinemia, and hyperlipidemia, factors strongly associated with both aging and cardiovascular disease. In addition to skeletal muscle, we isolated vascular smooth muscle cells (VSMC) from aortas of 6-, 12-, and 17-month-old rats and exposed them to 5-, 25-, 62-, and 100-mM glucose or a combination of hypoxanthine (100 [mu]M) and xanthine oxidase (0.025 U/ml) to generate reactive oxygen species in separate cultures. Long- and short-fragment and nested polymerase chain reaction was used to detect mutations in the common deletion region. The data demonstrate that aging and the cp genotype confer susceptibility to mtDNA deletions in vivo and
that high glucose concentrations can induce mtDNA mutations in vitro. Accordingly, aging and glucose-related oxidative stress and possibly hyperinsulinemia may contribute to alterations in mitochondrial gene integrity and the cp genotype appears to increase the susceptibility of muscle to the age-related accumulation of mtDNA mutations.


http://www.sciencedirect.com/science/article/B6T38-46NWYSG-1/2/ac12f8a8a29df8d4a7963dcd8ab5e3dc

Ascorbic acid (AA) metabolism in streptozotocin (STZ)-induced diabetic rats was determined by examining urinary excretion, renal reabsorption, reductive regeneration, and biosynthesis of AA at 3 and 14 days after STZ administration. AA concentrations in the plasma, liver, and kidney of the diabetic rats were significantly lower than those of controls on d 3, and decreased further as the diabetic state continued. Hepatic AA regeneration significantly decreased in the diabetic rats on d 3 in spite of increased gene expressions of AA regenerating enzymes and was further reduced on d 14. Hepatic activity of L-gulono-gamma-lactone oxidase, a terminal enzyme of hepatic AA biosynthesis, also decreased significantly on d 3 and decreased further on d 14. Urinary excretion of AA was significantly increased on d 3, with an increase in urine volume but no change in gene expressions of renal AA transporters (SVCT1 and SVCT2). Urinary excretion of AA was normalized on d 14. The results suggest that impaired hepatic and renal regeneration, as well as increased urinary excretion and impaired hepatic biosynthesis of AA, contributed to the decrease in AA in plasma and tissues of STZ-induced diabetic rats.


http://www.sciencedirect.com/science/article/B6T38-4B3JMTS-1/2/daa42d85f1c962b5021bb1c0a112f57

Ionizing radiation-induced adverse biological effects impose serious challenges to astronauts during extended space travel. Of particular concern is the radiation from highly energetic, heavy, charged particles known as HZE particles. The objective of the present study was to characterize HZE particle radiation-induced adverse biological effects and evaluate the effect of selenomethionine (SeM) on the HZE particle radiation-induced adverse biological effects. The results showed that HZE particle radiation can increase oxidative stress, cytotoxicity, and cell transformation in vitro, and decrease the total antioxidant status in irradiated Sprague-Dawley rats. These adverse biological effects were all preventable by treatment with SeM, suggesting that SeM is potentially useful as a countermeasure against space radiation-induced adverse effects. Treatment with SeM was shown to enhance ATR and CHK2 gene expression in cultured human thyroid epithelial cells. As ionizing radiation is known to result in DNA damage and both ATR and CHK2 gene products are involved in DNA damage, it is possible that SeM may prevent HZE particle radiation-induced adverse biological effects by enhancing the DNA repair machinery in irradiated cells.

Because reactive oxygen species have been implicated in the pathogenesis of various hyperproliferative and inflammatory diseases, the mRNA expression of the antioxidant enzyme superoxide dismutase was studied in psoriatic skin tissue. By using reverse transcription-PCR we found similar expression of copper, zinc superoxide dismutase (CuZnSOD) in the involved vs. uninvolved psoriatic skin. In contrast, the level of the manganese superoxide dismutase (MnSOD) mRNA message was consistently higher in lesional psoriatic skin as compared to adjacent uninvolved skin and healthy control skin. Parallel investigation of those cytokines that are thought to be direct or indirect inducers of the MnSOD activity revealed an increased mRNA expression of IL-1[beta], TNF-[alpha], and GM-CSF in lesional psoriatic skin. To study if these cytokines exert a direct effect on dismutase expression in epidermal cells, human keratinocytes in culture were challenged with IL-1[beta], TNF-[alpha], and GM-CSF. It was found that IL-1[beta] and TNF-[alpha], but not GM-CSF, induced the mRNA expression of MnSOD, and an additive effect was demonstrated for the two former cytokines. Further, the expression of both CuZnSOD and MnSOD transcripts was similar in cultured keratinocytes maintained at low differentiation (low Ca2+ medium) and cells forced to terminal differentiation (by high Ca2+ medium). Our results indicate that the abnormal expression of MnSOD mRNA in lesional psoriatic skin is not directly linked to the pathologic state of keratinocyte differentiation in the skin. It seems more likely that the cutaneous overexpression of MnSOD in psoriatic epidermis represents a protective cellular response evoked by cytokines released from inflammatory cells invading the diseased skin.


http://www.sciencedirect.com/science/article/B6T38-4668X49-G/2/8af966e6ad94f360d0b8bd58b9f249a5

Chronic ethanol consumption is associated with increased protein oxidation and decreased proteolysis in the liver. We tested the hypothesis that even single-dose treatment with ethanol or bromotrichloromethane causes increased protein oxidation and a distinct proteolytic response in cultured hepatocytes. HepG2 cells were treated for 30 min with ethanol, H2O2 and bromotrichloromethane at various nontoxic concentrations. Protein degradation was measured in living cells using [35S]-methionine labeling. Protein oxidation, and 20S proteasome activity were measured in cell lysates. Oxidized proteins increased immediately after ethanol, H2O2, and bromotrichloromethane exposure, but a further significant increase 24-h after exposure was observed only following ethanol and bromotrichloromethane treatment. All three reagents caused a significant increase of the overall intracellular proteolysis at rather low concentrations, which could be suppressed by the proteasome inhibitor lactacystin. A decline of proteolysis observed at higher--subtoxic--concentrations was not related to decreased proteasome activity. Preincubation with ketoconazole or 4-methylpyrazole completely prevented the ethanol- and bromotrichloromethane-induced but not the H2O2-induced protein oxidation and proteolysis, suggesting strongly an enzyme-mediated generation of reactive oxygen species. In conclusion single-dose exposure with ethanol or haloalkanes causes increased protein oxidation followed by an increased proteasome-dependent protein degradation in human liver cells.


http://www.sciencedirect.com/science/article/B6T38-4BS08TP-
Scavenger receptors recently have been related to Alzheimer's disease, although it is still unclear whether they contribute to the pathogenesis of the disease or reflect an inflammatory response to the deposition of amyloid [beta]-protein (A[beta]). In this study we demonstrate that CD36, a class B scavenger receptor, is highly expressed in the cerebral cortex of Alzheimer's disease patients and cognitively normal aged subjects with diffuse amyloid plaques compared with age-matched amyloid-free control brains. Moreover, in vitro experiments indicated that A[beta] is able to induce CD36 expression in neuronal cells after 24 h treatment. The interaction between CD36 and A[beta] has been reported to trigger oxidant production by macrophages and microglia. In line with this observation, we found an increased presence of nitrated proteins in brains showing A[beta] loads and CD36 overexpression, independent of the occurrence of Alzheimer's disease pathologic features.


http://www.sciencedirect.com/science/article/B6T38-451MV67-6/2/042b5622b9da28e7f61475899a32770b

The amounts of superoxide and hydrogen peroxide generated by mitochondria under physiological conditions can be enhanced by cellular stress. This study tested the hypothesis that the response to hemin-induced stress, which includes heme oxygenase-1 (HO-1) induction, predisposes to oxidative damage of mitochondrial DNA (mtDNA). Hepatic mitochondria from control, hemin-, and CO-exposed rats were incubated with tert-butyl hydroperoxide (tert-BH) or the NO donor 1,2,3,4-oxatriazolium, 5-amino-3-(3,4-dichlorophenyl)-chloride (GEA 3162). Mitochondrial total and oxidized glutathione (GSH and GSSG), total and free iron, and 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-OHdG) were determined with and without oxidants. As expected, oxidation by tert-BH induced significant GSH depletion and increased amounts of free iron and 8-OHdG. Oxidant exposure rapidly produced a large mtDNA deletion involving the coding regions for cytochrome c oxidase (COX 1) and NADH dehydrogenase (ND1 and ND2). Hemin and CO greatly exacerbated susceptibility to the deletion of mtDNA by tert-BH, and this was attenuated by preincubation with GSH methyl ester. Analysis of mitochondria-associated proteins Bax and Bcl-xl in hemin- and CO-exposed rats showed significant responses, revealing interactions with apoptotic pathways. Thus, hemin-induced mitochondrial events sensitize a specific region of the mitochondrial genome to deletion, which is related to depletion of GSH and is not explained by effects of CO. This mtDNA damage is associated with altered expression of mitochondrial cell death proteins, thereby suggesting a novel mechanism for systemic or environmental pro-oxidants to influence apoptosis.


http://www.sciencedirect.com/science/article/B6T38-4FM9KJW-1/2/3c9846725a287ca0b39af7c786d085c4

Vitamin E is the primary lipophilic antioxidant in mammals. Lack of vitamin E may lead to an increase of cytotoxic phospholipid-peroxidation products (PL-Ox). We could previously show that alimentary vitamin E-depletion in rats did not change the concentrations of dienes, hydroperoxides, and platelet-activating factor-related oxidation products in alveolar type II cells (TII cells). We hypothesized that vitamin E deficiency increases the activity of enzymes involved in the degradation of PL-Ox. Degradation of PL-Ox may be catalyzed by phospholipase A2, PAF-acetylhydrolase, or peroxiredoxins (Prx's). Alimentary vitamin E deficiency in rats increased the
expression of Prx-1 at the mRNA and protein levels and the formation of Prx-SO₃, but it did not change the expression of Prx-6 or the activity of phospholipase A2 and PAF-acetylhydrolase in TII cells. H₂O₂-induced oxidative stress in isolated TII cells activated protein kinase C[alpha] (PKC[alpha]) and increased the expression of Prx-1 and Prx-6. Inhibition of PKC[alpha] in isolated TII cells by long-time incubation with PMA inhibited PKC[alpha] and Prx-1 but not Prx-6. We concluded that the expression of Prx-1 and -6 is selectively regulated in TII cells; PKC[alpha] regulates the expression of Prx-1 but not Prx-6. Prx-6 expression may be closely linked to lipid peroxidation.

Fundamental and Molecular Mechanisms of Mutagenesis (60)


http://www.sciencedirect.com/science/article/B6T2C-47PCN7M-41/2/3157a48d39359d02b082784374179db8

Bleomycin-induced 6-thioguanine-resistant mutants pretreated with or without TRIEN (triethylene-tetramine), a superoxide dismutase (SOD) inhibitor, or TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), an SOD mimic, were analyzed by polymerase chain reaction (PCR)-based deletion screening in a Chinese hamster ovary (CHO) clone K1-BH4 and its derivative AS52 cells. As we proposed earlier, TRIEN would decrease and TEMPOL would increase the intracellular level of hydroxyl radical leading to a higher and lower recovery of deletion mutants. We found that the proportion of the deletion mutants induced by bleomycin at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus in K1-BH4 cells was 45.5%. The proportion of deletion HPRT- mutants induced by bleomycin pretreated with TRIEN was 31.0% and with TEMPOL was 50.0%. The proportion of deletion mutants induced by bleomycin on the xanthine-guanine phosphoribosyltransferase (gpt) gene in AS52 cells was 61.0%. The proportion of deletion GPT- mutants induced by bleomycin pretreated with TRIEN was 56.8% and with TEMPOL was 61.4%. The trend of the change of the proportion of bleomycin-induced deletion mutants as affected by TRIEN and by TEMPOL provides molecular evidence for the involvement of reactive oxygen species (ROS) in bleomycin mutagenesis in mammalian cells, in which deletion is a major type of induced mutation.


http://www.sciencedirect.com/science/article/B6T2C-3SY8DSV-1/2/dceccc39de58bdd5e7786012ed9cb14

A survey of glycoporphin A (gpa) in vivo somatic cell mutation in a population of 394 healthy people from 8 to 77 years of age (mean age +/- SD 41 +/- 15 years) revealed a subset of 37 individuals with stably elevated allele-loss and/or allele-loss with duplication variant erythrocyte
frequencies (Vf) exceeding \(30 \times 10^{-6}\). These 37 individuals with gpa outlier Vf are significantly older (hpprt mutant frequencies (Mf) in the peripheral blood T-lymphocytes of 27 of these individuals, together with 15 matched control individuals with unremarkable gpa Vf, was undertaken to determine if these subjects also displayed elevated mutation frequencies at this independent locus indicative of globally elevated somatic mutation. The hprt Mf in these 27 subjects (geometric mean \(11.5 \times 10^{-6}\) (dispersion interval \(5.8 \times 10^{-6}\) to \(22.8 \times 10^{-6}\)) was not significantly different from that observed in the 15 controls (geometric mean \(12.1 \times 10^{-6}\) (dispersion interval \(5.7 \times 10^{-6}\) to \(25.5 \times 10^{-6}\)). These Mf are higher than typically reported values reflecting the older age distribution of these individuals (arithmetic mean age+/-SD 53+/-12 and 50+/-16 years for the subjects and controls, respectively). Taken together, these data suggest that several genetic mechanisms may be responsible for producing the gpa outlier Vf observed in these subjects. The observation that hprt Mf were not increased indicates that the majority did not arise by a genome-wide increased rate of somatic mutation detectable at both loci. The fixation and subsequent expansion of 'jackpot' mutations at the gpa locus occurring early in embryonic/fetal development also does not appear to be a predominant mechanism. Some cases may result from a stable over-representation of gpa variant cells, perhaps associated with a marked age-dependent decrease in the number of contributing erythroid stem cells in the bone marrow. The subset that displays elevated allele-loss with duplication Vf involving both gpa alleles may represent individuals with increased rates of somatic recombination. Elevations arising by this mechanism are not detected in the hprt assay, but could be confirmed using an autosomal locus in vivo somatic cell mutation endpoint such as the hla-a assay. Of primary biological significance, these results demonstrate that genetic/stochastic processes leading to the loss of heterozygosity of somatic cells occur ubiquitously in humans and in some individuals this level of somatic mosaicism can approach a frequency of 10^-3 at the gpa locus in erythroid lineage cells.


http://www.sciencedirect.com/science/article/B6T2C-40PRW3G-F/2/cad6907bf4b24fae3fb31320e5a520c7

Chromosomal aberrations in human lymphocytes were analyzed by fluorescence in situ hybridization (FISH) in the first 3 postirradiation (0 and 2 Gy) divisions. Cells were grown in the presence of BrdU, collected at different sampling times (47, 70 and 91 h) and analyzed using an alphoid centromeric probe and PCR amplified DNA libraries for chromosomes 2 and 8. Following differential staining of sister chromatids, the analyzed cells were identified to be either in the first, second or third mitosis after irradiation. The frequencies of both dicentrics and fragments showed a reduction of about 50% after each cell generation, whereas translocations were more persistent. Cells within the same postirradiation division showed higher aberration frequencies when derived from later sampling times, indicating a delay in progression of aberrant cells. As a result, the frequencies for dicentrics and fragments remained rather constant at different sampling times if the cell cycle parameter was not taken into account. Thus, the average generation time of the lymphocytes had a clear effect on the obtained aberration frequencies. The described method allows the study of the persistence of chromosome damage using the FISH technique during 3 subsequent cell divisions in vitro.

Folic acid deficiency acts synergistically with alkylating agents to increase DNA strand breaks and mutant frequency at the hprt locus in Chinese hamster ovary (CHO) cells. To elucidate the mechanism of this synergy, molecular analyses of hprt mutants were performed. Recently, our laboratory showed that folate deficiency increased the percentage of clones with intragenic deletions after exposure to ethyl methanesulfonate (EMS) but not N-nitroso-N-ethylurea (ENU) compared to clones recovered from folate replete medium. This report describes molecular analyses of the 37 hprt mutant clones obtained that did not contain deletions. Folate deficient cells treated with EMS had a high frequency of G>A transitions at non-CpG sites on the non-transcribed strand, particularly when these bases were flanked on both sides by G:C base pairs. Thirty-three percent of these mutations were in the run of six G’s in exon 3. EMS-treated folate replete cells had a slightly (but not significantly) lower percentage of G>A transitions, and the same sequence specificity. Treatment of folate deficient CHO cells with ENU resulted in predominantly T>A transversions and C>T transitions relative to the non-transcribed strand. These findings suggest a model to explain the synergy between folate deficiency and alkylating agents: (1) folate deficiency causes extensive uracil incorporation into DNA; (2) greatly increased utilization of base excision repair to remove uracil and to correct alkylator damage leads to error-prone DNA repair. In the case of EMS, this results in more intragenic deletions and G:C to A:T mutations due to impaired ligation of single-strand breaks generated during base excision repair and a decreased capacity to remove O6-ethylguanine. In the case of ENU additional T>A transversions and C>T transitions are seen, perhaps due to mis-pairing of O2-ethylpyrimidines. Correction of folate deficiency may reduce the frequency of these types of genetic damage during alkylator therapy.


Folic acid deficiency acts synergistically with alkylating agents to increase genetic damage at the HPRT locus in Chinese hamster ovary cells in vitro and in rat splenocytes in vivo. The present studies extend these observations to human cells and, in addition, investigate the role of p53 activity on mutation induction. The human lymphoblastoid cell lines TK6 and WTK1 are derived from the same parental cell line (WI-L2), but WTK1 expresses mutant p53. Treatment of folate-replete or deficient WTK1 and TK6 cells with increasing concentrations (0-50 [μg/ml] of ethyl methanesulfonate (EMS) resulted in significantly different HPRT mutation dose-response relationships (PG>A transition on the non-transcribed strand. These transitions were mainly at non-CpG sites, particularly when these bases were flanked 3’ by a purine or on both sides by G:C base pairs. A smaller number of G>A transitions occurred on the transcribed strand (C>T=14%), resulting in 79% total G:C>A:T transitions. There were more genomic deletions in folate-deficient (15%) as compared to replete cells (4%) of both cell types. Mutations that altered RNA splicing were common in both cell types and under both folate conditions, representing 33% of the total mutations. These studies indicate that cells expressing p53 activity exhibit a higher rate of mutation induction but are more sensitive to the toxic effects of alkylating agents than those lacking p53 activity. Folate deficiency tends to reduce toxicity but increase mutation induction after EMS treatment. The p53 gene product did not have a major influence on the molecular spectrum after treatment with EMS, while folate deficiency increased the frequency of deletions in both cell types.

http://www.sciencedirect.com/science/article/B6T2C-47PCN50-2R/2/557d85d561bc6f79e3c1ce737a92e2bb

Spontaneous null mutations represent low frequency events that irreversibly and completely inactivate a gene, and can often consist of major gene alterations. To study the molecular mechanisms leading to recessive spontaneous null mutations in the human genome, we designed and tested a selection procedure in cell culture to enrich for this rare class of spontaneous mutations. The KT cell line contains the herpes simplex virus type 1 (HSV-1)_thymidine kinase (tk) gene and the neomycin-resistance gene (neo), from plasmid pSV2neoKT, integrated as a single-copy in the human tk- cell line 143B. The HSV-1 tk gene was the target for spontaneous gene inactivation, and antiviral drugs (acyclovir, trifluorothymidine and ganciclovir) were used, in combination, to provide a selective enrichment for null mutations over the background of more frequent and revertible point mutations. The tk- mutations obtained with this multiple drug selection assay appeared at a very low frequency, rarely reverted to wild-type (tk+), and the TK protein was observed only in 4.8% of these null mutants. Deletions of the entire tk gene, or its 3' region, constituted the major class of DNA rearrangements seen in the null mutations. Additionally, one of the null mutants contained an intragenic 106-bp duplication within a 43-bp deleted region of the tk gene. We propose this mutation to be the outcome of an intragenic gene conversion event which may have been facilitated by short regions of junctional homology.


http://www.sciencedirect.com/science/article/B6T2C-42YW37B-5/2/1486006039ca23f1f5b58112cd3868a3

Mutations in the HPRT gene cause a spectrum of diseases that ranges from hyperuricemia alone to hyperuricemia with profound neurological and behavioral dysfunction. The extreme phenotype is termed Lesch-Nyhan syndrome. In 271 cases in which the germinal HPRT mutation has been characterized, 218 different mutations have been found. Of these, 34 (13%) are large- (macro-) deletions of one exon or greater and four (2%) are partial gene duplications. The deletion breakpoint junctions have been defined for only three of the 34 macro-deletions. The molecular basis of two of the four duplications has been defined. We report here the breakpoint junctions for three new deletion mutations, encompassing exons 4-8 (20 033 bp), exons 4 and 5 (13 307 bp) and exons 5 and 6 (9454 bp), respectively. The deletion breakpoints were defined by a combination of long polymerase chain reaction (PCR) amplifications, and conventional PCR and DNA sequencing. All three deletions are the result of non-homologous recombinations. A fourth mutation, a duplication of exons 2 and 3, is the result of an Alu-mediated homologous recombination between identical 19 bp sequences in introns 3 and 1. In toto, two of three germinal HPRT duplication mutations appear to have been caused by Alu-mediated homologous recombination, while only one of six deletion mutations appears to have resulted from this type of recombination mechanism. The other five deletion mutations resulted from non-homologous recombination. With this admittedly limited number of characterized macro-mutations, Alu-mediated unequal homologous recombinations account for at least 8% (3 of 38) of the macro-alterations and 1% (3 of 271) of the total HPRT germinal mutations.

http://www.sciencedirect.com/science/article/B6T2C-47PCNK6-8F/2/c0a5dc38c987023d4c455a109a7fcec9c

In the present study we have introduced 19 activating base pair substitutions into N-ras cDNA by use of an in vitro site-directed mutagenesis system. Six mutants were constructed for N-ras codon 12 (exon 1), six for codon 13 (exon 1), and seven for codon 61 (exon 2). Fifteen out of 19 PCR-amplified mutation sequences showed a clear separation from the wild type of denaturing gradient gel electrophoresis runs as homoduplex band, and the rest could be separated after heteroduplex formation with wild-type DNA. These constructs can be used as controls in many screening systems for analyzing activating point mutations of the N-ras gene.


http://www.sciencedirect.com/science/article/B6T2C-41P18S3-2/2/f724e51e501aa29c3bd92e563fe9e9eb

Initiation of skin tumors in mice is associated with the formation of oncogenic mutations in the H-ras gene. Mice treated on the dorsal skin with the potent polycyclic aromatic hydrocarbon (PAH) carcinogen dibenzo[a,l]pyrene (DB[a,l]P) form papillomas carrying the H-ras codon 61 (CAA to CTA) mutations. These mutations are induced in early preneoplastic skin within 1 day after DB[a,l]P treatment (Oncogene 16 (1998) 3203-3210) and appear to be related to DB[a,l]P-Ade-depurinating adducts (Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 10422-10426). The rapid kinetics of mutation induction suggests that abasic sites generated from base depurination may undergo error-prone excision repair in pre-S-phase cells to induce these mutations. Analysis of mutations in the H-ras exon 1 and 2 region in DB[a,l]P-treated early preneoplastic skin indicated great changes in mutation spectra in the preneoplastic period. The initial spectra contained abundant A->G mutations, which frequently occurred 3' to a putative conserved sequence (TGN-doublet). These mutations appeared to be induced initially as mismatched (G.T) heteroduplexes and then converted into double-stranded mutations by one round of replication. Unlike the A->G mutations found in DB[a,l]P-treated skin (which forms 99% depurinating adducts), A->G mutations found in anti-DB[a,l]P-diol epoxide-treated skin (forms 97% stable adducts) did not appear to be G.T heteroduplexes. These results, therefore, suggest that under these conditions, the repair errors occurred only from abasic sites but not from stable adducts. Initiated cells carrying specific oncogenic mutations, formed presumably by misrepair, underwent rapid clonal expansion and regression (transient clonoplasia). The multiplication of initiated stem cells during transient clonoplasia may be a factor determining the tumor-initiating potential of some PAH carcinogens.

The dose rate at which cells are exposed to X-rays may influence the nature of induced mutations. To investigate this, the molecular spectra were determined at the HPRT gene in a hamster (V79) and a human (WI-L2-NS) cell line after the same total dose of X-rays has been administered at either a low dose rate (LDR; 3.33 mGy/min) or a high dose rate (HDR; 1.24-1.4 Gy/min) X-irradiation. Mutational spectra appeared similar, the fraction of mutants carrying deletions ranging between 59%-66% for the V79 strain and between 64%-75% for the WI-L2-NS strain, and independent of the irradiation conditions. The data indicate no effect of ongoing repair processes under LDR conditions on the kind of induced mutations in mammalian cells.


Genotoxic effects linking cigarette smoking with lung cancer have not been consistently demonstrated, therefore claims for the cause-effect relationships are vigorously contested. Using matched populations of 22 lung cancer patients who have been cigarette smokers (LCP), 22 non-cancerous cigarette smokers (SC) and 13 non-smokers (NSC), we have applied the fluorescence in situ hybridization (FISH) tandem probe assay to elucidate the frequency of chromosome breakage among the participants. Two probes were used, a classical satellite probe which hybridizes to the large heterochromatin region of chromosome 1, and an [alpha]-satellite probe which targets a small region adjacent to the heterochromatin probe. The highest frequency of structural aberrations was observed in LCP (1.4+/−0.1) followed by SC (1.25+/−0.1) and NSC (0.4+/−0.1). Aberration frequencies were not significantly different between LCP and SC (p>0.05), however, a statistically significant difference was detected between the smoker populations combined (LCP and SC) and the NSC (pr=0.5; p0.05). In addition, the aberration frequencies were influenced by the inheritance of polymorphic glutathione S-transferase (GST) genes. LCPs missing one or the other GST (GSTM1 or GSTT1) genes were found to have significantly higher chromosome breaks compared to LCPs with both genes present (p<0.05). Our data indicate that genetic predisposition and chromosome aberrations may be mechanistically related to the initiation of lung carcinogenesis; therefore, they may be useful biomarkers for lung cancer among cigarette smokers.


For the analysis of mutation in human T-cell lymphocytes, it is crucial to determine the clonal relationship between isolated mutants, particularly if they harbour identical mutations. Here we report an efficient method to determine the clonal relationships between these cells. The method is based on the analysis of restriction fragment length polymorphisms of a polymerase chain reaction amplified, rearranged T-cell receptor [gamma]-gene. As few as 600 cells are sufficient, regular agarose gels can be used for the separation of the restriction fragments, no radioactive
label is required, and results can be obtained in 2 days.


http://www.sciencedirect.com/science/article/B6T2C-47PCNHS-7H/2/5099fc5b1fcd2a2c543ea452d628c8fb

An experimental system has been developed to study in vivo autosomal mutations in murine splenic lymphocytes. Mutant lymphocytes were isolated by immunocytotoxicity using monoclonal antibodies directed against the k and d alleles of the K and D H-2 histocompatibility loci and were enumerated using limiting-dilution cloning. Genomic allele loss in mutant clones was detected using allele-specific primers in a polymerase chain reaction. Mutant clones were classified on the basis of phenotypic and genotypic criteria into "no change", deletion or recombination mutants.

The geometric mean mutation frequency in 102 mice was 2.42 x 10^{-4}. Detailed phenotypic and genotypic study of 87 mutant clones from 4 mice revealed "no change" mutants in 83%, mutants due to deletion in 7% and mutants due to recombination in 7%. Anomalous results were obtained in 3% of mutant clones. The development of an animal model for study of in vivo mutations at an autosomal locus will further advance study of mutations, particularly those involving chromosomal changes such as mitotic recombination.


http://www.sciencedirect.com/science/article/B6T2C-3W9D0FD-4/2/1534373a823c488dbeec1e5f85d64788

Aflatoxin B1 (AFB1) is a mutagenic and carcinogenic mycotoxin which may play a role in the etiology of human liver cancer. In vitro studies have shown that AFB1 adducts form primarily at the N7 position of guanine. Using quantitative PCR (QPCR) and ligation-mediated PCR (LMPCR), we have mapped total AFB1 adducts in genomic DNA treated with AFB1-8,9-epoxide and in hepatocytes exposed to AFB1 activated by rat liver microsomes or human liver and enterocyte microsomal preparations. The p53 gene-specific adduct frequencies in DNA, modified in cells with 40-400 [mu]M AFB1, were 0.07-0.74 adducts per kilobase (kb). In vitro modification with 0.1-4 ng AFB1-8,9-epoxide per microgram DNA produced 0.03-0.58 lesions per kb. The adduct patterns obtained with the epoxide and the different microsomal systems were virtually identical indicating that adducts form with a similar sequence-specificity in vitro and in vivo. The lesions were detected exclusively at guanines with a preference towards GpG and methylated CpG sequences. The methods utilizing QPCR and LMPCR thus provide means to assess gene-specific and sequence-specific AFB1 damage. The results also prove that microsomally-mediated damage is a suitable method for avoiding manipulations with very unstable DNA-reactive metabolites and that this damage can be detected by QPCR and LMPCR.


http://www.sciencedirect.com/science/article/B6T2C-4CTTR00-
Effluents from pulp and paper mills that historically have used elemental chlorine in the bleaching process have been implicated in inhibiting reproduction in fish. Compounds with estrogenic and androgenic binding affinities have been found in these effluents, suggesting that the impairment of reproduction is through an endocrine-related mode of action. To date, a great deal of attention has been paid to phytoestrogens and resin acids that are present in mill process streams as a result of pulping trees. Estrogen and estrogen mimics interact directly with the estrogen receptor and have near immediate effects on gene transcription by turning on the expression of a unique set of genes. Using differential display (DD) RT-PCR, we examined changes in gene expression induced by exposure to paper mill effluents. Largemouth bass were exposed to 0, 10, 20, 40, and 80% paper mill effluent concentrations in large flow-through tanks for varied periods of time including 7, 28 or 56 days. Plasma hormone levels in males and females and plasma vitellogenin (Vtg) in females decreased with dose and time. Measurements of changes in gene expression using DD RT-PCR suggest that the gene expression patterns of male fish do not change much with exposure, except for the induction of a few genes including CYP 1A, a protein that is induced through the action of the Ah receptor in response to dioxin and similar polyaromatic hydrocarbons. However, in the case of females, exposure to these effluents resulted in an up-regulation of CYP 1A that was accompanied by a generalized down-regulation of genes normally expressed during the reproductive season. These antiestrogenic changes are in agreement with previous studies in bass exposed to these effluents, and could result in decreased reproductive success in affected populations.


http://www.sciencedirect.com/science/article/B6T2C-3YYMKKC-15/2/301ea52a5cae420814cc5afe84e64d0c

Sequence analysis of the tyrosinase (TYR) coding region from one albino rhesus monkey (Macaca mulatta) family revealed that the two monkeys with phenotype similar to human TYR-negative oculocutaneous albinism (OCA) were homozygous for a missense mutation (S184TER) in exon 1 at codon 184. The offspring of one of the albino monkey ("Kangkang") are all heterozygous for the S184TER mutation, but the S184TER mutation was not observed in 93 control individuals. We conclude that the point mutation is responsible and sufficient to generate the albino rhesus monkey phenotype. The rough age of the S184TER nonsense mutation may be about 0.8 million years using a rate of 0.16% per million years.


http://www.sciencedirect.com/science/article/B6T2C-3SY9PXBM/2/46098cfd7bc8353bb62157461a93c55f

Tissues from nine species of plants and fungi were treated separately with eight solutions, including seven cytological fixatives (3.7% formaldehyde at pH 3.0 and 7.0, FAA at pH 3.0 and 7.0, 1% glutaraldehyde at pH 3.0 and 7.0, and Lavdowsky's fluid at pH 3.0) and one storage buffer (SED=NaCl-EDTA-DMSO, pH 7.0). DNA from untreated tissue and SED-treated tissue was of high molecular weight (>50 kb). DNA from glutaraldehyde-treated tissues averaged 20 kb in length, while DNA from all other treatments averaged less than 8 kb in length. Each DNA was subjected to amplification using the polymerase chain reaction, followed by sequencing of 250 bp
near the 3' end of the nuclear rRNA small subunit gene. Glutaraldehyde treatments (at pH 3.0 and 7.0) produced damaged bases at rates of 0.0% to less than 0.1%. Treatments with Lavdowsky's fluid (containing mercuric chloride), FAA at pH 7.0, and SED produced rates of 0.0% to 3.6%. FAA at pH 3.0 produced rates of 7.6% to 15.6%. Nearly 100 attempts to amplify from specimens treated with 3.7% formaldehyde (at pH 3.0 and 7.0) failed, indicating extreme damage to the DNA.


http://www.sciencedirect.com/science/article/B6T2C-3YKM47Y-26/2/20df78dd8d0e0fc228287446c06bed3e

Human pancreatic malignancies originating from duct cells most frequently demonstrate activation of Ki-ras gene by G-to-A transition at codons 12 and 13. Rat pancreatic exocrine tumors more frequently and almost exclusively derive from acinar cells and thus differ morphologically from human pancreatic neoplasms. Male Wistar rats fed with 2% gabapentin (1-aminomethyl)cyclohexane acetic acid) in diet for 2 years developed pancreatic exocrine adenomas and adenocarcinomas. To study the mutations in Ki-ras gene, rat pancreatic proliferative lesions induced by gabapentin were retrospectively analyzed by PCR amplification of DNA isolated from paraffin sections of formalin-fixed rat pancreatic adenomas and adenocarcinomas. The amplified 110-bp fragments of exon 1 and exon 2 were analyzed for mutations at codon 12/13 and 61. The results showed Ki-ras mutations at codon 12 in human pancreatic carcinomas. Novel mutations GGT-to-TGT and GGT-to-CGT were detected at codon 12 in 1/5 and 2/5 human pancreatic tumors. Rat adenomas or carcinomas induced by gabapentin expressed wild type sequences at codons 12, 13 and 61. These findings were confirmed by allele-specific oligonucleotide hybridization, single-strand confirmation polymorphism of exon 1 and direct sequencing of exon 1 and exon 2. The absence of mutations in these rat pancreatic tumors suggests that these tumors do not correspond to the human tumors, and that the pathogenesis of this rodent tumor formation may follow different molecular mechanisms.


http://www.sciencedirect.com/science/article/B6T2C-4864J8P-3R/2/c0f18dc78dd6a730ec3d07965a4c8913

Molecular alterations were examined in the hypoxanthine guanine phosphoribosyltransferase (hprt) gene of 41 independent X-ray-induced thioguanine-resistant (TGR) Chinese hamster ovary (CHO) cell clones. Rapid screening of the clones by multiplex polymerase chain reaction (PCR) revealed that the presence or absence of exons revealed that the causes of the mutant phenotype were total gene deletion (26/41), partial gene deletion (4/41), and an insertion (1/41). No alterations of exon number or sizes were apparent in 10 of the mutants. Southern blot analysis confirmed the deletion data and revealed an additional class of mutants that had a gene disruption but retained all hprt exons (2/41). Therefore, at least 80% of the ionizing radiation-induced mutations were due to mechanisms involving DNA breakage are rejoining. The distribution of deletion sizes that two DNA breaks required for a deletion are not independent events. A possible mechanism is presented. In addition, the DNA sequence of the insertion mutation was determined. The insertion (229 bp) is coupled with a deletion (31 bp). An imperfect inverted repeat with flanking hprt DNA
was identified and may be involved in the insertion event.


http://www.sciencedirect.com/science/article/B6T2C-4CNGT3D-2/2/4b2ae19decc7430beb1affcd3f44cf98

The p16-cyclin D-Cdk4(6)-pRB-E2F and p73 pathways are involved in the control of cell-cycle progression, and genetic lesions in both pathways frequently occur in breast carcinomas and other human cancers. The p16INK4a gene is involved in regulation of the G1/S transition, and when overexpressed, the p73 gene activates transcription of p53-responsive genes and promotes apoptosis. These pathways are related, for instance, p73 is also downstream of E2F-1, since E2F-1 induces p73-mediated apoptosis in the absence of p53. We studied 93 breast cancer patients to identify alterations in the expression of p16INK4a and p73 by semiquantitative RT-PCR analysis and possible interactions between them and correlations with clinicopathological parameters. p73 was overexpressed in 24 cases. Overexpression of p16INK4a was detected in 17 cases and underexpression in 32 cases. A significant correlation was observed between the overexpression of both genes (P = 0.05). Concurrent overexpression of p73 and p16INK4a was significantly correlated with metastases in three or more lymph nodes (P = 0.0007), positive immunohistochemistry for p53 (P = 0.014), vascular invasion (P = 0.048) and negative progesterone receptors (P = 0.004). These results indicate that concomitant overexpression of p16INK4a and p73 may be involved in breast cancer and associated with poor tumor characteristics.


http://www.sciencedirect.com/science/article/B6T2C-3WF7M86-2/2/d8615633f131d8db01097f8fd7923a88

The two distinct mucAB and samAB operons originally isolated from the plasmids of Salmonella typhimurium encode proteins engaged in induced mutagenesis. They represent two extreme cases among the so far characterized members of the enterobacterial umuDC family in respect to both the strength and the specificity of their effect. It is suggested that the MucA and SamA proteins are post-translationally processed to MucA' and SamA', respectively, which lack the N-terminal 25 amino acids and are the active species in mutagenesis. For the purpose of characterizing the individual activities of these proteins, we developed a new system for their SOS-independent separate and controllable expression in enterobacteria. Besides the matured forms of MucA', SamA' as well as MucB and SamB proteins we also expressed hybrid HisTag-MucA' and HisTag-SamA' proteins in which a synthetic 24 amino acid HisTag region replaces the natural 25 amino acid N-terminal leader present in the MucA and SamA precursors. In this study, we analyzed the effect of the mutagenesis proteins on the UV mutability of S. typhimurium YG5144. None of the proteins, if expressed alone, promoted UV mutagenesis. Different combinations of the proteins promoted mutagenesis to different extents in the order MucA' > MucB > SamA' + SamB > HisTag-MucA' + MucB > SamA' + MucB > MucA' + SamB > HisTag-SamA' + SamB. The mutagenesis enhancing potential of the combinations with MucB protein decreased as the expression of the proteins increased while the mutagenesis enhancing potential of the combinations with SamB protein increased together with the increase in the expression.
The artificially expressed MucA’ + MucB proteins were as active as their MucAB counterparts expressed from the plasmid pKM101 in promoting UV mutagenesis, but they were remarkably more efficient than their pKM101-born counterparts in promoting spontaneous mutagenesis. We conclude that the MucA’B and SamA’B proteins are partly interchangeable and the functionality of the resulting A’ + B complex is largely dependent on the appropriate B-protein.


http://www.sciencedirect.com/science/article/B6T2C-3W0FDH0-3/2/1024eb01232b72a4588fa063626eae4b

Two immortal fibroblastic cell strains (substrains) were established by culturing healthy skin cells obtained from a high-dose atomic bomb survivor (female, age 76 years, 5.14 Gy) for more than 4 years. Designated FM-U and FM-M, the two substrains share the same marker chromosome, t(5q-;6p+), but are karyotypically different, possessing hypodiploid chromosome numbers (39-43) in the former and hypertriploid (69-76) in the latter. Thus far, the two strains have passed through 117 and 156 subcultures or more than 230 and 310 cumulative population doublings, respectively, each passage requiring 4-6 days in the former and 3-4 days in the latter. In the process of immortalization, sequential rearrangement among various chromosomes presumably due to telomeric and interstitial telomeric fusions took place following the telomere shortening, particularly in the senescence and post-senescent phase cells. Of particular interest is the fact that loss of heterozygosity (LOH) of the p53 gene was demonstrated in these immortalized cell populations. In addition, the allelic patterns of the LOH of p53 differed. Further evidence indicative of infinite proliferation was demonstrated in both strains, such as the telomere elongation and the significantly low frequency of cells possessing dicentric chromosomes.


http://www.sciencedirect.com/science/article/B6T2C-4378X0V-F/2/9742cd25f3c74cbd4321540005f1c061

In this report we describe a simple and rapid protocol for reliable quantitation of mitochondrial DNA (mtDNA) mutations, which is basically a modification of the traditional polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) analysis technique. Up to now, the PCR/RFLP method has been of limited use for the accurate determination of ratios of mutant and wild type molecules, largely owing to the formation of heteroduplex molecules by PCR and incompleteness of restriction digestion. In order to overcome this problem, we have introduced a single-step primer extension reaction using VentR(R)(exo-) DNA polymerase and a fluorescence-labeled primer to the standard assay. The labeled homoduplex molecules are then digested with a restriction endonuclease, and the nucleic acids fractionated on an automated DNA sequencer equipped with GENESCAN(TM) analysis software. The amount of mutant mtDNA is readily estimated from fluorescence intensities of the wild-type and mutant mtDNA fragments corrected for incomplete digestion as monitored by a homologous control fragment. The accuracy of the improved protocol was determined by constructing standard curves obtained from defined mixtures of genomic DNA containing homoplasmic wild-type and mutant mtDNA. The expected values were obtained, with an observed correlation coefficient of 0.997 and a typical variability of +/-5% between repeated measurements. Further validation of the protocol is provided by the screening of five patients and unaffected subjects carrying the guanine to adenine transition at
the nucleotide 3460 of the mitochondrial genome responsible for the mitochondrial disorder of Leber's hereditary optic neuropathy.


http://www.sciencedirect.com/science/article/B6T2C-4864J4D-2D/2/608dd291595aba97e09f1f5a7494fb27

In this paper, the cloning and nucleotide sequence of the cDNA of the rat gene coding for hypoxanthine-guanine phosphoribosyltransferase (hprt) is reported. Knowledge of the cDNA sequence is needed, among other reasons, for the molecular analysis of hprt mutations occurring in rat cells, such as skin fibroblasts isolated according to the granuloma pouch assay. The rat hprt cDNA was synthesized and used as a template for in vitro amplification by PCR. For this purpose, oligonucleotide primers were used, the nucleotide sequences of which were based on mouse and hamster hprt cDNA sequences. Sequence analysis of 1146 bp of the amplified rat hprt cDNA showed a single open reading frame of 654 bp, encoding a protein of 218 amino acids. In the predicted rat hprt amino acid sequence, the proposed functional domains for S'-phosphoribosyl-1-pyrophosphate (PRPP) and nuceotide binding in phosphoribosylating enzymes as well as a region near the carboxyl terminal part were highly conserved when compared with amino acid sequences of other mammalian hprt proteins. Analysis of hprt amino acid sequences of 727 independent hprt mutants from human, mouse, hamster and rat cells bearing single amino acid substitutions revealed that a large variety of amino acid changes were located in these highly conserved regions, suggesting that all 3 domains are important for proper catalytic activity. The suitability of the hprt gene as target for mutational analysis is demonstrated by the fact that amino acid changes in at least 151 of the 218 amino acid residues of the hprt protein result in a 6-thioguanine-resistant phenotype.


http://www.sciencedirect.com/science/article/B6T2C-44J3V88-5/2/732aec0fd7730e34ad7c3ab27703d9fb

In our previous studies, we have shown the mutagenicity of bleomycin (BLM) at the nuclear hprt locus. In the present study we have analyzed mutagenic effects of BLM in mitochondrial DNA (mtDNA) using short extension-PCR (SE-PCR) method for detection of low-copy deletions. Fisher 344 rats were treated with a single dose of BLM and total DNA preparations from splenic lymphocytes were processed in SE-PCR assay. Spontaneous deletions were typically flanked by direct repeats (78.5%), while the in BLM-treated group, direct repeats were found in only 46.6% of breakpoints. The ratio between deletions based on direct repeats and random sequence deletions changed from 3.67 in control group to 0.87 in BLM-treated animals, which corresponds to an approximate 1.7-fold increase in the deletion mutation frequency. Furthermore, 62.5% of deletions not flanked by direct repeats in the treated group contained cleavage sites for BLM. The localization of breakpoints was not entirely random. We have found four clusters containing deletions from both groups indicative of deletion hot spots. The results indicate that BLM exposure may be associated with the induction of mtDNA mutations, and suggest the utility of SE-PCR method for evaluating drug-induced genotoxicity.

CHO cells were exposed to 11 different restriction endonucleases by electroporation and their mutagenicity was measured. Nine of them have one or more recognition sites within exons of the HPRT gene, whereas the remaining two cut in introns only. The mutagenic efficiency of the various enzymes varied markedly; mutagenicity of Sau3AI was considerably higher than that of the other enzymes. Neither cytotoxicity nor mutagenicity could be related to the number or location of recognition sites within the cDNA. A total of 417 independent restriction enzyme induced mutant clones were isolated from 20 separate experiments for molecular analysis; all nine exons of the HPRT gene were analyzed by a modified multiplex deletion screening method with polymerase chain reaction (PCR) amplification. Among spontaneously arising mutants, 70.8% showed no change in PCR pattern, indicating a small scale change (point mutation), whereas partial deletions were observed in 24.7%, and total deletions in 4.5% of mutant clones. In contrast, approximately 70% of restriction enzyme induced mutants showed partial or total deletions. There was no obvious relationship between type of break (blunt versus staggered ends), and the DNA structure of the mutations induced. For partial deletions, the distribution of breakpoints within introns appeared to occur at random, and did not correlate with the mutagenicity of a given enzyme. Thus, though DNA double-strand breaks appear to be important mutagenic lesions that can induce a high frequency of deletion mutants, no specific relationship of mutagenic potential to the type of breaks, their sites within the HPRT gene or the molecular structure of the mutations induced could be identified.


Several gpt+ transgenic cell lines were derived from hprr- V79 cells to study mutagenesis mechanisms in mammalian cells. The G12 cell line was previously shown to be hypermutable by X-rays and UV at the gpt locus compared to the endogenous hprr gene of the parental V79 cells (Klein and Rossman, 1990), and is now shown to be highly mutable by the clastogenic anti-tumor agent bleomycin sulfate. A second trasgenic cell line G10, which has a different gpt insertion site, was studied in comparison with G12. Both G12 and G10 cell lines carry the stable gpt locus at a single integration site in the Chinese hamster genome, and neither spontaneously deletes the integrated gpt sequence at a high frequency. Although spontaneous mutation to 6-thioguanine resistance in G10 cells is 3-4 times higher than in G12 cells, the cell lines differ to a much greater extent when mutated by clastogens. In comparison to G12 cells, the gpt locus in G10 cells is up to 13 times more sensitive to bleomycin mutagenesis and 5 times more responsive to X-ray mutagenesis. In contrast, there is much less difference in UV-induced mutagenesis and no differences in mutagenesis induced by alkylating agents such as N-methyl-N’-nitro-N-nitrosoguanidine (MNNG). The dose-dependent decrease in survival of the transgenic cells is the same for all mutagens tested, and does not differ from that of V79 cells. Neither transgenic cell line is generally hypermutable, since mutagenesis at an endogenous gene, Na+K+/ATPase, is similar to that of the parental V79 cell line. Although both cell lines can be induced to delete the transgene following clastogen exposure, deletions are not the only recovered mutations, and the
cell lines can also be used to study mutations within the PCR recoverable gpt gene. The utility of these transgenic cells to investigate genome position effects related to mammalian mutagenesis mechanisms is discussed.


http://www.sciencedirect.com/science/article/B6T2C-41TMPSB-1/2/c2e563316c5998c6ef91e3852004b8cb

Recent phylogenetic analysis of the superfamily of lesion-replicating DNA polymerases suggest that they can be broadly divided into four sub-groups comprised of UmuC-like, DinB-like, Rev1-like and Rad30-like proteins. The UmuC-like sub-family is best characterized at the genetic level and sequence analysis of eleven umu orthologs, residing on bacterial chromosomes or on self-transmissible R-plasmids allows further subdivision into five sub-groups (UmuDC, MucAB, ImpAB, RumAB and RulAB) based on amino acid sequence conservation. Some of these orthologs are apparently inactive in situ, but may promote increased mutagenesis and survival when subcloned and expressed from high-copy number plasmids. We were, therefore, interested in devising an assay that would identify umuC-like genes in situ in the absence of a functional assay. To this end, degenerate primers directed towards conserved amino acid regions within the UmuC-like sub-family of DNA polymerases were designed and used to identify mucAB-like operons on the IncT plasmids, R394 and Rts-1. Interestingly, DNA sequence analysis of an ~7 kb region of R394 identified two LexA-regulated genes immediately downstream of mucAB(R394) that are similar to the chromosomally-encoded Escherichia coli tus gene and the IncI plasmid-encoded impC gene, respectively. Analysis of the R394 and Rts-1 mucB genes revealed that both contain insertions which result in the expression of a truncated inactive MucB protein. While R394 was unable to restore mutagenesis functions to a [Delta]umuDC E. coli strain, Rts-1 surprisingly promoted significant levels of MMS-induced SOS mutagenesis, raising the possibility that Rts-1 encodes another, yet unidentified, umu-like homolog.


http://www.sciencedirect.com/science/article/B6T2C-490RJBG-1/2/69817d8631e91f775c86e51b2020965b

Adaptation is a complex process by which populations of organisms respond to long-term environmental stresses by permanent genetic change. Here we present data from the natural "open-field" radiation adaptation experiment after the Chernobyl accident and provide the first evidence of the involvement of epigenetic changes in adaptation of a eukaryote-Scots pine (Pinus silvestris), to chronic radiation exposure. We have evaluated global genome methylation of control and radiation-exposed pine trees using a method based on cleavage by a methylation-sensitive HpaII restriction endonuclease that leaves a 5’ guanine overhang and subsequent single nucleotide extension with labeled [3H] dCTP. We have found that genomic DNA of exposed pine trees was considerably hypermethylated. Moreover, hypermethylation appeared to be dependent upon the radiation dose absorbed by the trees. Such hypermethylation may be viewed as a defense strategy of plants that prevents genome instability and reshuffling of the hereditary material, allowing survival in an extreme environment. Further studies are clearly needed to analyze in detail the involvement of DNA methylation and other epigenetic mechanisms in the complex process of radiation stress and adaptive response.

http://www.sciencedirect.com/science/article/B6T2C-4BYC4M8-3/2/00d73c092cb48b51b50548c52e91420a

The biological and genetic effects of chronic low-dose radiation (LDR) exposure and its relationship to carcinogenesis have received a lot of attention in the recent years. For example, radiation-induced genome instability, which is thought to be a precursor of tumorigenesis, was shown to have a transgenerational nature. This indicates a possible involvement of epigenetic mechanisms in LDR-induced genome instability. Genomic DNA methylation is one of the most important epigenetic mechanisms. Existing data on radiation effects on DNA methylation patterns is limited, and no one has specifically studied the effects of the LDR. We report the first study of the effects of whole-body LDR exposure on global genome methylation in muscle and liver tissues of male and female mice. In parallel, we evaluated changes in promoter methylation and expression of the tumor suppressor gene p16INKa and DNA repair gene O6-methylguanine-DNA methyltransferase (MGMT). We observed different patterns of radiation-induced global genome DNA methylation in the liver and muscle of exposed males and females. We also found sex and tissue-specific differences in p16INKa promoter methylation upon LDR exposure. In male liver tissue, p16INKa promoter methylation was more pronounced than in female tissue. In contrast, no significant radiation-induced changes in p16INKa promoter methylation were noted in the muscle tissue of exposed males and females. Radiation also did not significantly affect methylation status of MGMT promoter. We also observed substantial sex differences in acute and chronic radiation-induced expression of p16INKa and MGMT genes. Another important outcome of our study was the fact that chronic low-dose radiation exposure proved to be a more potent inducer of epigenetic effects than the acute exposure. This supports previous findings that chronic exposure leads to greater genome destabilization than acute exposure.


http://www.sciencedirect.com/science/article/B6T2C-3W258J8-Y/2/e3ee92e432039f07f56b9fc1d38c5f41

To date, eight closely related homologs of the Escherichia coli UmuC protein have been identified. All of these homologs appear to play critical roles in damage-inducible mutagenesis in enterobacteria. Recently, a distantly related UmuC-homolog, DinB, has also been identified in E. coli. Using the polymerase chain reaction together with degenerate primers designed against conserved regions found in UmuC-like proteins, we have identified a new member of the UmuC-superfamily in the archeon Sulfolobus solfataricus. This new homolog shows high sequence similarity to DinB and a lower level of similarity to UmuC. As a consequence, we have called this new gene dbh(dinB homolog). Analysis of approximately 2.7 kb DNA encompassing the dbh region revealed several open reading frames (orfs). One, encoding a putative ribokinase, was located immediately upstream of dbh. This orf overlaps the dbh gene by 4 bp suggesting that both proteins might be coordinately expressed. Further upstream of the ribokinase-dbh locus was another orf encoding a potential ATPase homologous to two uncharacterized S. cerevisiae proteins (YD9346.02c and SC38KCXVI_20) and another E. coli DNA repair protein, RuvB. While this is the first report of a UmuC-like homolog in an archeon, we detected additional homologs using protein sequence comparisons in Gram-positive bacteria, cyanobacteria, and among potential human EST products, indicating that UmuC-related proteins comprise a ubiquitous

http://www.sciencedirect.com/science/article/B6T2C-47P87R7-94/2/0820b78a542e569ba5938eafec4bcb22

A DNA fragment including most of the tyrA gene from E. coli B/r strain WU (Tyr-, Leu-) was amplified in vitro by polymerase chain reaction. The sequence was determined, first, for essentially all of the fragment to locate an ochre nonsense defect, and second, repeatedly for a region of the fragment from several independent isolates containing backmutations at the ochre codon (spontaneous and UV-induced). There were 20 single base differences in the tyrA gene region from the analogous wild-type E. coli K12 sequence: an ochre codon at amino acid position 161, 18 silent changes (1 at the first codon base and 17 at the third) and one replacement of valine by alanine. Different backmutations at the ochre codon encoded lysine, glutamine, glutamic acid, leucine, cysteine, phenylalanine, serine or tyrosine. The diversities of base substitutions at the ochre codon after UV mutagenesis or after mutagenesis where targeting by dimers was reduced or eliminated (after photoreversal of irradiated cells treated with nalidixic acid to induce SOS functions or after UV mutagenesis of cells containing amplified DNA photolyase) were similar (with two notable exceptions). The overall differences between the gene sequences for E. coli K12 or B/r seemed consistent with the neutral theory of molecular evolution.


http://www.sciencedirect.com/science/article/B6T2C-45CW865-2/2/132c7bd724c1d23729c0e34b129c7513

In response to ionizing radiation and other agents that damage DNA, the p53 tumor suppressor protein activates multiple cellular processes including cell cycle checkpoints and programmed cell death. Although loss of p53 function is associated with radiation-induced genetic instability in cell lines, it is not clear if this relationship exists in vivo. To study the role of p53 in maintenance of genetic stability in normal tissues following irradiation, we have measured mutant frequencies at the adenine phosphoribosyltransferase (Aprt) and hypoxanthine-guanine phosphoribosyltransferase (Hprt) loci and examined mechanisms of loss of heterozygosity (LOH) in normal T cells of p53-deficient, Aprt heterozygous mice that were subjected to whole-body irradiation with a single dose of 4 Gy X-rays. The radiation-induced mutant frequency at both the Aprt and Hprt loci was elevated in cells from mice with different p53 genotypes. The radiation-induced elevation of p53-/- mice was significantly greater than that of p53+-/ or p53+/+ mice and was caused by several different kinds of mutational events at the both chromosomal and intragenic levels. Most significantly, interstitial deletion, which occurs rarely in unirradiated mice, became the most common mechanism leading to LOH in irradiated p53 null mice. These observations support the idea that absence or reduction of p53 expression enhances radiation-induced tumorigenesis by increasing genetic instability at various loci, such as those for tumor suppressor genes.

Transcription increases DNA repair efficiency and modulates the distribution of certain types of DNA damage. Furthermore, increased transcription level stimulates spontaneous mutation rate in yeast. We explored whether transcription level affects spontaneous mutation rate in human cells. We first developed two thymidine kinase (tk) inducible human cell lines using the Gal4-Estrogen receptor system. In our TK6i-G3 and G9 tk heterozygous cell lines, the active tk allele is linked to an inducible promoter element. Tk mRNA is induced following treatment with estrogen. Spontaneous mutation rate was significantly decreased in human cell lines after induction in contrast to the report in yeast. Thus, humans may have evolved different or additional mechanisms to deal with transcription related spontaneous mutagenesis.


We have used a polymerase chain reaction (PCR)-based exon screening assay to determine the spectrum of spontaneous hypoxanthine phosphoribosyltransferase (hprt) gene mutations occurring in an aphidicolin-resistant V79 Chinese hamster cell line (designated Aphr-4-2) that contains a mutant DNA polymerase-[alpha] and displays a spontaneous mutator phenotype. PCR analyses of 71 independent, 6-thioguanine (TG)-resistant sublines isolated from Aphr-4-2 or parental V79-743X cells using hprt exon 3- and exon 9-specific oligonucleotide primer pairs revealed the loss of exon 3 or 9 from 6 of 60 Aphr-4-2 derived-, and from 1 of 11 parental V79-derived, TG-resistant mutants. Exons 3 and 9 were both lost from 5 of 60 Aphr-4-2-derived mutants, while none of the 11 V79-derived mutants had lost both exons. The results of these PCR-screening assays were further corroborated by Southern and Northern blot hybridization analyses of 28 mutants: 22 of 28 mutants contained an intact hprt gene by Southern analysis; of these 22 mutants 6 of 11 Aphr-4-2-derived mutants contained either reduced or undetectable steady state mRNA levels in contrast to all 11 V79-derived mutants that contained normal amounts of a normal-sized hprt mRNA. The results of our PCR and blot hybridization analyses indicate that the rates of base substitution and deletion mutagenesis are elevated in Aphr-4-2 cells, and suggest that DNA polymerase-[alpha] may play a role in determining the rate of different molecular types of spontaneous mutations in vivo.

detoxification of certain food-borne carcinogenic-heterocyclic amines. To determine the importance of UDP-glucuronosyltransferase 1A1 (UGT1A1) in the biotransformation of the cooked-food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), genetically modified CHO cells that are nucleotide excision repair-deficient, and express cytochrome P4501A2 (UV5P3 cell line) were transfected with a cDNA plasmid of human UGT1A1 to establish the UDP-glucuronosyltransferase 1A1 expressing 5P3hUGT1A1 cell line. Expression of the UGT1A1 gene was verified by screening neo gene expressing clonal isolates (G-418 resistant) for their sensitivity to cell killing from PhIP exposure. Five of 11 clones were chosen for further analysis due to their resistance to cell killing. Western blot analysis was used to confirm the presence of the UGT1A1 and CYP1A2 proteins. All five clones displayed a 52-kDa protein band, which corresponded to a UGT1A1 control protein. Only four of the clones had a protein band that corresponded to the CYP1A2 control protein. Correct fragment size of the cDNAs in the remaining four clones was confirmed by RT-PCR and quantification of the mRNA product was accomplished by real-time RT-PCR. Expression of UGT1A1 in the transfected cells was 104-105-fold higher relative to the UV5P3 parental cells. One clone (#14) had a 10-fold higher increase in expression at 1.47 X 105 over the other three clones. This clone was also the most active in converting N-hydroxy-PhIP to N-hydroxy-PhIP glucuronide conjugates in microsomal metabolism assays. Based on the D50 values, the cytotoxic effect of PhIP was decreased [not, vert, similar]350-fold in the 5P3hUGT1A1 cells compared to the UV5P3 control cells. In addition, no significant increase in mutation frequency was observed in the transfected cells. These results clearly indicate that UGT1A1 plays a critical role in PhIP biotransformation, providing protection against PhIP-mediated cytotoxicity and mutagenicity.


http://www.sciencedirect.com/science/article/B6T2C-3W2TBF1-9/2/9e04a1a315d32d55e3bcacfb2b101be5

Benzo[a]pyrenediol-epoxide (BPDE), a metabolite of the ubiquitous environmental carcinogen benzo[a]pyrene (B[a]P), has been implicated as a point mutagen. However, as mutational events other than point mutations are also often associated with cancer, we have investigated whether BPDE can induce other classes of mutation. This was done by analyzing mutation at the aprt and hprt loci, both in hemizygous (D422) and heterozygous (D423) Chinese hamster ovary (CHO) cell strains. Southern blotting analysis indicated that BPDE is not an effective producer of either deletions or insertions in the hemizygous environment. The analysis of mutation in the aprt heterozygote was done to investigate the frequency of loss of heterozygosity (LOH) events following BPDE treatment. Using PCR to produce an artificial restriction fragment length polymorphism in the functional aprt allele, BPDE was found to induce LOH in about one-quarter of the mutants recovered. While the precise mechanism of this phenomenon remains obscure, it is likely to have important implications, since similar events involving homologous recombination in somatic cells may have an impact in tumorigenesis.


http://www.sciencedirect.com/science/article/B6T2C-3X3KGB-8/2/8d885d8245209e8e478f647d794ff218

The species specific response to 1,3-butadiene (BD), an important industrial chemical, was
investigated by determining the influence of exposure duration and exposure concentration on the mutagenicity of BD in mice and rats and by defining the spectra of mutations in the Hprt gene T-cell mutants from control and BD-exposed mice. Female B6C3F1 mice and F344 rats (4-5 weeks old) were exposed by inhalation to 0, 20, 62.5, or 625 ppm of BD for up to 4 weeks (6 h/day, 5 days/week). Groups of control and exposed animals (n=4-12/group) were necropsied at multiple time points after exposure and the T-cell cloning assay was used to measure Hprt mutant frequencies in lymphocytes isolated from spleen. Mutant clones collected from control and BD-exposed mice were propagated and analyzed by RT-PCR to produce Hprt cDNA for sequencing. In animals necropsied 4 weeks after 2 or 4 weeks of BD exposure (0 or 625 ppm), the rate of accumulation of mutations was greater in mice than in rats. Supra-linear dose-response curves were observed in BD-exposed mice, indicating a higher efficiency of mutant induction at lower concentrations of BD. The mutagenic potency estimates (represented by the differences in the areas under the mutant T-cell 'manifestation' curves of treated vs. control animals) in mice were 11 and 61 following 4 weeks of exposures to 62.5 and 625 ppm of BD, respectively, while mutant frequencies (Mfs) in rats were significantly increased only at 625 ppm BD (mutagenic potency of 7). Molecular analysis of Hprt cDNA from expanded T-cell clones from control and BD-exposed mice demonstrated an increased frequency of mutants in exposed animals that likely contain large deletions in the Hprt gene (P=0.016). These data indicate that both exposure duration and exposure concentration are important in determining the magnitude of mutagenic response to BD, and that mutagenic and carcinogenic properties of BD in mice may be related more to the ability of its metabolites to cause chromosomal deletions than to produce point mutations.


http://www.sciencedirect.com/science/article/B6T2C-47PCNN3-8N/2/596f779ee8fd98d9c99d8c2c10a0cee52

The distribution of O6-meG in the rat H-ras gene sequence was studied using PCR by transition of O6-meG to adenine during the reaction. In order to study the transition mutations the PCR product was cloned in a replicative form of phage M13mp18 and sequenced. The use of PCR for detection of O6-meG was validated by using oligonucleotides (61 bases) containing one O6-meG residue at a defined site. After treatment of rat liver DNA by N-methyl-N-nitrosourea in vitro, a striking nonrandom sequence distribution of O6-meG was observed. Sixty-eight per cent of O6-methylated Gs were found in the middle G of the sequences GGT and GGA in the H-ras gene whereas no methylation was found in the middle G of the sequences AGG, GGG, TGT, TGC, CGA and CGC. No O6-meG adduct was found in the 12th codon of H-ras (sequence GGA). The frequency of O6-meG formation as a function of two flanking nucleotides on each side of the target guanine was calculated as an approach to understanding more distant sequence effects. It was found that in the DNA sequence studied the formation of O6-meG was highest if the G was flanked by PyPu or PuPu on the 5' side (Py, pyrimidine and Pu, purine) whereas PuPu on the 3' side showed maximal inhibition of O6-meG formation.


http://www.sciencedirect.com/science/article/B6T2C-42YW37B-1/2/1db32d0f59afece3dd23e3bf9b9b3d2be

The lacI transgene used in the Big Blue(TM) (BB) mouse and rat mutation assays typically
displays spontaneous mutation frequencies in the 5 x 10-5 range. Recently, the bone marrow and bladder of the Big Blue(TM) rat were reported to have, by an order of magnitude, the lowest spontaneous mutation frequencies ever observed for lacI in a transgenic animal, approaching the value for endogenous targets such as hprt (~10-6). Since spontaneous mutations in transgenes have been attributed in part to deamination of 5-methylcytosine in CpG sequences, we have investigated the methylation status of the lacI transgene in bone marrow of BB rats and compared it to that present in other tissues including liver, spleen, and breast. The first 400 bases of the lacI gene were investigated using bisulfite genomic sequencing since this region contains the majority of both spontaneous and induced mutations. Surprisingly, all the CpG cytosines in the lacI sequence were fully methylated in all the tissues examined from both 2- and 14-week-old rats. Thus, there is no correlation between 5-methylcytosine content at CpG sites in lacI and the frequency of spontaneous mutation at this marker. We also investigated the methylation status of another widely used transgenic mutation target, the cII gene. The CpG sites in cII in BB rats were fully methylated while those in BB mice were partially methylated (each site approximately 50% methylated). Since spontaneous mutation frequency at cII is comparable in rat and mouse, the methylation status of CpG sequences in this gene also does not correlate with spontaneous frequency. We conclude that other mechanisms besides spontaneous deamination of 5-methylcytosine at CpG sites are driving spontaneous mutation at BB transgenic loci.


http://www.sciencedirect.com/science/article/B6T2C-47PG5TS-2/2/d84bc50795489e01e624363fe5355e08

DNA was isolated from the liver of young B6C3F1, C3H/He and C57BL/6 mice, 6-9 weeks old. A portion of exon 2 of Ha-ras was amplified by PCR allele-specific amplification. The PCR product was identified by (a) size, (b) presence of a diagnostic restriction site, and (c) direct sequencing. Our results indicate that nascent mouse liver bears a subpopulation of cells which contain a mutation in codon 61 of Ha-ras-, specifically an A to G transition at position 2. Therefore, the detection of this mutation in chemically induced mouse liver tumors does not demonstrate that the chemical in question acts as a mutagen. It might act by a nongenotoxic mechanisms, i.e., by facilitating a clonal expansion of cells bearing this spontaneous mutation.


http://www.sciencedirect.com/science/article/B6T2C-45C06N4-1/2/2ea0ca60724cf9aa331d90de3e6f0d2

We have studied mutagenic specificities of DNA lesions in vivo in yeast CYC1 oligonucleotide transformation assay. We introduced two lesions into oligonucleotides. One was a nucleoside analog, 3,4-dihydro-6H,8H-pyrimido[4,5-c][1,2]oxazin-7-one 2'-deoxyriboside (dP), which is highly mutagenic to bacteria. It is supposed to be a miscoding, but otherwise good template for DNA polymerases. The other lesion was the TT pyrimidine(6-4)pyrimidone photoproduct, one of the typical UV lesions, which blocks DNA replication. These oligonucleotides were used to transform yeast cyc1 mutants with ochre nonsense mutation to Cyc1+. As expected from its templating properties in vitro, the transforming activity of dP-containing oligonucleotides was similar to those of unmodified oligonucleotides. Results indicated that dP may direct incorporation of guanine and adenine at a ratio of 1:20 or more in vivo. An oligonucleotide containing the photoproduct showed
the transforming activity of as low as 3-5% of that of the corresponding unmodified oligonucleotide. This bypass absolutely required REV1 gene. The sequence analysis of the transformants has shown that the lesion was read as TT and TC at a ratio of 3:7, indicating its high mutagenic potential.


Chronic dietary insufficiency of the lipotropic nutrients choline and methionine is hepatocarcinogenic in male rats and certain mouse strains. Despite the fact that DNA hypomethylation is a hallmark of most cancer genomes, the tissue-specific consequences of this alteration with respect to tumorigenesis remain to be determined. In the present study, the folate/methyl deficient model of multistage hepatocarcinogenesis was used to evaluate in vivo alterations in DNA methylation in the liver, the carcinogenesis target tissue, and in non-target tissues, including pancreas, spleen, kidney, and thymus, of male F344 rats. By utilizing the HpaII/MspI-based cytosine extension assay, we demonstrated that the percent of CpG sites that lost methyl groups on both strands progressively increased in liver tissue after 9, 18, and 36 weeks of folate/methyl deficiency. The endogenous activity of DNA methyltransferase in liver of rats fed with folate/methyl deficient diet for the 36-week period gradually increased with time. In contrast, non-target tissues displayed no changes in DNA methylation level or activity of DNA methyltransferase. The failure of DNA methyltransferase to restore and maintain DNA methylation patterns in preneoplastic liver tissue may lead to the establishment of tumor-specific DNA methylation and DNA methyltransferase profiles that are not expressed in normal liver. These results provide additional information about alterations in DNA methylation during early preneoplastic stages of carcinogenesis. They also demonstrate that DNA hypomethylation is localized to tissue that undergoes carcinogenesis, and is not altered in non-target tissues.


In the yeast, Saccharomyces cerevisiae, the Rad52 gene is important for both mitotic and meiotic recombination. Homologs of the Rad52 gene have been identified in several eukaryotic organisms, ranging from yeast to man. As reported here, human Rad52 protein binds to both single- and double-stranded DNA; and acting on a pair of single-stranded and partially duplex substrates it promotes annealing of complementary strands of DNA, which is followed by branch migration.

Molecular analysis of cDNA derived from a papillary thyroid carcinoma (PTC) (follicular variant of papillary thyroid carcinoma on histology) which developed in an externally irradiated patient 4 years after exposure identified a portion of the 5' region, exons 1-3, of the rfp gene juxtaposed upstream of the fragment encoding the tyrosine kinase (TK) domain of the ret gene. The fusion gene, termed [Delta]rfp/ret, was the result of a balanced chromosomal translocation t(6;10) (p21.3;q11.2) confirmed by interphase FISH painting, with breakpoints occurring in introns 3 and 11 of the rfp and ret genes, respectively. Both [Delta]rfp/ret and reciprocal ret/rfp chimeric introns had small deletions around breakpoints consistent with presumed misrepair of a radiation-induced double-strand DNA break underlying the rearrangement. No extensive sequence homology was found between the fragments flanking the breakpoints. The fusion protein retained the propensity to form oligomers likely to be mediated by a coiled-coil of the RFP polypeptide as assessed by a yeast two-hybrid system. NIH 3T3 fibroblasts stably transfected with a mammalian expression vector encoding full-length [Delta]RFP/RET readily gave rise to the tumors in athymic mice suggestive of high transforming potential of the fusion protein. Thus, the [Delta]rfp/ret rearrangement may be causatively involved in cancerogenesis and provides additional evidence of the role of activated ret oncogene in the development of a subset of papillary thyroid carcinoma.


Microsatellites are regions of DNA containing tandem repeats of a core 2-6 bp nucleotide sequence. To test the hypothesis that microsatellite mutation can be directed by exposure to specific external cues, control and treatment groups of resistant and susceptible wheat varieties were grown under controlled conditions and genotyped at a number of microsatellite loci that map to chromosomes known to contain Fusarium head blight (FHB) resistance/susceptibility loci. Genotyping was undertaken both prior to and following exposure to Fusarium graminearum, the FHB pathogen. Within a month of inoculation of inflorescences, 58% of experimental plants, and no control plants, had acquired a novel allele at the locus Xgwm112.1. This allele was detected only in head blight affected tissue. Uninoculated control plants, and leaf samples from inoculated plants, showed no mutation. Cloning and sequencing of PCR products indicates that the new allele was generated by contraction of the (CT)n repeat motif. Observation of the same deletion-based mutation in all varieties, its absence in control plants not exposed to the head blight pathogen, and the detection of no similar mutational events in a control panel of loci not expected to show mutation, indicates that this example of microsatellite mutation is induced and/or caused by FHB infection.


The radiosensitive mutant xrs-5, a derivative of the Chinese hamster ovary (CHO) K1 cell, is
defective in DNA double-strand break rejoining ability and in V(D)J recombination. The radiosensitivity and defective repair phenotype are complemented by the 80-kDa subunit of the Ku protein. We determined the nature of the mutations that develop spontaneously at the hprr locus in this cell line using both multiplex PCR deletion screening and DNA sequencing. Ninety-two independent spontaneous mutants were analyzed and the results were compared to the mutation spectrum of 64 previously analyzed hprr spontaneous mutants isolated from the parental CHO-K1 cell line. More than 50% of the spontaneous xrs-5 mutants had lost one or more exons while less than 25% of spontaneous CHO-K1 mutants had lost one or more exons. Most of the deletions in xrs-5 cells involved the loss of multiple exons while single exon deletions predominated in CHO-K1. There was also a nonrandom distribution of breakpoints in both CHO-K1 and xrs-5. Most of the deletion breakpoints were 3’ to exon 9, around exons 4-6, or near exon 1. Although the frequency of base substitutions was lower in xrs-5, the spectrum of base substitutions was qualitatively similar to that of CHO-K1. There was no significant difference in the spontaneous mutant frequency in xrs-5 and CHO-K1. The results suggest that in certain regions of the hprr gene, base alterations can be converted to large deletions, and that alterations in the Ku protein complex can influence this process.


http://www.sciencedirect.com/science/article/B6T2C-4864JF7-5F/2/e7ad8636964b42049c7fcb1147824103

Previously, we reported the modification of denaturing gradient gel electrophoresis called constant denaturant gel electrophoresis (CDGE). CDGE separates mutant fragments in specific melting domains. CDGE seems to be a useful tool in mutation detection. Since the hypoxanthine phosphoribosyltransferase (HPRT) gene is widely used as target locus for mutation studies in vitro and in vivo, we have examined the approach of analyzing human HPRT cDNA by polymerase chain reaction (PCR) and CDGE. All nine HPRT exons are included in a 716-bp cDNA fragment obtained by PCR using HPRT cDNA as template. When the full-length cDNA fragment was examined by CDGE, it was possible to detect mutations only in the last part of exon 8 and exon 9. However, digestion of the cDNA fragment with the restriction enzyme Aval prior to CDGE enabled us to detect point mutations in most of exon 2, the beginning of exon 3, the last part of exon 8 and exon 9. With the use of two internal primer sets, including a GC-rich clamp on one of the primers in each pair, a region containing most of the exon 3 through exon 6 was amplified and we were able to resolve fragments with point mutations in this region from wild-type DNA. The approach described here allows for rapid screening of point mutations in about two thirds of the human HPRT cDNA sequence. In a test of this approach, we were able to resolve 12 of 13 known mutants. The mutant panel included one single-base deletion, one two-base deletion and 11 single-base substitutions.


http://www.sciencedirect.com/science/article/B6T2C-47GJ2V7-1/2/fa46efb6cf1d189ccf4a902f81286faf

Mannose-binding lectin (MBL) is a constituent of the human innate immune system which may play an important role in combating a variety of infectious diseases. We investigated the
distribution of MBL gene mutations in a Vietnamese population, using polymerase chain reaction and DNA sequence analysis, and sought associations with the outcome of hepatitis B virus (HBV) infection. For this purpose we used samples from a total of 123 patients with confirmed, well-defined HBV infections, representing a full spectrum of clinical presentation from acute to chronic to malignant states, as well as from 112 healthy controls. The only MBL gene mutation found in this population, that at codon 54 of exon 1, was present at an overall frequency of 0.12, with a trend towards a higher frequency in the HBV-infected group compared with controls (0.15 versus 0.08, P=0.079). Within the HBV-infected group there was a non-significant trend towards higher viral loads in those with this mutation, accompanied by significantly higher serum transaminase levels in the same individuals. Segregation according to clinical presentation showed that the mutation was present at a significantly higher frequency in the group with acute hepatitis B (AHB) compared with the healthy control group (0.25 versus 0.08, P=0.01), and was associated with higher serum transaminase levels. Our results indicate that a mutation of the MBL gene might influence the clinical outcome of HBV infection in Vietnamese patients.


http://www.sciencedirect.com/science/article/B6T2C-4DK6CXX-2/2/5ccfffa94e65a97772c005aabdd0fe02

5-(2-Chloroethyl)-2'-deoxyuridine (CEDU) is a pyrimidine nucleoside analogue formerly in development for the treatment of herpes simplex virus infections. The compound proved clearly mutagenic in the mouse spot test and exhibited weak activity in the Salmonella reverse mutation test, which led to the termination of the compound's development. In another study, CEDU, administered orally to beta-galactosidase (lacZ) transgenic mice (MutatrademarkMouse) for five days, induced a clear increase in lacZ mutant frequencies in spleen, lung, and bone marrow [1]. In the present follow-up study, we analyzed 32 of those lacZ mutants isolated from the bone marrow of the MutatrademarkMouse animals of the experiments mentioned above, in order to obtain further information on the type of mutations induced by CEDU. CEDU induced a pronounced increase in A:T to G:C transitions. The distribution of A:T to G:C transitions was clearly non-random, showing a bias towards T to C substitutions in the coding DNA strand and a preference to occur in the sequence motif 5'-(G or C)-T-G-3'. Our data support the hypothesis that CEDU, after being phosphorylated, is incorporated into cellular DNA in place of thymidine, which leads to mispairing with guanosine during subsequent DNA replication. As a result, the compound is thought to exert its mutagenicity by inducing mismatches leading to T to C transitions. Our findings point towards a mode of mutagenic action of CEDU that differs fundamentally from that of other antiviral antinucleosides whose clastogenic and recombinogenic activities prevail.


http://www.sciencedirect.com/science/article/B6T2C-3R3PRN6-9/2/792baf05326045c34557ea226bddd3e9a

Frequencies of spontaneously occurring and X-ray induced, stable and unstable types of chromosome aberrations in peripheral blood lymphocytes from two groups of radiosensitive
patients, i.e., aplastic anemia (AA) and Diamond-Blackfan anemia (DBA), were determined. Two types of staining methods, i.e., chromosome painting with two cocktails of chromosome-specific DNA libraries (Nos. 1, 3, X and Nos. 2, 4 and 8), as well as conventional Giemsa staining, were employed. Chromosome painting was done with single and multicolor fluorescence in situ hybridization (FISH). The frequencies of spontaneously occurring chromosome aberrations in AA and DBA patients were not significantly different from healthy individuals. Hypersensitivity to X-rays was seen both in G0 as well as in G2 phase of the cell cycle in lymphocytes from AA and DBA patients, confirming our earlier findings using micronucleus (DBA) and G2 radiosensitivity (AA) assays.


In this review we describe the principles, protocols, and applications of two commercially available SNP genotyping platforms, the TaqMan[trademark] SNP Genotyping Assays and the SNPlex[trademark] Genotyping System. Combined, these two technologies meet the requirements of multiple SNP applications in genetics research and pharmacogenetics. We also describe a set of SNP selection tools and validated assay resources which we developed to accelerate the cycle of experimentation on these platforms. Criteria for selecting the more appropriate of these two genotyping technologies are presented: the genetic architecture of the trait of interest, the throughput required, and the number of SNPs and samples needed for a successful study. Overall, the TaqMan assay format is suitable for low- to mid-throughput applications in which a high assay conversion rate, simple assay workflow, and low cost of automation are desirable. The SNPlex Genotyping System, on the other hand, is well suited for SNP applications in which throughput and cost-efficiency are essential, e.g., applications requiring either the testing of large numbers of SNPs and samples, or the flexibility to select various SNP subsets.


The 4977 bp deletion in mitochondrial DNA (mtDNA) is known to accumulate with age in various human tissues. Findings regarding its accumulation in blood, however, have so far been contradictory. We investigated the levels of the 4977 bp deletion in mtDNA from 100 intravital and postmortem blood samples. Applying an improved version of a PCR plus silver staining of polyacrylamide gels, we could detect the 4977 bp deletion in blood of healthy individuals over 20 years of age. While the 4977 bp deletion in blood is subject to a certain age dependence, it appears to be influenced by additional factors. A Primer-Shift-Assay amplifying four different deletion-specific fragments showed that the smaller fragments were amplified with a higher amplification efficiency than the larger fragments. The deletion-specific 389 bp fragment was demonstrated in 73% of individuals over 80 years of age, but in only 46% of individuals between 21 and 30 years old whereas the largest 802 bp deletion-specific fragment was detectable in 38% of subjects over 80 years of age, and in only 15% of individuals under 30 years of age. Deletion-specific fragments were not detected in a single individual under 20 years old, nor in fetal blood. In this work, we demonstrate for the first time the detection of 4977 bp specific fragments in blood...
of healthy individuals without the necessity of using a nested PCR. The deletion is detectable in postmortem and intravital blood, so that the occurrence of the 4977 bp deletion seems to be a physiological and not only a postmortem process.


http://www.sciencedirect.com/science/article/B6T2C-47PG610-2N/2/2339058089135955862f3c991b955fa8

We have developed an approach for determining mutational spectra in exon 3 of the hypoxanthineguanine phosphoribosyl transferase (hprt) gene in splenic T-lymphocytes of B6C3F1 mice. Hprt mutants from treated animals were isolated by culturing splenic T-cells in microtiter dishes containing medium supplemented with IL-2, concanavalin A, and 6-thioguanine. DNA was extracted from 6-thioguanine-resistant colonies and amplified by the polymerase chain reaction (PCR) using primers flanking the exon 3 region of hprt. Identification of samples containing mutant exon 3 sequences and purification of mutant DNA from contaminating wild-type hprt DNA was accomplished using denaturing gradient gel electrophoresis. Purified mutant sequences were then sequenced. This approach is being used to study the sequence specificity of ethylene oxide (ETO). 12-day-old mice were given single i.p. injections of 100 mg ETO/kg every other day or 30, 60, 90 or 120 mg ETO/kg daily for 5 days to achieve different cumulative doses of this compound. In mice exposed every other day, cumulative doses of 200, 600 and 900 mg ETO/kg produced average mutant frequencies of 15 +/- 12.8, 45 +/- 13.2, and 73 (70, 75) x 10^{-6}, respectively, 8 weeks after the first treatment. In mice exposed daily, cumulative doses of 150, 300, 450 and 600 mg ETO/kg produced average mutation frequencies of 4.2 +/- 10.4, 8.2 +/- 10.4, 11.1 +/- 1.0 and 15.5 +/- 10.7 x 10^{-6}, respectively, 20 weeks after the first treatment. The mutant fraction in control mice was less than 3 x 10^{-6}. 123 hprt mutants from mice exposed to 600 or 900 mg ETO/kg were isolated and analyzed for mutations in exon 3. 18 were located in exon 3 (14.6%). DNA sequencing revealed that 11/18 mutations were base-pair substitutions at 8 different sites in exon 3. Four AT transversions, three AT transitions, two GC transversions, and two GC transitions were observed. Three of the substitutions (2 AT -> CG, 1 AT -> GC) occurred at one base (203) in a single animal. The remaining 7 mutations, isolated from 4 different animals, were the same + 1 frameshift mutation in a run of 6 consecutive guanine bases (207-212) in exon 3. These results suggest the involvement of both modified guanine and adenine bases in ETO mutagenesis. The mouse T-cell cloning/sequencing assay for hprt described here represents a useful system for studying the molecular mechanism of chemically-induced mutation occurring in vivo at an endogenous gene.


http://www.sciencedirect.com/science/article/B6T2C-4BP9P00-1/2/3d559b5d466bd0f25d793730179f8f24

Tth MutS, a mismatch repair protein from Thermus thermophilus, was reported to effectively recognize all eight possible types of base pair mismatches and insertions or deletions up to three base pairs at a wide temperature range up to 60 [deg]C. Here a procedure for directly fishing out subtle unknown mutations in bacterial genome with Tth MutS was described. Wild type genomic DNA and mutant one were mixed, digested with restriction enzymes, denatured and re-annealed. Hetero-duplex DNA carrying mispaired bases were bound to Tth MutS and recovered through Ni-
NTA His-Bind(R) Resin. The recovered DNA was cloned into plasmids, producing a mini-library with inserts of the mutated regions. Further DNA sequencing and genetic complementation demonstrated that the method was extremely efficient in fishing out the mutations from total genomic DNA. Using this method, the mutations existed in a Psedomonas aeruginosa mutant strain were screened, indicating that A/G transitions at nt 181 and nt 314 in chloramphenicol acetyltransferase (catB7) gene conferred this strain with a high chloramphenicol dosage resistant. Compared with those reported previously, this protocol can screen the mixed mutations more easily.


http://www.sciencedirect.com/science/article/B6T2C-47MJ4X7-1/2/7553ae296cff6ed22a402d00e781fb93

Mitochondrial DNA (mtDNA) is the only extrachromosomal DNA in human cells. The mitochondrial genome encodes essential information for the synthesis of the mitochondrial respiratory chain. Inherited defects of this genome are an important cause of human disease. In addition, the mitochondrial genome seems to be particularly prone to DNA damage and acquired mutations may have a role in ageing, cancer and neurodegeneration. We wished to determine if radiotherapy and chemotherapy used in the treatment of cancer could induce changes in the mitochondrial genome. Such changes would be an important genetic marker of DNA damage and may explain some of the adverse effects of treatment. We studied samples from patients who had received radiotherapy and chemotherapy for point mutations within the mtDNA control region, and for large-scale deletions. In blood samples from patients, we found a significantly increased number of point mutations compared to the control subjects. In muscle biopsies from 7 of 8 patients whom had received whole body irradiation as well as chemotherapy, the level of a specific mtDNA deletion was significantly greater than in control subjects. Our studies have shown that in patients who have been treated for cancer there is an increased level of mtDNA damage.


http://www.sciencedirect.com/science/article/B6T2C-45215VR-1/2/7bbc9d27c6067f5befc51293b213b901

We have implemented a technique combining allele-specific PCR (AS-PCR) and denaturing high-performance liquid chromatography (DHPLC) to identify new polymorphic variants within an intragenic region in the [beta]-globin cluster. This technique is applicable to the detection of new variants in genomic regions where variation is apportioned into distinct classes of haplotype. Duplexes for DHPLC analysis were created by denaturation and re-annealing of a mixture of two AS-PCR products of known and unknown sequence from the same haplotypic class, permitting detection of new haplotypes in each class. A 454 bp fragment 3.5 kb 5’ to the human [delta]-globin gene, which may have a gene regulatory function, was analysed in 840 chromosomes from a global sampling of human populations using this method. Two divergent haplotypes were found to predominate in all populations studied, possibly as a result of balancing selection.
Recurrent mutations in vivo in T-lymphocytes identify clonally restricted genomic instabilities in some individuals. Cell-based assays allow initial recognition of clones with mutator phenotypes, but genotypic selection is required to determine frequencies and temporal sequences of potentially independent mutational events isolated only as complex changes in the same allele. The present work illustrates how two single-base insertions in the HPRT gene recovered only as a double event in a cell-based assay were shown to arise as separate in vivo mutations, being individually present at frequencies of -4 and -5, respectively, in peripheral blood. Full characterizations of mutator clones will allow elucidation of the earliest events in the emergence of genomic instability in human somatic cells.


http://www.sciencedirect.com/science/article/B6T2C-47PCNN3-8V/2/f15d99bffd31d39aa76a76c07989e236

We report here the development of multiplex in vitro DNA amplification and solid-phase direct exon sequencing for the analysis of mutations at the hypoxanthine-guanine phosphoribosyltransferase (hprt) locus in Chinese hamster cells. 18 representative HPRT-deficient mutants, derived either spontaneously, or after exposure to UV light or ionizing radiation, were analyzed. All 9 hprt exons were simultaneously amplified via the polymerase chain reaction (PCR) for rapid deletion detection. 5 mutants involve single or multiple-exon deletions. Altered multiplex PCR patterns were detected in mutants Bsp-040, Bsp-065 and BGR-606. Subsequent direct sequence analysis reveals that Bsp-040 and Bsp-065 carry a 52-bp and a 13-bp intragenic DNA deletion in exon 3, respectively. BGR-606 contains a 223-bp insertion accompanied by a 10-bp deletion of intron sequence within exon 4 fragment. Other subtle DNA alterations identified by direct exon sequence analysis include single-base substitutions, small deletions and insertions, adn RNA splicing mutations.


http://www.sciencedirect.com/science/article/B6T2C-3VNR7B0-3/2/cb271a1c523d55b16bfe/f7cbb6d4f811

We have determined the mutational specificity of S9-activated benzo[a]pyrene (B[a]P) at the endogenous aprt locus in a hemizygous Chinese hamster ovary cell line. The aprt gene of recovered mutants was amplified using the polymerase chain reaction (PCR) and directly sequenced. This spectrum was then compared to mutations recovered following treatment with the B[a]P metabolite, benzo[a]pyrene diol-epoxide (BPDE). No significant difference between the two spectra in the types of mutations produced, or their distribution was observed. This
observation supports the hypothesis that BPDE is the reactive metabolite of B[a]P, responsible for the significant biological effects caused by this ubiquitous polycyclic aromatic hydrocarbon. The major mutation recovered was the G:C=>T:A transversion, and mutations were primarily localized within runs of guanines. We also confirmed our previous finding that mutation by B[a]P is non-random, targeting events in runs of guanines flanked by adenine residues. This same target hotspot region is found in codon 61 of the human c-Ha-ras1 proto-oncogene. This may help explain the selective activation of this codon by BPDE.

Fungal Genetics and Biology (8)


http://www.sciencedirect.com/science/article/B6WFV-46P3W8J-C/2/9b5a34e997f3537ef29dc92daaaaa45ba


http://www.sciencedirect.com/science/article/B6WFV-4DW98YY-3/2/265f1d644ab17c87ad9265abaf2be78f

A gene for the Alternaria major allergen, Alt a 1, was amplified from 52 species of Alternaria and related genera, and sequence information was used for phylogenetic study. Alt a 1 gene sequences evolved 3.8 times faster and contained 3.5 times more parsimony-informative sites than glyceraldehyde-3-phosphate dehydrogenase (gpd) sequences. Analyses of Alt a 1 gene and gpd exon sequences strongly supported grouping of Alternaria spp. and related taxa into several species-groups described in previous studies, especially the infectoria, alternata, porri, brassicicola, and radicina species-groups and the Embellisia group. The sonchi species-group was newly suggested in this study. Monophyly of the Nimbya group was moderately supported, and monophyly of the Ulocladium group was weakly supported. Relationships among species-groups and among closely related species of the same species-group were not fully resolved. However, higher resolution could be obtained using Alt a 1 sequences or a combined dataset than using gpd sequences alone. Despite high levels of variation in amino acid sequences, results of in silico prediction of protein secondary structure for Alt a 1 demonstrated a high degree of structural similarity for most of the species suggesting a conservation of function.


http://www.sciencedirect.com/science/article/B6WFV-49TRKD3-1/2/56463dc6d856569b5f84383b5d0457cb
We constructed and characterized a bacterial artificial chromosome (BAC) library for Epichlo' festucae, a genetically tractable fungal plant mutualist. The 6144 clone library with an average insert size of 87 kb represents at least 18-fold coverage of the 29 Mb genome. We used the library to assemble a 110 kb contig spanning the putative ornithine decarboxylase (odc) ortholog and subsequently expanded it to 228 kb with a single walking step in each direction. Furthermore, we evaluated conservation of microsynteny between E. festucae and some model filamentous fungi by comparing sequence available from a 43 kb region at the end of one BAC to publicly available fungal genome sequences. Orthologs to the 13 contiguous open reading frames (ORFs) identified in E. festucae are syntenic in Neurospora crassa and Magnaporthe grisea occurring in small sets of two, three or four colinear ORFs. This library is a valuable resource for research into traits important for the development and maintenance of a plant-fungus mutualistic symbiosis.


http://www.sciencedirect.com/science/article/B6WFV-49MDYXV-3/2/4b840452dc2cad662b694e6db23ee1fb

Different species of the lichen-forming ascomycete fungus Teloschistes were found to contain group IB introns at position S1506 in the small subunit ribosomal RNA gene. We have characterized the structural organization and phylogeny of the Teloschistes introns Tco.S1506, Tla.S1506, and Tvi.S1506. Common features to all the introns are a small size, a compact RNA structure, and an atypical catalytic ribozyme core sequence motif. Variations in intron sizes, due to sequence extensions in the P1 and P8 loop segments, were observed in different species and isolates. Phylogenetic analyses based on the ITS1-5.8S-ITS2 region as well as the introns show that the Teloschistes S1506 introns represent a distinct evolutionary isolated cluster among the nuclear group I introns. Furthermore, introns from different lineages of Teloschistes villosus appear not strictly vertically inherited probably due to horizontal transfer in one of the lineages.


http://www.sciencedirect.com/science/article/B6WFV-4C4WXRJ-1/2/ccdf2640e0859cbeade1089bae502fba

Species limits were investigated within the Fusarium graminearum clade (Fg clade) through phylogenetic analyses of DNA sequences from portions of 11 nuclear genes including the mating-type (MAT) locus. Nine phylogenetically distinct species were resolved within the Fg clade, and they all possess contiguous MAT1-1 and MAT1-2 idiomorphs consistent with a homothallic reproductive mode. In contrast, only one of the two MAT idiomorphs was found in five other species, four of which were putatively asexual, and the other was heterothallic. Molecular evolutionary analyses indicate the MAT genes are under strong purifying selection and that they are functionally constrained, even in species for which a sexual state is unknown. The phylogeny supports a monophyletic and apomorphic origin of homothallism within this clade. Morphological analyses demonstrate that a combination of conidial characters could be used to differentiate three species and three species pairs. Species rank is formally proposed for the eight unnamed species within the Fg clade using fixed nucleotide characters. Index Descriptors: Fusarium head blight; Mating-type; Histone H3; Homothallic; Heterothallic; Gene trees; Species trees; Species limits; Phylogeny; Reciprocal monophyly

http://www.sciencedirect.com/science/article/B6WFV-46P3W8J-7/2/47512785770fe0db07710ac5a0f896d1


http://www.sciencedirect.com/science/article/B6WFV-49NVFHF-4/2/1c7267dcf57c35f50cf7502799c29cae

Guest is a transposable element of the Tc1/mariner superfamily with 30-40 bp terminal inverted repeats and a TA dinucleotide target site duplication. Guest was originally discovered in the St. Lawrence 74A laboratory strain of the filamentous fungus Neurospora crassa. In this report, Guest iterations subcloned from a cosmid library of the Oakridge 74A strain were used to design PCR primers that permitted the detection of Guest in wild isolates of *N. crassa*. Guest is present in *N. crassa* as multiple copies ranging between 100 bp and 2.4 kb and is present in the mating type locus of several Neurospora species. Bioinformatic analysis of the entire *N. crassa* genome (Oakridge 74A strain) detected 48 Guest iterations. All iterations appeared to have been inactivated either by repeat-induced point mutation or sequence deletion, with the majority being remnants less than 400 bp in length. The possible involvement of Guest in the evolution of the variable region that flanks the mating type idiomorphs in several Neurospora species is discussed.


http://www.sciencedirect.com/science/article/B6WFV-46BMVNH-9/2/7d449928b69e5b073d6755cd937d2115


http://www.sciencedirect.com/science/article/B6T39-4019DM2-C/2/ef52f0cce86dc7252a55a1b46c319537

http://www.sciencedirect.com/science/article/B6T39-3XD3S7F-9/2/3746e0b3da06dd1abc3764a254391645


http://www.sciencedirect.com/science/article/B6T39-3VGR20J-8/2/b37ec53646b50c5d06a8473649360686

To isolate cDNAs encoding Kruppel-like zinc finger proteins consisting of several hundred members, most of which are yet to be identified, from a limited number of available cells, we developed a rapid and efficient zinc finger gene cloning method based on reverse transcription-polymerase chain reaction (RT-PCR) using tagged, degenerate oligonucleotide primers corresponding to the conserved H/C link followed by the reverse blue selection to identify clones containing properly amplified fragments. More than $5 \times 10^3$ blue colonies were obtained from only 1 ng of total RNA. Eighty-eight out of 89 clones, which were randomly picked up from blue colonies and sequenced, encoded 60 different zinc fingers with the expected structure, and among them, only four have been previously described. Furthermore, it was possible to rapidly select clones that were differentially expressed in a tissue and stimulation-specific manner by a differential screening of the zinc-finger cDNA library using probes consisting of distinct sets of the zinc-finger PCR products. These results indicate that our PCR-based method is quite efficient and suitable for analyzing not only zinc finger genes but also other large gene families, especially when the available cells are very limited.


The cDNA for the [beta]3-subunit of cone-specific transducin (T[beta]3) was cloned and characterized from wild type dogs, and used in linkage studies as a candidate gene for cone degeneration. Sequence analysis of the T[beta]3 cDNA revealed an open reading frame of 1020 bp, potentially coding for a protein of 340 amino acids (aa). The deduced aa sequence of canine T[beta]3 shares 97% identity with the previously identified human T[beta]3, and 82% identity with bovine rod-specific transducin (T[beta]1). RT-PCR and sequencing of the amplified products demonstrated that the retinal canine T[beta]3 gene is expressed in two different transcripts which can be generated by alternative splicing of the intron in the 3'-untranslated region (UTR). The short and the long mRNAs differ in the length of their 3'-UTR by 456 nt. We have also determined the genomic organization of the canine T[beta]3 gene; it consists of ten exons and the first exon is in the 5'-UTR. The cDNA encoding T[beta]3 from cd-affected dogs was also cloned and sequenced. We found no differences at the nucleotide level between the cDNAs isolated from normal and diseased retinas. The level of transcription of T[beta]3 mRNA in the cd dog retina appeared to be normal. Linkage analysis of a crossbred informative pedigree showed five obligate recombinants out of nine informative offspring. These results suggest that T[beta]3 is not a candidate gene for the cone degeneration of the cd mutant.

http://www.sciencedirect.com/science/article/B6T39-47PNWHM-1W/2/23b1c8b090601438894747b4ce247262

Four types of cDNAs encoding the GTPase-activating protein-related domain (GRD) of the mouse neurofibromatosis type-1 gene (NF1) have been cloned. One of these isoforms was a newly identified form termed type IV. Analysis of the genomic structure of the mouse NF1-GRD revealed two exons (23A and 23B) between exons 23 and 24, leading to the production of four types of NF1-GRD cDNAs by an alternative splicing mechanism. Amino-acid sequences encoded by NF1-GRD are highly conserved between human and mouse. Analysis of the expression of these transcripts in various tissues of adult mouse revealed that the type-1 transcript is predominantly expressed in neural tissues such as brain and spinal cord. Other forms, termed types II, III and IV, are also expressed in various tissues. The type-1 and type-II transcripts are expressed equivalently in undifferentiated P19 mouse teratocarcinoma cells, whereas type-1 expression becomes predominant during neuronal differentiation by retinoic acid treatment. Expression of type I is also shown to be correlated with cessation of cell proliferation in P19 cells, but not in NIH3T3 cells. These, together with other results, suggest that the four types of NF1-GRD transcripts generated by alternative splicing have some important biological roles in cell differentiation and proliferation.


http://www.sciencedirect.com/science/article/B6T39-48KFP56-1/2/768bfa64b247d76014db2d4a7ea8507

Monitoring of differential gene expression is an important step towards understanding of gene function. We describe a comparison of the representational difference analysis (RDA) subtraction process with corresponding microarray analysis. The subtraction steps are followed in a quantitative manner using a shotgun cloning and sequencing procedure that includes over 1900 gene sequences. In parallel, the enriched transcripts are spotted onto microarrays facilitating large scale hybridization analysis of the representations and the difference products. We show by the shotgun procedure that there is a high diversity of gene fragments represented in the iterative RDA products (92-67% singletons) with a low number of shared sequences (<9%) between subsequent subtraction cycles. A non redundant set of 1141 RDA clones were immobilized on glass slides and the majority of these clones (97%) gave repeated good fluorescent signals in a subsequent hybridization of the labelled and amplified original cDNA. We observed only a low number of false positives (<2%) and a more than twofold differential expression for 32% (363) of the immobilized RDA clones. In conclusion, we show that by random sequencing of the difference products we obtained an accurate transcript profile of the individual steps and that large-scale confirmation of the obtained transcripts can be achieved by microarray analysis.


http://www.sciencedirect.com/science/article/B6T39-44YF890-7/2/4cc5217b609439ff067ae69a10204dc7

Hu-Surf5 is included within the Surfeit locus, a cluster of six genes originally identified in mouse.
In the present study, we have cloned and characterized the Hu-Surf5 gene and its mRNA multiple transcripts. Comparison of the most abundant cDNA and genomic sequence shows that the Hu-Surf5 is spread over a region of approximately 7.5 kb and consists of five exons separated by four introns. The nucleotide sequence of the genomic region flanking the 3'-end of the Hu-Surf5 gene revealed the presence of a processed pseudogene of human ribosomal protein L21 followed by Hu-Surf6 gene. Only 110 bp separate the transcription start site of Hu-Surf5 and Hu-Surf3/L7a gene and the transcription direction is divergent. Earlier studies defined the 110 bp region essential for promoter activity of Hu-Surf3/L7a. Here, we show that this region stimulates transcription with a slightly different efficiency in both directions. The bidirectional promoter lacks an identifiable TATA box and is characterized by a CpG island that extends through the first exon into the first intron of both genes. These features are characteristic of housekeeping genes and are consistent with the wide tissue distribution observed for Hu-Surf5 expression. Hu-Surf5 encodes three different transcripts, Surf-5a, Surf-5b, and Surf-5c, which result from alternative splicing. Two protein products, SURF-5A and SURF-5B have been characterized. Production of chimaeras between the full-length SURF-5A or SURF-5B and the green fluorescent protein (GFP) allowed to localize both proteins in the cytoplasm.


http://www.sciencedirect.com/science/article/B6T39-47T1JJN-36/2/dcd93bc3906a9ee77ce75e67965fb71e

Thermostable DNA ligase has been harnessed for the detection of single-base genetic diseases using the ligase chain reaction [Barany, Proc. Natl. Acad. Sci. USA 88 (1991) 189-193]. The Thermus thermophilus (Tth) DNA ligase-encoding gene (ligT) was cloned in Escherichia coli by genetic complementation of a ligts7 defect in an E. coli host. Nucleotide sequence analysis of the gene revealed a single chain of 676 amino acid residues with 47% identity to the E. coli ligase. Under phoA promoter control, Tth ligase was overproduced to greater than 10% of E. coli cellular proteins. Adenylated and deadenylated forms of the purified enzyme were distinguished by apparent molecular weights of 81 kDa and 78 kDa, respectively, after separation via sodium dodecyl sulfate-polyacrylamide-gel electrophoresis.


http://www.sciencedirect.com/science/article/B6T39-3Y5MNGT-2V/2/c711a4b871f86599651bb0508338c89e

Using the polymerase chain reaction (PCR) and primers based on regions of homology between the human and murine interleukin 7 (IL-7)-encoding cDNAs, we have amplified an ovine (ov) IL-7 cDNA from reverse-transcribed RNA extracted from concanavalin A (Con A)-activated ovine lymph-node cells. The nucleotide sequence of the cDNA and the predicted amino acid (aa) sequence showed significant homology to those of the human and murine molecules. The ovIL-7 cDNA encodes a 176-aa polypeptide that, based on analysis of murine IL-7, is processed to a protein of 151 aa. The cDNA was demonstrated to encode a protein with IL-7 biological activity. Supernatants from COS or CHO-K1 cells transfected with an expression vector containing the ovIL-7 cDNA were able to synergise with a suboptimal level of Con A to induce proliferation of ovine thymocytes. In addition, both supernatants were able to induce thymocyte proliferation, albeit at a reduced level, in the absence of Con A. Further experiments demonstrated that for induction of ovine thymocyte proliferation, recombinant (re)-ovIL-7 was able to synergise with re-human (h) IL-2 but not re-hIL-6 or tumor necrosis factor-[alpha] (re-hTNF[alpha]).

http://www.sciencedirect.com/science/article/B6T39-47T2TG2-1R/2/4bce72e1a879ba4095c931aac05ceab0

KlenTaq DNA polymerase is an N-terminally truncated *Thermus aquaticus* (Taq) DNA polymerase I. As expressed from a gene construct in *Escherichia coli*, translation initiates at Met236, bypassing the 5'->3' exonuclease domain of the DNA polymerase-encoding gene. A sensitive forward mutation assay was used to measure the relative number of mutations introduced into the entire lacZ gene by the polymerase chain reaction (PCR) under various conditions which allow the amplification of such a large DNA span. Two selectable markers, one at each end of the test lacZ fragment, were employed to avoid the plating and scoring of PCR artefacts such as primer initiation in the midst of the lacZ gene, and cloning artefacts such as empty vector plasmid. The measured relative mutation rate was twofold lower for KlenTaq as compared to the full-length Taq DNA polymerase.


http://www.sciencedirect.com/science/article/B6T39-3Y6HGWH-3C/2/97da7b07f2dc5b484b9e3fec6617491f

During bone resorption, osteoclasts acidify the extracellular bone resorbing compartment via a vacuolar H+-ATPase (V-ATPase), which resides in the ruffled-border membrane. In an effort to characterize the composition of the osteoclast V-ATPase catalytic domain, we have isolated a cDNA clone that encodes the V-ATPase B-subunit from a cDNA library constructed from highly purified chicken osteoclasts. Comparison of the predicted amino-acid sequence with the published sequences of isoforms of V-ATPase B-subunits from other sources revealed that the chicken osteoclast B-subunit is brain type and not kidney type. Furthermore, only clones encoding the brain type isoform of subunit B could be generated by PCR from a cDNA library prepared from human osteoclastoma osteoclast-like cells. Northern blot analysis revealed that two B-subunit mRNAs, approx. 1.7 and 3.5 kb in length, are expressed in chicken bone marrow mononuclear cells, brain and kidney, although the relative amounts of these two transcripts were different in each tissue. In brain, the 3.5-kb mRNA was predominantly expressed. In bone marrow cells, the levels of the 1.7-kb mRNA were higher than in other tissues and expression of this message was increased by 1,25-dihydroxyvitamin -3, suggesting that this mRNA is specifically upregulated during osteoclast differentiation. These results indicate that the B-subunit isoforms present in the catalytic domains of the osteoclast and kidney V-H+-ATPases are different and further suggest that selective expression of isoforms of the B-subunit in these two tissues could provide a structural basis for some of the differences we have reported in the pharmacology and catalytic properties of these two enzymes.


http://www.sciencedirect.com/science/article/B6T39-45CNFV2-12/883b408ef0205a8fd87ee2ead8c6391b
Deoxyribonuclease (DNase) I has been implicated in the induction of DNA fragmentation and cell death, however little is known about its regulation in vivo. In the present study, we describe that DNase I messenger RNA (mRNA) is alternatively spliced in rat kidney, and the activity of the DNase I correlates with the alternative splicing during the course of renal ischemia/reperfusion. Northern blot analysis with mRNA from control rat kidneys and kidneys subjected to ischemia/reperfusion in vivo yielded two bands of approximately 1.3 and 1.5 kb, suggesting the possibility of alternative splicing. However, prolonged reperfusion up to 16 h resulted in the predominant expression of 1.3 kb transcript. The disappearance of the 1.5 kDa band was associated with the increased DNase I activity in the kidney during ischemia/reperfusion. To study the alternative splicing of the DNase I mRNA, rat kidney cortex DNA complementary to RNA library was screened using rat DNase I probe. Twenty-one positive clones were obtained and were compared with the reported DNase I mRNA transcript cloned from rat parotid gland. All clones showed 100% homology with the reported DNase I coding sequence and part of 5'-untranslated region (5'-UTR), named exon 1a by us. Twelve out of 21 isolated clones had longer 5'-UTR than previously described, and DNase I pre-mRNA was alternatively spliced in this region. Six out of these 12 clones contained extra up to 153 bp in extreme 5' end, whereas, in six other clones, an internal 132 bp segment (exon 1b) of this additional sequence was absent, and only the extreme 5'UTR sequence (exon 1c) was found in these clones. The nucleotide analysis showed that alternating exon 1b has the possibility of a secondary structure with high internal homology and potential for at least one major stable stem-loop. Both newly identified segments, exons 1b and 1c, were also identified in genomic DNA. The long splice variant, containing exon 1b, is expressed only in the kidney among different tissues tested. Exon 1b inhibited translational activity of DNase I mRNA in vitro. Our data suggest that alternative splicing in 5'-UTR in the kidney may provide a prompt DNA-independent regulation of DNase I activity when DNA is damaged during ischemic injury.


http://www.sciencedirect.com/science/article/B6T39-3V7WV9H-F/2/0831c88d386e5dc0e95d45b6f57e934a

Protein phosphatase 2A (PP2A) holoenzyme plays a critical role in cell-cycle control and growth-factor signaling, and is implicated in tumorigenesis. Because the protein phosphatase 2 regulatory subunit A beta isoform gene (PPP2R1B) maps within the critical region of hereditary paraganglioma (PGL1) on chromosomal band 11q23, we characterized its genomic structure and evaluated it as a candidate gene for PGL1. PPP2R1B has 15 exons spanning approx. 27 kb genomic distance. We placed the exons on genomic EcoRI fragments and identified their flanking intronic sequences. The gene was oriented from telomere to centromere. Splice acceptor and donor sites of all introns conformed to the GT/AG rule. Northern analysis with a cDNA probe identified 2.5 kb and 5.0 kb transcript sizes. We identified an ATG initiation codon in a favorable context and mapped two transcription start sites 15 bp and 66 bp upstream of it. We also mapped a 3'-polyadenylation site 504 bp downstream of the TGA stop codon, consistent with the 2.5 kb transcript size. We did not detect germ-line mutations by single-stranded conformational polymorphism (SSCP) analysis or major rearrangements by Southern analysis in a set of PGL1 patients. In conclusion, we precisely mapped and characterized the structure of PPP2R1B and evaluated it as a candidate gene for PGL1.

We propose a model for the expansion of short tandem repeats (ESTR), a phenomenon which has been found to occur in human DNA and is associated with a dozen of neuromuscular diseases. The model is based mainly on theoretical considerations and recovers experimental data from the literature; it also finds support in preliminary results obtained by us in multiprimed polymerase chain reactions designed to assess the effects of a downstream primer on the fidelity of the elongation of an upstream one. The model links the occurrence of the ESTR to a defective maturation of the Okazaki fragments (OF), and in particular to an improper processing of their 3' termini. This may occur when the last OF approaches the 5' terminus of the previous one in a susceptible region of the template. It is postulated here that when a growing OF has progressed past the priming region and its main portion has been synthesized, upon approaching its conclusion, the final elongation may take place in a region of the template where certain triplets are repeated: in that case a series of aberrations on the elongation mechanism may occur. These aberrations could involve (a) the displacement of the 5' terminus of the penultimate, properly matured OF, enacted by the incoming 3' terminus of the last OF, (b) the switch of the latter to the displaced strand of the former as template, (c) the fold-back on itself of the growing 3' terminus of the last OF, (d) its assumption of an unusual structure because of the repetition, and (e) some impairment of its removal by structure-specific exo-endonuclease(s). Derangements of this last part of the process may trigger the ESTR.


A 3.2-kb BamHI genomic DNA fragment containing the pyrG gene of Mucor circinelloides was isolated by heterologous hybridization using a pyrG cDNA clone of Phycomyces blakesleeanus as the probe. The complete nucleotide sequence of the M. circinelloides pyrG gene encoding orotidine-5'-monophosphate decarboxylase (OMPD) was determined and the transcription start points (tsp) were mapped by primer extension analysis. The predicted amino acid sequence showed homology with the OMPD sequences reported from other filamentous fungi, with 96% similarity with the OMPD of *P. blakesleeanus*. Analysis of the sequence revealed the presence of two short introns whose length and location were confirmed by sequencing a cDNA clone and comparing this with its genomic counterpart. The intron splice sites and the 5'- and 3'-noncoding flanking regions show general features of fungal genes. Northern-blot hybridization revealed the pyrG transcript to be approx. 1.0 kb. The M. circinelloides pyrG cDNA clone was able to complement the pyrF::Mu-1 mutation of *Escherichia coli* when inserted between bacterial expression signals. Additionally, the genomic clone complemented the M. circinelloides pyrG4 mutation. When an M. circinelloides autonomous replication sequence was included in the transforming plasmid, the average transformation frequency obtained was 600 to 800 transformants per [mu]g DNA and per 106 viable protoplasts.

The 2-5A/RNase L system is one of the pathways induced by interferon (IFN). It plays a major role in the antiviral and antiproliferative activities of IFNs. Recently, we have shown that the activity of the RNase L could be inhibited by a proteic inhibitor, the RNase L Inhibitor (RLI). Human RLI (Hu-RLI) was cloned and characterized. We describe here the isolation and characterization of the cDNA encoding the murine RLI (Mu-RLI). Hu-RLI and Mu-RLI protein have 98% amino acid identity. Mu-RLI is functionally homologous to Hu-RLI, and all the structural features and amino acid sequence motifs of Hu-RLI are conserved in Mu-RLI. Moreover, reticulocyte lysate translated Mu-RLI protein is also able to inhibit 2-5A binding on 2-5A-dependent RNase-L. Northern blot analysis revealed that Mu-RLI cDNA hybridizes with one mRNA of 3.5 kb except for the testis where two mRNA of 3.5 and 2.1 kb, respectively, are detected, suggesting a tissue-specific regulation.


http://www.sciencedirect.com/science/article/B6T39-47PNXV0-PV/2/0223bbb53c03db0da991e3cda4e34ea

A cDNA from human brain poly(A) + RNA with significant similarity to the gene encoding yeast L17A large subunit ribosomal (r) protein (L17A) was isolated using the polymerase chain reaction. The deduced amino acid (aa) sequence of 140 aa (calculated pI of 10.79) exhibits a 78% similarity to that of the yeast L17A r protein (88% when conservative aa replacements are considered as well). This indicates that L17A is one of the best conserved r-proteins and therefore may play a critical role in ribosome function. In contrast to its eubacterial and chloroplast counterparts, human L17A contains an N-terminal extension of 19 aa which may be involved in nuclear targeting of the r-protein. Approximately five to seven genes in mammalian genomes give strong hybridization signals when probed with the human L17A homologue cDNA. Whereas the LIT A homologue was found to be expressed at similar levels in several human tissues as a transcript of 600 nucleotides, a several-fold higher transcript level was detected in the rapidly growing neuroblastoma cell line, SK-N-BE.


http://www.sciencedirect.com/science/article/B6T39-3S1PY3W-2/2/54284a1e0ab23bda4055cf17a9644c1c

The degradation of 1,2-dichloroethane (DCE) by Xanthobacter autotrophicus GJ10 proceeds via chloroacetaldehyde (CAA), a toxic intermediate in the cells if it is not metabolized further by the NAD+-dependent CAA dehydrogenases. Here, we describe the cloning, sequence and expression in Escherichia coli of aldA, a plasmid-located CAA dehydrogenase-encoding gene of GJ10 as well as a chromosomal homolog, designated aldB. The DNA-predicted amino acid (aa) sequences of the two proteins (505 aa in AldA and 506 aa in AldB) are 84% identical. The cloned aldA and aldB genes were verified by their expression in the E. coli T7 polymerase/promoter and the pUC lac promoter systems. The expression level of AldA and its enzymatic activity towards CAA were both higher than those of AldB. In a hybrid construct, the 3’end of aldB was able to complement, although not completely, the corresponding portion of aldA to produce a functional gene. Both AldA and AldB proteins of GJ10 share the highest degree of sequence identity with an acetaldehyde dehydrogenase (ALDH) encoded by acoD of Alcaligenes eutrophus (77.3-78% identity). Together with at least three other ALDHs of prokaryotic origin, these proteins apparently form a special class of ALDHs whose expressions are dependent on RpoN factors. By pulsed-
field gel electrophoresis the 225-kb pXAU1 plasmid encoding aldA was shown to be linear.


http://www.sciencedirect.com/science/article/B6T39-3Y5FPYJ-2/2/e65e3250fdd68f7989c13c26e85f50fc

Transcripts for cysteine protease cathepsin B (CTSB) were found to be highly variable in the 5'-UTR (untranslated region). In cDNA clones from a human gastric adenocarcinoma cDNA library, we have identified two new exons (designated 2a and 2b) between exons 2 and 3 in the 5'-UTR of the gene. All of the exons of the 5'-UTR could be alternatively spliced to produce several transcript species. In addition, transcription was initiated from more than one promoter region. Using RT-PCR (reverse transcription-polymerase chain reaction) and primer extension assays, CTSB mRNA species were found to differ among tissues and between a glioblastoma sample and a cell line derived from it. Exons 2a and 2b were detected more frequently in tumor samples than in matched normal samples. Thus, factors related to the cell differentiation and environment seem likely to determine the types of transcripts that are expressed which in turn could influence the overall steady-state level of CTSB mRNAs and their rate of translation. Interestingly, at least three upstream translation initiation codons were observed and could constitute a means of controlling translation initiation.


http://www.sciencedirect.com/science/article/B6T39-43HT1F4-B/2/b158eb08f395045df02ebad8925c210f

The fast evolving progress of the human genome mapping and sequencing efforts facilitate the detection of genes also for complex traits. We focus on the detection of susceptibility loci for autism, a prototypical pervasive developmental disorder. Five genome screens worldwide have identified several putative locations of susceptibility genes thus far, with the most common region on chromosome 7q. In order to identify new candidate genes for infantile autism we constructed a physical map of bacterial artificial chromosome, P1-derived artificial chromosome and yeast artificial chromosome clones of a 3 Mb region between D7S1575 and D7S500, including a complete contig of the ~1.2 Mb region around D7S2533, the marker with the most significant association result. We developed 16 novel sequence tag sites and mapped 23 genes/expressed sequence tags to the contigs. As this map contains a putative autistic disorder locus this integrated physical and transcript map provides a valuable resource for identification of candidate gene(s).


http://www.sciencedirect.com/science/article/B6T39-47PH7G4-1NX/2/53188ea07d65237b15a35070153f0b51

The construction of a new plasmid vector, devoid of all MboI (GATC) and TspEI (AATT) restriction sites, is described. The lack of these two frequent-cutting restriction sites is a unique
A feature among plasmids. This new plasmid, pBRkanf1-, allows selective fragmentation of a cloned insert. As a result, the vector offers an alternative strategy to create overlapping and sequentially deleted subclones. In addition, the construction of the new plasmid required the development of a rapid and accurate multiple site-directed mutagenesis procedure. The mutagenesis method uses a combination of DNA amplification and chain extension by DNA polymerase. By this method, mutations are created progressively from one end of a DNA molecule to the other.


http://www.sciencedirect.com/science/article/B6T39-47PH64N-13N/2/1557a3bff5af73d5c381a3e3c3b58523

SV2 is a secretory vesicle-specific protein produced by all neurons and by endocrine cells. The deduced amino acid (aa) sequence of this protein indicates that it is a transmembrane transporter [Bajjalieh et al., Science 257 (1992) 1211-1213; Feany et al., Cell 70 (1992) 861-867; Gingrich et al, FEBS Lett. 312 (1992) 115-122]. To determine the regions of the protein that are the most highly conserved throughout evolution, and might therefore be essential for the function of SV2, we isolated a cDNA clone encoding SV2 from the elasmobranch fish, Discopyge ommata, and compared the deduced aa sequence to two isoforms from rat, SV2A and SV2B [Bajjalieh et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2150-2154]. The comparison indicates that although the N-terminal cytoplasmic domain of SV2 is the most divergent region, it contains a highly conserved sequence that is predicted to be the epitope for a monoclonal antibody that crossreacts with all species and two isoforms of SV2 [Buckley and Kelly J. Cell Biol. 100 (1985) 1284-1294; Bajjalieh et al., Proc. Natl. Acad. Sci. USA 90 (1993) 2150-2154]. The remainder of the protein is highly conserved: 62% of the aa in SV2 from D. ommata are identical to the rat SV2A sequence, and 12% are conservative substitutions. The high degree of conservation of this protein throughout evolution and across species indicates that it mediates a critical function of synaptic vesicles.


http://www.sciencedirect.com/science/article/B6T39-4BK2JHH-5/2/ca78c51e54ec726d53a1a0feec077e42

The cytotoxin-associated gene (cag) pathogenicity island (PAI) is important for the virulence of Helicobacter pylori. In this study, we have compared the complete nucleotide sequence of the cag PAI in four clinical isolates. These isolates were selected from patients matched for age and sex from the same geographical region. The patients suffered from either gastric cancer (Ca52 and Ca73) or duodenal ulcer (Du23:2 and Du52:2). All four strains induced an interleukin (IL)-8 response in AGS cells and translocated CagA into host cells where the protein was tyrosine phosphorylated, and thus harboured a functional type IV secretion system encoded by the cag PAI. The cagA gene contains a variable region close to its 3' end. Different compositions of this region has been proposed to exert various degrees of morphological changes in cultured gastric epithelial cells, and there are indications that the structure of the repetitive region is connected to the severity of disease. One of the studied strains (Du23:2) possessed five Western-type repeat regions while the other three strains harboured one Western-type repeat. Strain Du23:2 also contained a major rearrangement or large insertion/duplication in the intergenic region between HP0546 and HP0547 (cagA). Sequence similar to that of genes HP0510 and HP0509 was found in the 5' end of this region. The 3' end was similar to the corresponding region of strain ATCC 43504, including a mini IS605 element and a duplication of the 3' end of the cag PAI. Finally, a
novel gene was identified in the cag PAI in three of the sequenced strains at the position of HP0521. This gene, HP0521B, is present in approximately half of Swedish H. pylori isolates.


http://www.sciencedirect.com/science/article/B6T39-4019DM2-S/2/a7b9ca8d26cc05a862b73404b6ec2e7b


http://www.sciencedirect.com/science/article/B6T39-3YJ0JD-3/2/07b5d356ecc1c08eaddba48d9aa7901f


http://www.sciencedirect.com/science/article/B6T39-490H3SW-1/2/7c970c0ce47d30a5340758d19e3b808b

Satellite DNA sequence evolution has been studied in several insect species from the genus Pimelia (Tenebrionidae, Coleoptera). Low-copy number homologs of the previously characterized major satellite DNA from P. monticola (PMON) have been cloned and sequenced from six congeneric species belonging to two species groups: Ibero-Balearic and Moroccan. Sequence analysis of a sample of low-copy number repeats revealed two subfamilies, differing on average 17.5% due to randomly spread single point mutations. Each subfamily is specific for a group of taxa in congruence with their biogeography. Within each group, there is no significant species-specific clustering of the sequences. These results suggest that the two satellite subfamilies arose after the split of an ancestral lineage into the North African and Ibero-Balearic Pimelia species-groups, but before their subsequent radiation. Rate heterogeneity tests suggest that PMON sequences have evolved faster in the lineage leading to the Moroccan group. Comparison of sequence divergences between minor PMON and the previously characterized major PIM357 satellite obtained from the same taxa, points to similar evolutionary dynamics. Both sequences are evolving in parallel accumulating mutations in a gradual manner irrespectively of significant differences in abundance. These data show that copy number of the sequence families does not necessarily affect the sequence change dynamics of satellite repeats.

http://www.sciencedirect.com/science/article/B6T39-3Y6GVD3-6/2/d0f77a92fad7c8a5d6f7776f09531a73


http://www.sciencedirect.com/science/article/B6T39-3S1PY3W-T/2/924351bce01047098f0599c4e8953c09

The Drosophila protein functions critically in the determination of neuroblasts in the embryonic ectoderm and many other cell types. is a member of the MIP family of transmembrane channel proteins. The conserved channel domain of is flanked by amino- and carboxy-terminal cytoplasmic domains of unique sequence, which comprise over two-thirds of the protein. To determine whether the cytoplasmic domains of are important for function, we have cloned and sequenced the bib gene of D. virilis and compared it with that of D. melanogaster. Here we report that the channel domain and both cytoplasmic domains are highly conserved between the two species. The conservation of the cytoplasmic domains indicates that they are critical to function. bib transcripts are found in similar patterns in both species, indicating that the developmental function(s) of have also been conserved.


Campylobacter jejuni (Cj) and C. coli (Cc) clinical isolates, obtained from three different sources, were characterized using two Cj DNA probes, CJ01 and CJ02. These probes were selected at random by virtue of their stability in Escherichia coli (Ec). CJ01 hybridized specifically with DNA from Cj reference strains, but not with DNA from Cc, C. lari (Cl) nor C. fetus (Cf) reference strains. Using clinical isolates characterized by genome-genome hybridization and biotype, CJ01 hybridized with DNA derived from all Cj strains. However, DNA from four out of ten Cc strains, from three different sources, also hybridized with CJ01, suggestive of this region being heterogeneous between clinical isolates of both species. The nucleotide sequence analysis of CJ01 reveals two incomplete open reading frames (ORFs) that did not show significant homology with any other known sequences. CJ02 hybridized specifically with DNA from Cj and Cc reference strains, but not with DNA from Cl and Cf reference strains. The specificity and sensitivity were maintained upon hybridization with DNA from 31 clinical isolates. CJ02 has an uninterrupted ORF whose deduced amino-acid sequence showed extensive homology with the central region of the Ec and Salmonella typhimurium (St) RNA polymerase [beta] subunits (52 and 66% similarity, respectively). The most conserved segments correspond to putative functional domains.

Pectinases produced by Aspergillus flavus and A. parasiticus are believed to play a significant role in the ability of these fungi to spread in cotton bolls and other crops. Utilizing a DNA probe, generated by PCR, of the Aspergillus niger pgaII gene, we have isolated a novel, constitutively expressed polygalacturonase (PG)-encoding gene (pecA) from an A. parasiticus cDNA library. DNA sequence analysis and the deduced amino acid (aa) sequence of pecA demonstrated significant identity at the nucleotide and aa levels with other PG of fungal origin. Northern blot analysis of RNA isolated from A. parasiticus grown on either glucose or pectin as the sole carbon source showed that pecA was expressed during growth in both media.


This study reports the nucleotide (nt) sequence of the human [beta]B2-crystallin (h[Beta]B2-Cry)-encoding cDNA (h[Beta]B2-cry). The h[Beta]B2-cry gene encodes a major structural protein in the lens of the vertebrate eye. Sequence information obtained from mouse genomic clones and the mouse [beta]B2-cry cDNA (m[Beta]B2-cry) sequence was used to design oligodeoxyribonucleotide primers, corresponding to exon 1, transcription start points, and termination and polyadenylation sites, that were used in the polymerase chain reaction (PCR) to generate full-length cDNA clones from total human lens RNA. In addition, cDNA libraries were made with [lambda]gt11 from both human fetal and adult lens tissue RNAs, and screened with a m[Beta]B2-cry cDNA clone. The full-length h[Beta]B2-cry cDNA is 721 bp and contains an open reading frame of 612 nt. It codes for a 23-kDa protein of 205 amino acid residues. Comparison of the overall nt and deduced aa sequences shows a greater similarity of h[Beta]B2-cry to bovine [Beta]B2-cry than to m[Beta]B2-cry or rat [Beta]B2-cry.


With a combined approach of database search, heterologous polymerase chain reaction (PCR), reverse transcription-PCR, rapid amplification of complementary DNA ends and genomic library screening, we have successfully cloned a mouse RING finger gene, mouse RING finger 1 (Mrf1). The Mrf1 gene has two exons of 63 and 2665 bp, respectively, and one intron of over 13 kb. An open reading frame was identified exclusively in exon 2, which encodes a putative protein of the RING-B box-coiled coil or the tripartite motif type of 403 amino acids. Mrf1 is moderately expressed in the spleen, brain and heart as a single 3.0 kb product and very highly expressed in the testis as two transcripts of 3.0 and 1.5 kb, respectively. The Mrf1 gene was mapped to mouse chromosome 3, between markers D3Mit70 and D3Mit277. Western blotting analysis indicated that an expected protein of approximately 44 kD was detected in the brain extracts of mouse, rat and human. The possible functions of Mrf1 are discussed in the contexts of protein-protein interactions, oncogenesis and ubiquitination.

[ gamma]-Glutamyl hydrolase (GGH) plays a central role in folate metabolism and antifolate action. Increased GGH activity has been found in rat hepatoma cells resistant to the cancer drug methotrexate (MTX). The aim of this study was to identify polymorphisms in the GGH gene that modulate GGH activity and that may affect methotrexate resistance. Exons of the human [gamma]-glutamyl hydrolase (hGGH) gene were amplified by polymerase chain reaction (PCR) from breast cancer tissue and leukemia cell lines. Single-stranded conformational polymorphism (SSCP) analysis was performed, and PCR products containing different patterns were cloned and sequenced. Six single nucleotide polymorphisms (SNPs) were identified, at bases -401C>T, -354G>T, -124T>G, +16T>C, +452C>T, and +1102A>G, relative to the A of the translation start codon being considered as +1. The SNP at +16, which changes codon -19 (relative to the start of the mature hGGH protein) in the endoplasmic reticulum targeting sequence of hGGH protein from cysteine to arginine, has previously been identified in this laboratory. The SNP at +452 changes the conserved hGGH protein codon 127 from threonine to isoleucine. The functions of SNPs in the promoter of the hGGH gene were studied by site-directed mutagenesis of a 516-bp region of the hGGH gene promoter in a luciferase reporter vector and transfection into HepG2 and MCF-7 cells. All of the promoter polymorphisms enhanced the production of luciferase compared to the wild-type hGGH gene promoter in HepG2 cells, and -401C>T and -124T>G enhanced luciferase expression in MCF-7 cells, suggesting that polymorphisms in the hGGH gene promoter may increase expression of hGGH protein.


Partial sequences corresponding to eleven novel Rab proteins and one new Rho protein have been isolated using a PCR-based cloning approach. These results confirm that the overall diversity of the Rab and Rho protein subfamilies account for more than thirty different members in mammalian cells.


A novel 114-kDa zinc finger protein, ZEC, has been found by cDNA cloning and characterized. ZEC was strongly expressed in the testis, liver and kidney, and also in embryonic stem cells. Epitope-tagged experiments indicated nuclear localization of ZEC. ZEC contained 18 C2H2 zinc fingers which were organized in two clusters. A ZEC binding DNA sequence, C/GA/TA/TGGTTGGTTGC, which we have designated the GT box, was identified by random oligonucleotide binding selection assay. The GT box did not contain binding sites for other previously characterized transcription factors and thus represented a potentially novel DNA target.
sequence. Electrophoretic mobility shift assay (EMSA) showed that both clusters of zinc fingers bound to the same DNA sequence. Site-directed mutagenesis revealed that the core sequence TTGGTT within the GT box was essential to ZEC binding, while DNA sequences outside of the core sequence enhanced this interaction. Furthermore, co-transfection assays demonstrated that ZEC could activate a reporter luciferase gene driven by this DNA sequence.


http://www.sciencedirect.com/science/article/B6T39-47P8KJR-1CP/2/840929109b1c535da59c4acac4405f1

Salmonella enterotoxin (Stn) is a virulence factor in S. typhimurium strain Q1 that causes both fluid secretion in ligated intestinal loops of rabbits and elongation of Chinese hamster ovary (CHO) cells. High-level expression systems are needed to provide Stn in soluble form for detailed study of the biological activity of Stn. To maximize the synthesis and solubility of Stn, we systematically compared the production of native Stn synthesized with a T7 RNA polymerase/promoter system to that of two fusion proteins: glutathione S-transferase:Stn (Gst:Stn) and thioredoxin A:Stn (TrxA:Stn). The latter fusion protein expression systems resulted in a 64-fold increase in Gst:Stn and TrxA:Stn antigen concentration, as measured by specific anti-peptide antibodies in an enzyme-linked immunosorbent assay (ELISA). Most of the toxin derived using these vector systems was insoluble; however, the solubility of the TrxA:Stn antigen increased by at least 50-fold, with a concomitant increase in CHO cell elongation activity. In addition, stn gene expression was enhanced more than 50-fold by addition of 0.2-0.4 M NaCl to Luria-Bertani medium. The biological activity of Stn also was increased in the high-osmolarity medium. Consequently, the expression of stn may be regulated by DNA supercoiling.


http://www.sciencedirect.com/science/article/B6T39-47PH7G4-1NJ/2/2751d14f42d7f4c978ae6066df43a836

Sequences of four new heat-shock (HS) genes of Escherichia coli organized into two operons were determined. The operon at 83 min specifies two proteins of 15.8 kDa (HslT) and 16.1 kDa (HslS), which are identical to IbpA and IbpB, respectively. Expression of mRNA from a [sigma]32-dependent promoter of the hslTS/ibpAB operon is stimulated 30-75-fold upon temperature upshift. The transcription start point (tsp) is located at a G, 96 bp upstream from the AUG start codon of hslT/ibpA. The deduced amino acid sequences of HslT/IbpA and HslS/IbpB are 48% identical to each other and were found to be remotely related to the chloroplast low-molecular-weight HS protein, which is highly conserved among plants. The second hs operon is much less actively stimulated by temperature upshift, although it has a hs promoter that perfectly matches the consensus of promoters recognized by [sigma]32. Located at 88.9 min, the hslIVU operon specifies proteins of 19.1 kDa (HslIV) and 49.6 kDa (HslIU). Multiple tsp were found in this operon. HslIV is remotely related to the eukaryotic proteasome proteins, and HslIU is very similar to a Pasteurella haemolytica protein of unknown function. Both HslU and the P. haemolytica protein share an ATP/GTP-binding motif near their N-termini. The two operons described here are transcribed counterclockwise on the standard genetic map.

http://www.sciencedirect.com/science/article/B6T39-49M0W0P-2/2/5a70ace97faaaabadcfftbd93b2f0c638

It is widely recognized that metal compounds may modify gene expression. In this context, we have searched for genes whose expression may be affected by cadmium and platinum ions within the context of a cell culture system. Cadmium is well known for its carcinogenic potential while platinum is destined to become more and more interesting because of its increasing use in the automotive industries. By applying differential display to cultures of mouse fibroblast, we have identified two transcripts (acute lymphoblastic leukemia-1, All-1, and a novel gene named metal-responsive gene, MERE-1) that were responsive to platinum and cadmium ions. Moreover, further experiments with a panel of metal compounds have shown that MERE-1 was strongly induced also by La(NO3)2 and Cr(NO3)3, and to a lesser extent, by Na2CrO4 and (NH4)2TeCl6.


http://www.sciencedirect.com/science/article/B6T39-40TY7W0-C/2/feae569ea962be3a0920a9447175799


http://www.sciencedirect.com/science/article/B6T39-46YJC6C-5/2/47e9476ab93c6aeaa080c2b7a5ec8137

Membrane-associated guanylate kinase (MAGUK) proteins are cell-cell contact organizing molecules that mediate targeting, clustering and anchoring of proteins at synapses and other cell junctions. MAGUK proteins may contain multiple protein-protein interaction motifs including PDZ, SH3 and guanylate kinase (GuK) domains. In this study, we performed a detailed analysis of the expression pattern of MPP4, a recently described member of the MAGUK protein family. We confirmed that this gene is highly expressed in retina, and demonstrate that it is also present, at lower levels, in brain. We identified a new retina specific isoform encoding a predicted protein lacking 71 amino acids. This protein region contains a newly identified L27 domain, another module playing a role in protein-protein interaction. By RNA in situ hybridization, Mpp4 expression was found to be localized to photoreceptor cells in postnatal retina. The MPP4 gene is localized to chromosome 2, in band 2q31-33, where a locus for autosomal recessive retinitis pigmentosa (RP26) has been mapped. Mutation analysis of the entire open reading frame of the MPP4 gene in a RP26 family revealed no pathologic mutations. In addition, we did not identify mutations in a panel of 300 unrelated patients with retinitis pigmentosa.


http://www.sciencedirect.com/science/article/B6T39-3YHWR69-R/2/e4898463d28e7a2e2a4b57f9a73d5114

http://www.sciencedirect.com/science/article/B6T39-45M6JMW-2/2/f5056758aab3f88d22a1e1f2bea843a59

We have isolated and characterized a novel differentially spliced gene predominantly expressed in the nervous system, which encodes protein isoforms with significant homology to the [alpha]-actinin protein superfamily, the Caenorhabditis elegans UNC-53 protein and weak homology to the nuclear membrane protein POM121. Similar to POM121 the primary structures show a hydrophobic region that is likely to form one or more adjacent transmembrane segment(s). Indirect immunofluorescence with antibodies against a synthetic peptide gave staining of the nucleus. Target experiments with EGFP (enhanced green fluorescent protein)-fusion proteins confirmed the nuclear localization. Two further members of this gene family could be isolated. All three pore membrane and/or filament interacting like (POMFIL) genes are differentially expressed in neuronal tumor cell lines. In 40% of tested primary neuroblastomas expression of POMFIL1 is strongly reduced and after brain injury POMFIL1 protein expression is upregulated, indicating that POMFIL1 is involved in the process of neuron growth and regeneration, as well as in neural tumorigenesis.


http://www.sciencedirect.com/science/article/B6T39-4C1NGYS-1/2/7c9950446a64bb96e3821e2791824ca8

Polymers of the HEX1 protein produce Woronin bodies in filamentous fungi. We have isolated and sequenced the hex1 gene and flanking regions from the industrially exploited fungus Trichoderma reesei. Multiple transcription start sites (TSS) and the 5’ untranslated region (UTR) were identified by 5’RACE PCR. There are three hex1 transcript types, two of which originate from two TSSs at approximately -320 and -1335 from the start codon, which are separated by a 500-bp intron within the 5’UTR. The third transcript type results from alternative splicing of the intron within the coding sequence at the 3’ end, which results in the inclusion or exclusion of an unconserved histidine-rich coding region. The three transcripts code for two forms of HEX1 protein. N-terminal sequencing of HEX1 separated by 2D gel electrophoresis confirms that there are two forms of HEX1 protein which are modified further by alternative cleavage of the N-terminus. The dominant form of HEX1 is coded by a cDNA with TSS at position -1335. Expression of hex1 on cellulase-inducing medium peaks strongly within 24 h of growth but the protein is expressed at a lower and more consistent level in medium containing glucose. This is the first investigation of expression of the hex1 gene encoding a protein unique to filamentous fungi.


http://www.sciencedirect.com/science/article/B6T39-44CMXX2-J/2/2b558dca9ac92b92bbcb8315c39131193
Diapause-associated gene expression was studied in Drosophila triauraria using subtractive hybridization. Two genes that were shown to be upregulated in diapausing flies by Northern hybridization have similarity to genes encoding antifungal peptides of Drosophila melanogaster, members of the drosomycin family (drosomycin, CG10812, CG10813, CG10815 and CG11520). In addition, a signal peptide and Knot 1 domain are shared with them. The genes cloned from D. triauraria are tentatively named drosomycin-like. However, the similarities between drosomycin-like in D. triauraria and the members of the drosomycin family in D. melanogaster are quite lower than those between other homologous genes in these species. In addition, neighbor-joining analysis revealed that drosomycin-like in D. triauraria is not closely related to known members of the family in D. melanogaster. Thus, it is most plausible that drosomycin-like is not a D. triauraria counterpart of known members of the family, but a novel member belonging to the family. The drosomycin-like gene is expected to have a few copies, because at least two sequences having unique 3'-ends were obtained in RACE, and multiple bands were observed in Southern hybridization. However, these sequences from RACE had the same ORF. Probes for genes encoding additional antimicrobial peptides were used to evaluate expression during diapause. Like drosomycin-like, drosomycin was upregulated during diapause, but defensin and drosocin were not.


http://www.sciencedirect.com/science/article/B6T39-3W07P0S-S/2/426cc12ab2124c3b40629972335e69b9


http://www.sciencedirect.com/science/article/B6T39-47PH7C3-1MP/2/5ad87b0d143f82b166270294865c00ab

Naturally occurring mutations in hypoxanthine-guanine phosphoribosyltransferase (HPRT) have been identified by amino acid sequencing, cDNA cloning, and direct nucleotide sequencing of PCR-amplified transcripts. To determine the effect these mutations have on the catalytic properties of the molecule, knowledge of the three-dimensional structure of HPRT is required. A prerequisite for this, however, is the availability of a large amount of purified product for crystallization and x-ray diffraction analysis. For these reasons we have developed an effective means of producing high levels of human HPRT in Escherichia coli using the expression cassette PCR. By taking advantage of a T7 polymerase/promoter system, we have expressed both normal and variant human hprt sequences in E. coli. The proteins synthesized from these sequences are immunologically and enzymatically active, and are physically indistinguishable from the HPRT in B-lymphoblasts derived from normal and three HPRT-deficient subjects.


http://www.sciencedirect.com/science/article/B6T39-41H3KVD-10/2/ab5ae7f0ae7d845e6e3f863905d4f198

http://www.sciencedirect.com/science/article/B6T39-4019DM2-M/2/94e988c05a529318d647406faf2ffc85


http://www.sciencedirect.com/science/article/B6T39-3Y51FGV-5/2/69d5f5fa2e5decadb7534f42a06872e8


http://www.sciencedirect.com/science/article/B6T39-3W2T6PW-G/2/a6272dc9fc5b64f10742a9951d4505f5

The cDNA encoding the equine copper/zinc superoxide dismutase (SOD1) was cloned from leukocyte total RNA from healthy horses and its nucleotide (nt) sequence was determined. We further sequenced the SOD1 gene from 16 horses diagnosed with equine motor neuron disease (EMND) and eight unrelated, clinically normal horses to determine if this disease, similar to amyotrophic lateral sclerosis (ALS) in humans, is linked to SOD1 mutations. The 465-bp SOD1 coding region in the horse encodes 153 amino acid (aa) residues. Equine SOD1 exhibited 81.8 and 79.9% sequence identity to the human homolog at the nt and aa levels, respectively, with only five distinct aa in the two loops that constitute the active site of the enzyme. None of the human SOD1 mutations found in the familial form of ALS were detected in SOD1 of the 16 affected horses. Although DNA sequence analysis identified three potential polymorphisms in equine SOD1, these were silent and were found in both normal and EMND-affected horses. At this time, there is no conclusive evidence for EMND linkage to SOD1 mutations.


http://www.sciencedirect.com/science/article/B6T39-3Y6HK1D-1T/2/ac867cfa6af185b131edfa26fe55ecd

The metabolism of the branched-chain amino acids (BCAA) isoleucine, leucine and valine is correlated to the production of polyketide antibiotics in many streptomycetes. Despite its significance, this biosynthetic pathway is poorly understood in Streptomyces. In order to develop a better understanding of Streptomyces BCCA biosynthesis, two genes, ilvBN and ilvC, encoding acetohydroxy acid synthase (AHS) and acetohydroxy acid isomeroreductase (IR), respectively, were cloned from Streptomyces avermitilis, a strain producing avermectins, potent antiparasitic compounds. The genes were isolated by applying a combination of PCR and genomic library screening. The deduced amino-acid sequences revealed significant homology to the AHS and IR proteins from other bacterial species. The ilvBN gene, expressed in Escherichia coli (Ec) by using the expression vector pGEX-4T-1, complemented the ilv- mutation of Ec PS1283. Ec transformants produced high levels of AHS, whose activity was feedback inhibited by valine.

http://www.sciencedirect.com/science/article/B6T39-47PH7G4-1NV/2/93dc9517c64ea3ac8529a29b225f5379

A natural population of the lichen-forming ascomycetous fungus, Cladonia chlorophaea, contained individuals with small subunit ribosomal DNA (SSU rDNA) of at least four different size classes and nine restriction-site patterns. The source of these differences was the variable occurrence of 200-400-nucleotide insertions, previously identified as small group-I introns, at five different positions within the SSU coding region. By specific amplification of the sequences flanking these five intron positions with the polymerase chain reaction (PCR), a minimum of nine types of rDNA repeats were defined that differ in number, position, restriction pattern and size of introns. The positions of the introns were verified by sequence analysis. The variable distribution of these introns suggests that they are currently mobile -- either by intron insertion, deletion or both -- within this species complex.


http://www.sciencedirect.com/science/article/B6T39-42JHDKP-S/2/086ca9f6bca34f5c6ae01e3fea3432b3

Queuosine (Q) is a 7-deazaguanosine found in the first position of the anticodon of tRNAs that recognize NAU and NAC codons (Tyr, Asn, Asp and His). Eukaryotes synthesize Q by the base-for-base exchange of queuine (Q base) for guanine in the unmodified tRNA, a reaction catalyzed by TGT. A search of the human EST database for sequences with significant homology to the well studied TGT from Escherichia coli identified several candidates for full-length (1.3-1.4 kb) cDNA clones. Three candidate cDNA clones, available from IMAGE Consortium, LLNL, (Lennon et al., 1996, Genomics 33, 151-152) were obtained: IMAGE Clone Id Nos. 611146, 1422928, and 72154. Here we report the complete sequences of these clones. IMAGE:72154 contains an ORF encoding a 44 kDa polypeptide with high homology to bacterial TGTs and was subcloned into the mammalian expression vector pMAMneo-Cat. When this construct was transfected into the TGT-negative cell line, GC3/c1 (Gunduz et al., 1992, Biochim. Biophys. Acta 1139, 229-238), it restored the ability of the cells to form Q-containing tRNA. This TGT cDNA sequence is encoded in human chromosome 19 clone CTC-539A10 (GenBank accession no. AC011475), enabling determination of the exon-intron boundaries for the TGT gene. The sequence of IMAGE:611146 is 5’-truncated by 76 bp compared to that from IMAGE:72154 and, except for two differences in the 3’-non-coding region, the remainder of the sequence is identical to that of IMAGE:72154. IMAGE:1422928 is a 1390 bp chimera: the 5’-portion, bp 1-708, is identical to a genomic DNA sequence from chromosome 15 (GenBank accession no. AC067805, bp 148976-149683); the 3’-end, bp 726-1390, is identical to the 3’-end of the TGT cDNA sequence from IMAGE:611146.


http://www.sciencedirect.com/science/article/B6T39-40XNXHB-6/2/8556e5f24978dba2a7a111fe68d40d77

http://www.sciencedirect.com/science/article/B6T39-3W0FCD6-7/2/f114b40e5310124ef56e99a1da8b7ee5

Aequorea victoria green fluorescent protein (GFP) is a promising fluorescent marker which is active in a diverse array of prokaryotic and eukaryotic organisms. A key feature underlying the versatility of GFP is its capacity to undergo heterocyclic chromophore formation by cyclization of a tripeptide present in its primary sequence and thereby acquiring fluorescent activity in a variety of intracellular environments. In order to define further the primary structure requirements for chromophore formation and fluorescence in GFP, a series of N- and C-terminal GFP deletion variant expression vectors were created using the polymerase chain reaction. Scanning spectrofluorometric analyses of crude soluble protein extracts derived from eleven GFP expression constructs revealed that amino acid (aa) residues 2-232, of a total of 238 aa in the native protein, were required for the characteristic emission and absorption spectra of native GFP. Heterocyclic chromophore formation was assayed by comparing the absorption spectrum of GFP deletion variants over the 300-500-nm range to the absorption spectra of full-length GFP and GFP deletion variants missing the chromophore substrate domain from the primary sequence. GFP deletion variants lacking fluorescent activity showed no evidence of heterocyclic ring structure formation when the soluble extracts of their bacterial expression hosts were studied at pH 7.9. These observations suggest that the primary structure requirements for the fluorescent activity of GFP are relatively extensive and are compatible with the view that much of the primary structure serves an autocatalytic function.


http://www.sciencedirect.com/science/article/B6T39-43KGNS7-F/2/a3f3a875b082b10f196f30cc59eb719c

NrCAM is one member of the L1 subfamily of cell surface recognition molecules implicated in nervous system development and function. Here we report the complete sequence of the human NRCAM locus. The gene comprises 34 exons and shows extensive conservation of exon/intron structure compared to L1, suggesting a common evolutionary ancestor. By human-chick sequence comparison we identified exons not previously found in mammalian NRCAM mRNAs. One of these encodes a premature stop codon that would give rise to an isoform of NRCAM lacking ankyrin-binding capacity. The availability of the complete sequence will allow an investigation of the potential role of these splice variants, and examination of the regulatory elements controlling NRCAM expression as well as the relationship of NRCAM to disorders involving 7q.


http://www.sciencedirect.com/science/article/B6T39-4292HH9-N/2/638ae1fd312dda3916730fd27199e4b2
The Interleukin Enhancer Binding Factor 3 (ILF3) gene has been mapped to chromosome 19 in humans and to chromosome 9 in mice. Several reported double-stranded RNA binding proteins including NF90, ILF3, MPP4 and DRBP76 have been suggested to be isoforms of the ILF3 gene but this has not been clearly established. We isolated several ilf3 transcripts from a melanoma cDNA library and two corresponding genomic fragments, and report alternative splicing and polyadenylation site selection in the human ILF3 gene. We show the existence of an alternative splice site responsible for the sequence divergence in the 3' part of the transcripts. Another alternative splicing event at a site between the two double-stranded RNA binding motifs leads to the additional presence in some cases of a four amino acids NVKQ peptide. We also describe the utilization of three distinct polyadenylation signals and the generation of an ilf3 transcript with a long extended 3' UTR. The expression of the different transcripts was evaluated. We used a GenBank sequence for the part of chromosome 19 corresponding to the ILF3 gene to determine the exon-intron organization of the entire gene which spans 38 kb and is divided into 21 exons.


http://www.sciencedirect.com/science/article/B6T39-3RM6VMT-9/2/d1e1fe60a32d7ffbf75a13fec1faec061

The molecular diversity of protein D of nonencapsulated Haemophilus influenzae strains isolated from persistently infected patients with chronic bronchitis was studied by sequencing the hpd gene of four independently obtained isolates. The nucleotide (nt) sequences of the hpd genes of two strains were identical. The other two hpd sequences showed nt substitutions which were mostly synonymous. As a consequence the deduced amino acid (aa) sequences differed from the consensus sequence only by a few aa. No changes in the hpd genes were observed among the four variants of the four strains persisting in chronic bronchitis patients for 9, 11, 8 and 3 months, respectively, although variation in their major outer membrane proteins P2 and P5 occurred. We conclude that the hpd gene is conserved during chronic infections of nonencapsulated H. influenzae.


http://www.sciencedirect.com/science/article/B6T39-3SRBHWR-1B/2/216a04cc45ec209a4cbce0c735171fee

Cellobiose dehydrogenase (CDH) is an enzyme produced under lignocellulose-degrading conditions by Trametes versicolor strain 52J (Tv) and several other wood-degrading fungi, including Phanerochaete chrysosporium (Pc). In order to understand better the nature and properties of this enzyme, we isolated a genomic clone of Tv cdh using heterologous probes derived from the sequence of Pc cdh. DNA sequence analysis revealed that Tv cdh consists of 3091 bp of coding sequence interrupted by 14 introns. Southern blotting showed that the gene was present in a single copy in the strain of Tv analyzed. Tv cdh was shown by Northern blot analysis to be expressed as a single transcript under cellulolytic conditions. RT-PCR of poly(A)+ RNA isolated under cellulolytic conditions was used to generate a near full-length cDNA copy of the cdh mRNA. The deduced protein encoded by Tv cdh consists of 768 amino acids (aa), including a predicted 19 aa signal peptide. The protein had 73% identity to the corresponding protein from Pc, which is the only other CDH-encoding gene that has been cloned. Based upon its deduced primary structure and alignment to similar sequences, Tv CDH shares a general structural organization with Pc CDH and other hemoflavoenzymes. Amino acid residues H-109 and M-61 in the N-terminal heme domain are hypothesized to function in heme binding; the C-
terminal flavin domain contained a consensus sequence for flavin binding between residues 217-222. Although the protein is known to bind to cellulose, no obvious homology to bacterial or fungal cellulose binding domains was observed. However, a strong homology was observed to a region of Pc CDH that is hypothesized to be involved in cellulose binding.


http://www.sciencedirect.com/science/article/B6T39-3RD1R45-3T/2/6361257328d462a07f05df0c6a5ea5b87

The role of ecdysteroids in modulating exoskeletal growth during the moult cycle of Crustacea has been well described. However, little is known about the action of ecdysteroids at the level of gene transcription and regulation in Crustacea. This paper reports the cloning of an ecdysteroid responsive gene, HHR3, a potential Manduca sexta MHR3 homologue in the American lobster, Homarus americanus. Levels of HHR3 expression are up-regulated in response to in vivo injections of premoult concentrations (10-6 M) of 20-hydroxyecdysone in the epidermal and muscle tissue of the lobster after 6 h. Maximal mRNA levels are observed after 21 h before returning to basal levels. In muscle tissue, elevated levels of HHR3 mRNA follow a time course similar to elevated actin mRNA expression in response to hormonal injection. In contrast, in eyestalk tissue, the HHR3 levels decline up to 21 h post-injection before rising to basal levels after 48 h. Eyestalk, epidermal and leg muscle tissue was extracted over the moult cycle to determine the levels of expression. In muscle, HHR3 is high during the premoult period that corresponds to the period of the moult cycle when the ecdysteroid titre is high. In the epidermis, HHR3 levels are also high during the premoult with elevated levels maintained into the postmoult period. In the eyestalk, mRNA levels of HHR3 show an opposite pattern of expression with low levels during premoult and postmoult and high levels found during the intermoult period. Our results provide novel evidence for an ecdysteroid responsive gene in a crustacean that has many similarities to MHR3 in Manduca and DHR3 in Drosophila melanogaster. This raises the question of whether a similar cascade of ecdysteroid responsive genes exist in other members of Arthropoda such as the Crustacea, as has been demonstrated in Drosophila. In addition, we provide further evidence for negative feedback regulation of ecdysteroids at the site of moult-inhibiting hormone (MIH) production in the lobster eyestalk.


http://www.sciencedirect.com/science/article/B6T39-3WBG1Y1-R/2/96924a772ebf72c7fb7a1a606ed4694a

Mouse-liver [gamma]-glutamyl hydrolase (GH) is a lysosomal endopeptidase with an acid pH optimum that is activated by sulfhydryl compounds and preferentially hydrolyzes the most proximal [gamma]-glutamyl linkage of longer chain polyglutamates of folates and their analogues. We describe the cloning of this mouse lysosomal cDNA enzyme from liver GH mRNA in the form of two cDNA variants (1.295 and 1.268 kb in length) differing 14-fold (Variant I versus Variant II) in relative frequency that exhibited 5'-end heterogeneity and encoded alternate leader peptides. The 5' UTR in these variants also differs in length by 27 nucleotides. Otherwise, the ORF and 3' UTR in each case are the same. These cDNAs encode a protein in which the deduced amino acid sequence shares 78.9 and 69.1% identity to rat and human GH sequences, respectively. Amino acid sequence comparisons among the three species identified three conserved Asn sites and two conserved Cys residues that may be sites of glycosylation and sulfhydryl compound
activation, respectively. Variant I GH mRNA was more abundant than Variant II GH mRNA in all mouse tissues examined. Variant I GH mRNA levels were extremely high in salivary gland, moderately high in kidney, liver, lung, stomach and uterus, low in small intestine, brain and fetal liver and relatively rare in thymus, spleen and skeletal muscle. Abundance of GH mRNA among tumors varied from low to high, with no discernible correlation with their tissue of origin.


http://www.sciencedirect.com/science/article/B6T39-47PH672-14V/2/30b8de2ba290dfe9e726e898585b609a

The attempts at identifying precise replication origins (ori) in mammalian DNA have been pursued mainly through physico-chemical and biochemical approaches, in view of the essential failure of the search for autonomously replicating sequences in cultured cells. These approaches involve the mapping of short stretches of nascent DNA, the identification of the regions where either leading or lagging strands switch polarity, or the localization of replication intermediates by two-dimensional gel electrophoresis. Due to the complexity of animal cell genomes, most of these studies have been performed on amplified domains and with the use of synchronization procedures. The results obtained have been controversial. In order to avoid the use of experimental procedures potentially affecting the physiological mechanism of DNA replication, we have developed a method for the localization of ori in single-copy loci in exponentially growing cells. This method entails the absolute quantification of the abundance of selected DNA fragments along a genomic region within samples of newly synthesized DNA by competitive polymerase chain reaction (PCR); the latter is immune to all the uncontrollable variables which severely affect the reproducibility of conventional PCR. The application of this method to SV40 ori-driven plasmid replication precisely identifies the known ori localization. Using the same approach, we have mapped an ori for bi-directional DNA replication in a 13.7-kb locus of human chromosome 19 encoding lamin B2.


http://www.sciencedirect.com/science/article/B6T39-4019DM2-6/2/3a60fcee44bc86600d08aee94c7508ba3


http://www.sciencedirect.com/science/article/B6T39-4CNJD7P-1/2/f5e8fa800aab3b441ccb3a569bd47c14

Because of its unusual high degree of compaction and paucity of repetitive sequences, the genome of the smooth pufferfish Tetraodon nigroviridis is the subject of a well-advanced sequencing project. An astonishing diversity of transposable elements not found in the human and the mouse has been observed in the genome of T. nigroviridis. Due to the difficulty of assembling repeat-rich regions, the whole genome shotgun sequencing approach will probably fail to reveal the general organisation of this compact vertebrate genome. Therefore, in order to
gain new insights into the global distribution pattern of repeated DNA in the genome of T. nigroviridis, we have reconstructed partial/complete repetitive sequences from data generated by the genome project and performed double-colour fluorescent in situ hybridization (FISH) analysis for representatives of three major categories of repeated sequences including two minisatellites (ms100 and ms104), two DNA transposons (Tol2 and Buffy1) and two non-long terminal repeat (LTR) retrotransposons (Rex3 and Babar). We show that DNA transposons and retroelements very frequently colocalize with minisatellites and mostly accumulate within heterochromatic regions. These results, which have not been reported so far for the fugu Takifugu rubripes, show that repeated elements are generally excluded from gene-rich regions in T. nigroviridis and underline the extreme degree of compartmentalization of this compact genome. The genome organization of the pufferfish is clearly different from that observed in humans, where repeated sequences make up an important fraction of euchromatic DNA, and is more similar to that observed in the fruit fly Drosophila melanogaster.


http://www.sciencedirect.com/science/article/B6T39-402KBCD-P/2/b88fc71c56e3700a684412ce292ebf65


http://www.sciencedirect.com/science/article/B6T39-4FC449B-5/2/08e436b9444deb163367b2c51b9c7613e

The involvement of clay surfaces in the origin of the first genetic molecules on Earth has long been suggested. However, the formation of these polymers was not sufficient by itself to initiate the evolutionary process leading to the appearance of life. These macromolecules had to persist in primeval habitats so that their biological potentiality could be expressed. In this study, we assess the possibility of development of the RNA world on a clay substrate by investigating the capacity of different RNA molecules adsorbed/bound on the clay minerals montmorillonite (M) and kaolinite (K) to persist in the presence of a degrading agent (RNase-A), to interact specifically with complementary RNA strands, and to transmit the information contained in their nucleotide sequences. The RNase-A degradation of clay-adsorbed 23S rRNA from Escherichia coli was significantly slower (75-80%) than that observed for free rRNA, and the complete digestion of nucleic acid in the presence of clay was obtained in 2 vs. 1 h. Clay-adsorbed Poly[A] homopolymer was able to recognize the complementary Poly[U] homopolymer present in the surrounding water solution and to establish a specific interaction (association) with it, possibly leading to the formation of double strands. Reverse transcription and amplification (RT-PCR) amplification of free and clay-adsorbed 16S indicated that the presence of clay particles partially reduced the efficiency and processivity of reverse transcriptase but did not inhibit its activity, demonstrating that clay-adsorbed RNA is still available for enzymatic replication. These findings indicate that primordial genetic molecules adsorbed on clay minerals would have been protected against degrading agents present in the environment and would have been in the right conditions to undergo evolutionary processes.

We have isolated the entire coding sequence of human FRAT2 (frequently rearranged in advanced T-cell lymphomas-2). It exhibits appreciable amino acid identity to FRAT1 (77%) which was initially isolated as frequently being overexpressed in a murine leukemia virus insertion model in murine tumors. FRAT proteins are thought to play a role in Wnt signaling. They can bind to glycogen synthase kinase-3 (GSK-3) and Dishevelled, two proteins involved in Wnt signal transduction. Both hFRAT1 and hFRAT2 are intronless genes localized to the same portion of chromosome 10q24.1 and separated by only 10.7 kb. In a broad range of human tissues FRAT1 and FRAT2 are readily detected and expressed in a near identical pattern. Both species are repressed when the human embryonal carcinoma cell line, NT2/D1, is induced to differentiate with all-trans retinoic acid (RA). This treatment had no appreciable effect on FRAT levels in two other RA-sensitive cell lines that were not of germ cell tumor origin. The overlapping expression patterns suggest these two genes share a regulatory region. Both FRAT genes exhibited three species of mRNA, which varied in representation between tissues. When transiently overexpressed in COS-1 cells, the FRAT proteins were detected in the cytosol and concentrated in the nucleus. Both hFRAT1 and hFRAT2 are implicated in the selective modulation of GSK-3 activity via the Wnt signaling pathway. This study provides a foundation from which to examine the role these proteins play in Wnt-dependent and -independent processes.


Since osteogenic sarcoma (OGS) predominantly affects children, its etiology and progression may be determined more by genetic than environmental factors. A few genes have been associated with OGS, however, their value in the diagnosis and/or prognosis of the disease remains poor. Evidently, more markers need to be identified for improving management of patients with OGS. To identify potential genetic markers for OGS, we have extended preferential amplification of coding sequences (PACS) to screen multiple samples simultaneously. The extended method is termed multi-PACS. Multi-PACS was applied between a normal osteoblast and four OGS-derived cell lines to identify differentially expressed coding sequence tags (dCST) that identified 145 dCSTs. Subsequently, differential mRNA expression was validated for a chosen subset of 22 dCSTs. These chosen dCSTs include among others cyclins D and E, two
cyclin dependent kinases, two other kinases, transcription factors E2F4, E2F5, and p130, a DNA repair gene, a gene for the signalosome subunit, and potential guanine nucleotide binding factors. We infer that these genes could be so easily identified because PACS preferentially identifies coding instead of non-coding sequences. We also infer that these genes identify signaling pathways pertinent to OGS. mRNA expression profile of these 22 genes/dCSTs generated distinct expression signature of the OGS-derived cell lines suggesting that further work on clinical samples with these dCSTs will yield valuable information for OGS. We conclude that these 22 genes/dCSTs are candidate markers for OGS.


http://www.sciencedirect.com/science/article/B6T39-3TVNRN6-G/2/623cda9603b5014f8c455b25d9355aa3

Tumor necrosis factor stimulated gene-6 (TSG-6) has been previously shown to be induced in vitro in several cell types by proinflammatory cytokines, and in vivo in pathological conditions such as rheumatoid arthritis. In this study, we report the complete coding sequence for the mouse TSG-6 protein, and the exon-intron structure and the chromosomal localization of the gene. We have identified a 1605 nt cDNA sequence from mouse cumulus cell-oocyte complexes (COCs) induced to expand in vivo. The sequence contains an open reading frame of 825 nt that codes for the 275 amino acid TSG-6 protein. The gene contains six exons separated by 1.1-5.8 kb introns and has been localized to the murine chromosome 2 by linkage analysis. Comparative reverse transcription-polymerase chain reaction studies have revealed that TSG-6 mRNA is specifically expressed after COC expansion induced in vivo, identifying the first non-pathological process in which TSG-6 may play an important role. Since TSG-6 binds to hyaluronan and interacts with inter-[alpha]-trypsin inhibitor ([alpha]I), molecules that are essential for matrix formation by COCs, this protein may have a structural role in the matrix or may enhance the antiproteolytic effect of [alpha]I to protect the matrix from degradation.


http://www.sciencedirect.com/science/article/B6T39-4F0GR5F-3/2/eac48deaffbd41945402580780a1d27b

Loci for several human genetic diseases including glaucoma have been mapped to q23 region on chromosome 8. We carried out homology search analysis of the genomic sequence of a bacterial artificial chromosome (BAC) clone, KB1590E11, on 8q23 region, and mapped a previously described cDNA, KIAA1472, to this BAC clone. In this study, we determined the complete genomic structure of the KIAA1472 gene and its expression in various tissues and cell lines. Four mRNA species (types 1a, 1b, 1c, and 2) were produced from this gene by use of alternative transcription start sites and alternative-splicing events. These mRNAs were expressed in various tissues, except for type 1a, which was found only in the brain. Further, type 1 mRNA could be translated into two protein isoforms with different N-terminal sequences; and type 2 mRNA, into another type of isoform. All three of these KIAA1472 gene products were localized in Golgi apparatus and contained a C-terminal hydrophobic segment characteristic of a transmembrane domain, thus indicating them to be Golgi membrane-bound proteins. Furthermore, these proteins were homologous to syntaphilin, a molecule involved in guiding vesicular transport. These results indicate that KIAA1472 gene products may play an important role in vesicular traffic in various tissues including the brain.

http://www.sciencedirect.com/science/article/B6T39-3YS34MW-5/2/9b74818782fd75c9df13fb653e1351c1


http://www.sciencedirect.com/science/article/B6T39-433NSPG-D/2/b0404bd0eca08cdc5ddced60c60956e1

We cloned the full-length cDNA of max gene from the common carp (Cyprinus carpio). The cDNA clone of carp max consists of 1209 bp and contained an ATG-initiated ORF consisting of 156 aa. The carp MAX share 76.7-93.8% aa identity with those of human, mouse, rat, chicken, Xenopus and zebrafish, respectively. The 15 bp alternative splicing was observed in the loop region of helix-loop-helix and is not previously described in mammalian max sequences. Transcripts of max gene were observed in all of the tissues of carp investigated in this study. The highest expression was found in the ovary, and the transcripts in hepatopancreas and heart were low. Two carp c-myc genes (CAM1 and CAM2) showed differential expression pattern. The expression of max was concomitant with CAM2 expression, but not with CAM1. It has been reported that MYC/MAX heterodimer as a regulator of gene expression has been maintained throughout vertebrate evolution, and the expression of c-myc has been concomitant with max expression. In addition, according to phylogenetic analysis, CAM1 is evolving faster than CAM2 after gene duplication. Therefore, this result suggests that CAM1 may evolve to obtain a new function different from c-myc.


A novel plasmid vector, composed of a 1.7-kb Bacillus thuringiensis (B.t.) replicon, a multiple cloning site, and an erythromycin-resistance marker gene from Bacillus subtilis, was constructed for use in B.t. Unlike other vectors which have been reported to be acceptable for B.t., this new B.t. vector was stably maintained in the absence of Er and did not displace host plasmids, some of which carry crystal protein-encoding genes (cry genes). The compatibility of this B.t. vector with native plasmids is highly desirable when introducing new cry genes into a wild-type B.t. strain. When a cryIIA gene of B.t. tenebrionis was cloned in this vector and introduced into B.t. kurstaki (kur) HD119, cryIIA was highly expressed without affecting the level of expression of native cry genes. The stability of this vector and its compatibility with native B.t. plasmids were achieved by subcloning only nucleotide sequences required for the vector to replicate in B.t. The origin of replication was first cloned on a 9.6-kb BglII fragment from a 75-kb plasmid of B.t. kur HD73 and then localized to a 2.4-kb region within the 9.6-kb fragment. Sequencing of the 2.4-kb region revealed the presence of an open reading frame (ORF), encoding a putative 312-amino acid (aa) protein. The deduced aa sequence of the ORF showed no homology to any published aa sequences. Deletion analysis indicated that the B.t. vector required at least the ORF and up to 300 bp surrounding the ORF, in order to replicate.

http://www.sciencedirect.com/science/article/B6T39-40XNXHB-H/2/51c323c80d584d639043dd2d0d0362de


http://www.sciencedirect.com/science/article/B6T39-4DW90R3-2/2/5d62df8ed8a21aba9c65b1b51177e2c6

Here we report the identification and expression analysis of two novel human genes—DIPLA1 (Differentially expressed in placenta 1) and DIPAS (DIPLA1 Antisense). These genes are located at chromosomal region 9q33.1, in opposite orientations, and are flanked by the pregnancy-associated plasma protein-A (PAPP-A) and astrotactin 2 (ASTN2) genes. The mRNA sequences of both genes contain several upstream AUGs (uAUG) and various potential open reading frames (ORFs). DIPLA1 mRNA is 1.8 kb long and contains a 285 nt ORF coding for a polypeptide designated as replicative senescence up-regulated (RSU) protein. Antisense DIPAS mRNA is 2.7 kb long and contains a 309 nt ORF coding for a protein with partial similitude to the [gamma] isoform variant of the human Ca2+/calmodulin (CaM)-dependent protein kinase II. Both genes are conserved in placental-species and are presumably transcribed from initiator (Inr) promoter elements located at opposite strands. In 20 human normal tissues tested, DIPLA1 mRNA expression was placenta-specific, whereas DIPAS mRNA expression was higher in placenta, brain, kidney and testis. In addition, DIPAS mRNA hybridizes with the 3'UTR region from PAPP-A mRNA, which spans over 4 kb more than previously reported, forming a potential sense-antisense double stranded RNA (dsRNA) duplex. Our results are of interest for placenta gene expression regulation and for the identification of novel genes in the human genome.


http://www.sciencedirect.com/science/article/B6T39-41N572W-D/2/25c21946afcb005fd86cd5c7a9341210


http://www.sciencedirect.com/science/article/B6T39-3Y6HGRC-F/2/07ce763d58b3fc99c5c551317853c388

An excellent model for studying heart development in vertebrates is the cardiac non-function lethal mutant (gene c) Mexican axolotl, Ambystoma mexicanum. In order to facilitate our analyses of the mutant system, we have undertaken a search for stage-specific molecular markers during embryonic development of the axolotl. We have concentrated on homebox genes ‘as suitable candidates for monitoring molecular changes during development. A 270-bp probe encoding a
portion of the axolotl homeobox gene Ahox-1 was generated by PCR from a stage-18 axolotl embryonic cDNA library. 32P-labelled PCR-amplified Ahox-1 DNA was used as the probe for screening a [lambda]AM18 cDNA library using moderately stringent conditions. We isolated six clones and determined their partial nucleotide (nt) sequences. One of the clones, which has very high homology to human, mouse and rat Hox A5 (83 and 99% at the nt and amino-acid levels, respectively, in the homeodomain region), was analyzed further. RT-PCR analyses show that the level of expression of HoxA5 is very low at stage 11 of embryonic development (gastrula). The level of expression reaches maximum at stage 25 (tailbud) and then plateaus at stages 30 and 35 (heartbeat onset). Although the expression of Ahox-1 was also found to start at stage 11, it reaches a maximum level at stage 25 and declines at stage 35. We have also studied, using RT-PCR, the tissue-specific expression of HoxA5 and Ahox-1 in juvenile axolotl.


http://www.sciencedirect.com/science/article/B6T39-3Y6HK60-52/2/998cedab88b0d2cf538df71cbd7ca4d9

Using a reverse transcription-polymerase chain reaction (RT-PCR) procedure that exploited the presence of a conserved 22-nucleotide spliced leader (SL) sequence that is trans-spliced to the 5' end of nematode transcripts, a novel Brugia malayi (Bm) infective-stage SL cDNA expression library was constructed and characterized. The library was immunoscreened with rabbit anti-infective-stage antibodies (Ab) and an immunodominant clone, BmG4-7, was identified and characterized. BmG4-7 contained a full-length cDNA that had significant sequence similarity to nucleoside diphosphate kinase (NDK)-encoding sequences reported from a number of species, including Drosophila melanogaster and humans. BmNDK was found to be constitutively transcribed during all stages of parasite development. An anti-BmNDK Ab was used to immunostain a Western blot of extracts from adult and larval parasites. The Ab specifically recognized a 17.5-kDa molecule in all of the parasite extracts. Molecular modeling of the BmNDK showed several regions surrounding the conserved catalytic site that may be important in the design of drugs specific for the disruption of NTP synthesis in filarial parasites.


http://www.sciencedirect.com/science/article/B6T39-44357HF-9/2/65f075b208ede1d2f2699ea599a99074

Mono(ADP-ribosyl)transferases regulate the function of target proteins by attaching ADP-ribose to specific amino acid residues in their target proteins. The purpose of this study was to determine the structure, chromosomal localization, and expression profile of the gene for mouse ecto-ADP-ribosyltransferase ART5. Southern blot analyses indicate that Art5 is a single copy gene which maps to mouse chromosome 7 at offset 49.6 cM in close proximity to the Art1, Art2a and Art2b genes. Northern blot and RT-PCR analyses demonstrate prominent expression of Art5 in testis, and lower levels in cardiac and skeletal muscle. Sequence analyses reveal that the Art5 gene encompasses six exons spanning 8 kb of genomic DNA. The 5' end of the Art5 gene overlaps with that of the Art1 gene. A single long exon encodes the predicted ART5 catalytic domain. Separate exons encode the N-terminal leader peptide and a hydrophilic C-terminal extension. Sequencing of RT-PCR products and ESTs identified six splice variants. The deduced amino acid sequence of ART5 shows 87% sequence identity to its orthologue from the human, and 37 and 32% identity to its murine paralogues ART1 and ART2. Unlike ART1 and ART2, ART5 lacks a glycosylphosphatidylinositol-anchor signal sequence and is predicted to be a
secretory enzyme. This prediction was confirmed by transfecting an Art5 cDNA expression construct into Sf9 insect cells. The secreted epitope-tagged ART5 protein resembled rat ART2 in exhibiting potent NAD-glycohydrolase activity. This study provides important experimental tools to further elucidate the function of ART5.


Insect odorant-binding proteins (OBPs) are thought to facilitate the delivery of hydrophobic odorants, such as sex pheromones or food odors, to receptors on sensory neurons. Increasingly, OBP family members are also being found in non-sensory tissues where they might carry other types of small hydrophobic molecules. They are identifiable by four or six conserved Cys residues and contain six [alpha]-helices which enclose a hydrophobic ligand-binding pocket. Through exhaustive BLAST searches we have increased the total number of OBPs identified in Drosophila melanogaster to 38, and have amplified the DNA complementary to RNA corresponding to 21 of these by reverse transcriptase polymerase chain reaction. Isoforms frequently share less than 30% amino acid identity and appear to have radically changed since the separation of the major insect orders. However, their sequences are consistent with known OBP structures. Most are located in clusters of between four and 14 genes and several were unusual in that they contained additions, deletions, or fusions. These hexa-helical insect OBPs are structurally unrelated to the functionally analogous lipocalin-like [beta]-barrel OBPs of vertebrates. As only two lipocalin-like proteins have been found in D. melanogaster, these helical proteins appear to be the dominant carrier of small hydrophobic molecules in insects.


http://www.sciencedirect.com/science/article/B6T39-45HWVR3-7/2/c6ade71ca6644518d4d2c973d3db9f7f

The cloning and sequencing of complementary DNAs corresponding to the two copies (a and b) of the Xenopus laevis gene for hnRNP E2 is presented. Comparison of the two sequences reveals that while they are somewhat divergent at the nucleotide level, they are very conserved at the amino acid level. The analysis also showed two transcripts of different length ([alpha] and [beta]), likely generated by alternative processing. There are indications that either gene copy can generate both type of transcripts. Northern blot analysis in oocytes and developing embryos showed that hnRNP E2 RNA is constantly present and that increases in amount at tadpole stage. A semiquantitative reverse transcriptase polymerase chain reaction analysis performed with RNA from developing embryos showed that long ([alpha]) transcript accumulation is constant during development, whereas the short one ([beta]) accumulation increases at later stages, thus determining the observed increase in total RNA. Nucleo-cytoplasm localization experiments indicated that in oocyte hnRNP E2 is exclusively cytoplasmic, whereas in somatic cells it is distributed in both compartments. Comparison of the amino acid sequence of the two X. laevis hnRNP E2 with the corresponding mammalian sequences shows a high homology along the molecule except for the region subjected to alternative splicing, which is completely different. Moreover, there are indications that the homologous of mammalian hnRNP E1 gene, very related to and derived from hnRNP E2 by retrotransposition, is not expressed or even not present in X. laevis, suggesting that mammalian hnRNP E1 gene may have originated after mammal/amphibia divergence.
In a study aimed at characterising, at the molecular level, the obligate biotrophic fungus *Blumeria graminis* f. sp. *hordei* (Bgh), we have identified a novel group of genes, the Egh16H genes, and shown that two of these are up-regulated during primary infection of barley leaves. The genes have partial homology to a previously characterised Bgh gene family, Egh16. Egh16 and Egh16H are subfamilies of a larger multigene family with presently about 15 members identified in Bgh. Egh16H has about ten members, and we show that five of these are expressed as highly conserved mRNAs that are predicted to encode proteins with a C-terminal variable region. Egh16H has high homology to sequences in *Magnaporthe grisea* and other plant pathogenic fungi, as well as sequences of both the insect pathogen *Metarhizium anisopliae* and the human pathogen *Aspergillus fumigatus*. No close homologues of Egh16H were found in the non-pathogenic fungi *Neurospora crassa* and *Aspergillus nidulans*. We predict that Egh16H plays a general role in the interaction between pathogenic fungi and their hosts. At present, the large number of gene family members with C-terminal variation appears to be unique for Bgh, and the Egh16/Egh16H gene family is to our knowledge the largest gene family so far characterised in this fungus.

The *Ngoll* restriction-modification (R-M) system of *Neisseria gonorrhoeae* recognizes the sequence 5'-GGCC-3'. This system is encoded by two separate genes, dcmB for the methyltransferase (MTase) and dcrB for the restriction endonuclease (ENase). Three strains that vary in their *Ngoll* phenotype were examined. Strain Pgh3-2 produced detectable levels of both enzymes, strain F62 lacked detectable levels of the dcrB gene product, and strain WR302 failed to produce either gene product. Strains that lacked either enzyme activity still possessed the genes that encode them. Transcriptional fusions of dcrB in strains F62 and Pgh3-2 indicate that this gene is transcribed at nearly identical levels in each strain. The DNA encoding the *Ngoll* R-M system was cloned from the three strains, and the nucleotide sequence was determined. The dcrB genes of WR302 and F62 possess the same frameshift mutation (base position 1435) which would result in a truncated protein. The WR302 dcmB was found to have a point mutation that changed Arg288 (a residue that is conserved in all prokaryotic and phage cytosine MTases sequenced to date) to Trp.

The polymerase chain reaction (PCR) and amplification of specific regions of DNA in vitro is a
widely used and powerful technique, and the optimization of conditions used to maximize PCR product yield has received much attention. We have shown that lengthy denaturation times of template DNA ranging from 1 to 7 min at pH 7.0-8.0, that are often employed prior to the start of a PCR reaction, result in marked degradation of the template. This can result in a significant reduction in the yield of PCR products larger than 500 bp, by up to 99%. This effect was demonstrated for both complex genomic template DNA, and also for a 2691-bp linear piece of template DNA using both a rapid hot-air thermocycler and a conventional block thermocycler. This decrease in product yield is likely due to the increased degradation of the template or target DNA as a result of pre-amplification denaturation (PAD). We therefore recommend that when amplifying larger pieces of DNA, the template DNA should not be exposed to PAD prior to a PCR reaction, irrespective of the starting pH of the template solution.


http://www.sciencedirect.com/science/article/B6T39-44KWSWR-1/2/dd8a2f2ea3cb776b8732c17a27f17664

cDNA for rat transcription factor IIIA (TFIIIA) was cloned by degenerate PCR and rapid amplification of cDNA ends. This cDNA coded for a protein with nine Cys2His2 zinc fingers and a non-finger C-terminal tail, 63% amino acid (aa) sequence identity was observed with the Xenopus TFIIIA zinc finger region. Recombinant rat protein containing only the nine fingers afforded DNase I protection of the identical nucleotides protected by Xenopus laevis native TFIIIA on the Xenopus 5S RNA gene internal control region. A putative mouse TFIIIA clone was identified in an expressed sequence tag database by sequence similarity to rat TFIIIA. Recombinant nine-finger protein from this clone afforded DNase I protection of the Xenopus 5S rRNA gene like the native frog protein as did a recombinant nine-finger-form of a putative human TFIIIA clone. These DNA binding results demonstrate that these clones code for the respective mammalian TFIIIA.


http://www.sciencedirect.com/science/article/B6T39-3Y9GDS5-R/2/2d3ff905043c104a2ebc76f0ee75c0096

Investigations into the mechanisms and properties of gene conversion in mammals are greatly restricted by the inability to recover all the products of a meiosis. Additionally, the study of this process has been hampered by the lack of visible markers to detect gene conversion, especially when the events are rare. In previous work, we developed a transgenic system for detection and quantitation of gene conversion events in the germline of mice (Murti, J.R., Bumbulis, M., Schimenti, J.C., 1992. High frequency germline gene conversion in transgenic mice. Mol. Cell. Biol. 12, 2545-2552) that could be exploited as an assay for recombinogenic chemicals (Murti, J.R, Schimenti, K.J., Schimenti, J.C., 1994. A recombination-based transgenic mouse system for genotoxicity testing. Mutat. Res. 307, 583-595). A specific intrachromosomal gene conversion
event between two complementarily defective lacZ genes resulted in the production of [beta]-galactosidase in spermatids, enabling a measurement of conversion frequency. Here, we report that the anticancer drug, cisplatin, increased gene conversion in meiotic stage cells in these transgenic mice. Furthermore, a method was developed for direct molecular analysis of transgene conversion events in single or pooled lacZ-positive spermatids. The ability to identify gametes that have undergone a rare gene conversion event, followed by molecular amplification of the recombinant gene, should make it possible to investigate the mechanisms of genetic recombination in mammals in greater detail than previously possible.


http://www.sciencedirect.com/science/article/B6T39-3W496YH-4/2/8de1a79cf220242b82fb17bad21d7f69

From libraries made from activated mouse T lymphocytes, we have isolated cDNAs encoding Wnt-10B, a new member of the Wnt family of developmental control genes. This protein appears to be the mammalian orthologue of Wnt-10B, first identified in several non-mammalian vertebrates and recently in mouse. The mRNA expression pattern of mouse Wnt-10B indicates that it is induced following activation of helper T cells, but is also expressed in a variety of other tissues and cells of fetal or adult origin. 93 bp at the 5’ end of the cDNA clone are identical to sequences previously reported as 3’ flanking genomic DNA adjacent to a mouse mammary tumor virus (MMTV) provirus in the MMTV-induced BR6 mammary tumor, W26. Sequence analysis of tumor-derived genomic DNA confirms that the entire Wnt-10B gene is immediately adjacent to the provirus, suggesting that MMTV integration drives transcription of Wnt-10B, possibly contributing to the oncogenic process. Consistent with this idea is the detection of hybrid MMTV-Wnt-IOB transcripts in BR6 tumor cells. T cells which produce abundant Wnt-10B mRNA were also found to produce protein.


A multi-gene family (Cetn1, Cetn2, and Cetn3) encodes the calcium-binding protein, centrin, in the mouse. This work characterizes the Cetn2 gene. Structurally, Cetn2 consists of five exons and four introns, and contains a classical TATA-less promoter. Cetn2 has two alternate transcription start sites, and a single length 3’ untranslated region. Fluorescence in situ hybridization demonstrates that Cetn2 is an X-linked gene whose alleles replicate asynchronously during S-phase. Cetn2 encodes a 172 amino acid protein, with a predicted molecular mass of 19,795 Da (pl=4.71), that contains all of the defining characteristics of centrin. Northern blot analysis indicates that Cetn2 is ubiquitously expressed in the tissues of adult mice. RT-PCR shows that Cetn2 and Cetn3, but not Cetn1, are expressed in NIH 3T3 cells. Immunofluorescence microscopy demonstrates that mouse centrin 2 protein localizes to the region immediately surrounding the centrioles in the centrosome of NIH 3T3 cells.

Highly degenerate primers to conserved regions of the eukaryotic phosphoinositol-specific phospholipase C (PLC) were used to amplify fragments of plant PLCs from Arabidopsis thaliana genomic DNA. Eight completely different fragment sequences that showed high homology to PLCs of both animals and plants were isolated. The variation between these putative PLCs was high and suggests that, like animals, plants have multiple isoforms of PLC. Using one of the PCR clones, we isolated a corresponding full-length Arabidopsis PLC gene (ATHATPLC1G), and sequence analysis indicated that it was most like a delta-type PLC. This gene is 2.5 kb and contains seven introns, all but one of which has intron/exon border sequences that conform to the Arabidopsis consensus. The structural complexity of the gene is relatively simple compared to mammalian [beta]-type PLCs that can be 15 kb long with up to 30 introns. The plant gene is a single copy and was mapped to four Arabidopsis YACs, one located on chromosome 2. The promoter region contained two TATA-like elements at -43 and -185 and other putative regulatory elements that suggest that this PLC is hormonally regulated. This is the first plant PLC gene and the first delta type-PLC gene from a higher organism to be sequenced.


Many proteins required for neurotransmission are homologous to proteins involved in the Golgi-to-plasma membrane stage of the yeast secretory pathway. A novel 17S complex composed of eight proteins including rsec6 and rsec8, the rat homologues of the yeast secretory proteins, Sec6p and Sec8p, has been identified in rat brain cytosol. Sec6p and Sec8p are components of a complex of at least seven proteins which are essential for secretion in yeast. While the complementary DNAs (cDNA) encoding rsec6 and rsec8 have been cloned [Ting et al. (1995) Proc. Natl. Acad. Sci. USA 92, 9613-9617], the other six components of the 17S complex remain undescribed. Using the peptide sequence obtained from p71, one of the subunits of the rat brain 17S complex, we isolated a full-length cDNA from a rat brain library. This cDNA is predicted to encode a hydrophilic protein of 82 kDa, similar in size to that observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for the endogenous rat brain rsec6/8 complex 71 kDa component. p71 contains domains of peptide sequence which display significant homology to regions of the tail domain of yeast type II myosin. Northern blot analysis of rat tissues indicates that messenger RNA transcripts of 3.1 and 4.4 kb encoding this protein are expressed broadly across several rat tissues in a pattern similar to that of rsec6 and rsec8 mRNA expression. A possible role for p71 as a point of interaction for proteins of the cytoskeleton and proteins involved in secretion is discussed.


Phenol sulfotransferases (PST) esterify hydroxylated aromatic compounds with sulfate, and therefore play a role in the metabolism of xenobiotics. In this investigation, a bovine PST gene of 2372 bp was amplified from genomic DNA. Sequence overlap with the cognate cDNA revealed
seven exons, with all introns containing GT/AG splicing donor/acceptor sites. This product was subcloned into an SV40-based expression vector and transfected into WI-26 human lung fibroblasts. Immunoblot analyses revealed production of the anticipated 32-kDa protein, and the active enzyme displayed steady-state kinetic properties consistent with the enzyme characterized in bovine lung (apparent Km=6.5 [μM] for 2-naphthol).


http://www.sciencedirect.com/science/article/B6T39-47T307S-1F/2/ae3de030d0e5e807b96cd6b7eaa02879

The constructions of deletions, insertions and point mutations in DNA sequences is a powerful approach to analysing the function and structure of genes and their products. Here, we present a fast and efficient method using the polymerase chain reaction to introduce mutations into cDNAs coding for the [alpha]-, [gamma]- and [epsiv]-subunit of the rat muscle acetylcholine receptor. Two flanking primers and one mutant oligo, in conjunction with supercoiled plasmid DNA and a fragment of the target DNA are sufficient to introduce the mutation by two PCR amplifications. Our method permits directing the location of mutations anywhere in the target gene with a very low misincorporation rate, as no substitution could be detected within 9600 bp. The utility of this approach is demonstrated by the rapid introduction and analysis of eleven mutations into three different cDNAs. Any kind of mutation can be introduced with an efficiency of at least 50%.


http://www.sciencedirect.com/science/article/B6T39-454TC74-7/2/94ee95d56e840ea093728c3194787805

The nasal epithelium is an important target site for chemically-induced toxicity and carcinogenicity in rodents. Gene expression profiles were determined in order to provide normal baseline data for nasal transitional/respiratory epithelium from healthy rats. Cells lining the rat nasal passages were collected and gene expression analysis was performed using Clontech(TM) cDNA Rat Atlas 1.2 arrays (1185 genes). The percentages of genes within specific average expression ranges were 4.2% at 45,000-1000, 14.8% at 1000-200, 25.0% at 200-68, and 56.0% below 68. Nine out of a subset of ten genes were confirmed for relative signal intensity using quantitative real-time RT-PCR. The most highly expressed genes included those involved in phase I (e.g. cytochrome P450s) and phase II (e.g. glutathione S-transferases) xenobiotic metabolism, bioenergetics (e.g. cytochrome oxidase), osmotic balance (e.g. Na+/K+ ATPase) and epithelial ionic homeostasis (e.g. ion channels). Such baseline data will contribute to further understanding the normal physiology of these cells and facilitate the interpretation of responses by the nasal epithelial cells to xenobiotic treatment or disease.


http://www.sciencedirect.com/science/article/B6T39-3TDHM52-D/2/ee75ea90215dabda335b2fb837468f43
A gene encoding a novel transmembrane protein was identified by DNA sequence analysis within the insulin-dependent diabetes mellitus (IDDM) locus IDDM4 on chromosome 11q13. Based on its chromosomal position, this gene is a candidate for conferring susceptibility to diabetes. The gene, termed low-density lipoprotein receptor related protein 5 (LRP5), encodes a protein of 1615 amino acids that contains conserved modules which are characteristic of the low-density lipoprotein (LDL) receptor family. These modules include a putative signal peptide for protein export, four epidermal growth factor (EGF) repeats with associated spacer domains, three LDL-receptor (LDLR) repeats, a single transmembrane spanning domain, and a cytoplasmic domain. The encoded protein has a unique organization of EGF and LDLR repeats; therefore, LRP5 likely represents a new category of the LDLR family. Both human and mouse LRP5 cDNAs have been isolated and the encoded mature proteins are 95% identical, indicating a high degree of evolutionary conservation.


Phosphomannomutases catalyze the reversible conversion of mannose 6-phosphate to mannose 1-phosphate. In humans, two different isozymes have recently been identified, PMM1 and PMM2. We have previously shown that mutations in the PMM2 gene cause the most frequent type of the congenital disorders of glycosylation, CDG-Ia. Here, we present data on the two mouse orthologous genes, Pmm1 and Pmm2. The chromosomal localization of the two mouse genes has been determined. We also present the gene structure and the exon-intron organization of Pmm1 and Pmm2. Pmm1 maps to mouse chromosome 15, Pmm2 to chromosome 16. These chromosomal regions are syntenic with regions on human chromosomes 22 and 16, respectively. The Pmm1 gene is composed of eight exons and spans approximately 9.5 kb. The genomic structure is extremely well conserved between the human and mouse gene. The Pmm2 gene consists of eight exons and spans a larger genomic region (ap20 kb). An alignment of the human and mouse protein sequences confirms the conservation among this family of phosphomannomutases. The two mouse genes are expressed in many tissues, but the expression pattern is slightly different between Pmm1 and Pmm2. The most striking difference is the high expression of Pmm1 in brain tissue, whereas Pmm2 is only weakly expressed in this tissue.


Extrachromosomal DNA forms of Drosophila retrotransposons (RTn) and retroviruses have been extensively analyzed. However, no such analysis with plant RTn has been reported. Here, we report the analysis of extrachromosomal forms of the tobacco RTn Ttol. Ttol is one of a few active RTn of plants and has been shown to be activated in tissue culture. Extrachromosomal circular DNA forms of Ttol, with one or two long terminal repeats (LTR), were found in cultured cells. Sequence analysis of the sites of circularization through joining two LTR showed that the junction between the LTR contains small deletions and/or insertions. The insertions are heterogeneous and do not show any homology to the Ttol sequence. Similar insertions have been detected in the extrachromosomal circular forms of the copia element of Drosophila and suggested to be the result of excision of genomic copia. The structural features of the junctions found in Ttol suggest
that the insertions are produced by a mechanism other than excision. The potential mechanism of production of the extrachromosomal circular forms of Ttol is discussed.


http://www.sciencedirect.com/science/article/B6T39-3Y5MNR1-7T/2/e8fc930799a698d93a793fada920dc22

We have identified and cloned a novel member of the nuclear receptor superfamily from murine testis, referred to as retinoid receptor-related testis-associated receptor or RTR. Degenerate PCR primers homologous to two conserved regions of the DNA-binding domain of members of this superfamily were employed to identify this gene. The amino-acid sequence of RTR is most closely related to that of the mouse RXRs with an overall identity of 32-34%; the highest similarity (61%) is observed in the DNA-binding domain. Northern blot analysis using RNA from multiple tissues showed that RTR is predominantly expressed in the testis. Northern blot analysis using RNA from different testicular cell types showed that RTR mRNA is not expressed in early germ cells or Sertoli cells but is most abundant in round spermatids. Our observations suggest that this putative transcription factor plays a role in the regulation of gene expression particularly during the post-meiotic phase of spermatogenesis.


http://www.sciencedirect.com/science/article/B6T39-47PNXG6-HG/2/00af5a9919995f88086ab24554a03b26

The gene (cht60) encoding N-acetyl-[beta]-glucosaminidase (Cht; EC 3.2.1.30) from the marine bacterium Alteromonas sp. strain O-7 was cloned into pUC18 in Escherichia coli JM109. The nucleotide (nt) sequence of cht60 was determined. A 1797-bp open reading frame encoded a polypeptide of 598 amino acids (aa) (Mr 64535). The aa sequence of the cloned enzyme (Cht60) deduced from the nt sequence showed no significant sequence homologies with available aa sequences from databases. Cht60 was purified from the periplasmic fraction of E. coli cells carrying pCHT982. The enzyme was most active towards ide(PNP-[beta]-GlcNAc) and diacetylchitobiose. The optimum pH and temperature of the enzyme were pH 7.5 and 37[deg]C, respectively. The N-terminal 11 aa residues of Cht60 were sequenced, and the location of the signal peptide cleavage site was clarified.


http://www.sciencedirect.com/science/article/B6T39-47P8H5B-K5/2/20735e327a7047aa633e3881add2b36

Human immunodeficiency virus type 1 (HIV1) integrase is cleaved from the gag-pol precursor by the HIV1 protease. The resulting 32-kDa protein is used by the infecting virus to insert a linear, double-stranded DNA copy of its genome, prepared by reverse transcription of viral RNA, into the host cell's chromosomal DNA. In order to achieve high levels of expression, to minimize an internal initiation problem and to facilitate mutagenesis, we have designed and synthesized a
gene encoding the integrase from the infectious molecular clone, pNL4-3. Codon usage was
optimized for expression in Escherichia coli and unique restriction sites were incorporated
throughout the gene. A 905-bp cassette containing a ribosome-binding site, a start codon and the
integrase-coding sequence, sandwiched between EcoRI and HindIII sites, was synthesized by
overlap extension of nine long synthetic oligodeoxyribonucleotides [90-120 nucleotides (nt)] and
subsequent amplification using two primers (28-30 nt). The cassette was subcloned into the
vector pKK223-3 for expression under control of a tac promoter. The protein produced from this
highly expressed gene has the expected N-terminal sequence and molecular mass, and displays
the DNA processing, DNA joining and disintegration activities expected from recombinant
integrase. These studies have demonstrated the utility of codon optimization, and lay the
groundwork for structure-function studies of HIV1 integrase.


http://www.sciencedirect.com/science/article/B6T39-44CMXX2-
M/2/1647d958db41fedbb828a682ef299a47

Triadin is a ryanodine receptor and calsequestrin binding protein located in junctional
cardiolipin reticulum of striated muscles. In the present study, mouse cardiac triadin cDNAs
have been identified by cDNA library screening and RT-PCR. The deduced aa sequences show
that the three isoforms consist of 277, 293 and 305 aa giving rise to the molecular weights of
approximately 31,414, 33,066, and 34,328, respectively. The isoforms have identical 262 aa N-
terminal sequences, whereas they have distinct C-terminal sequences. Northern blot analysis
using a cDNA probe representing the N-terminal common region of triadin revealed that the
mouse triadins were present both in heart and skeletal muscles. The estimated sizes of the
transcripts were approximately 1.3, 4.3 and 5 kb in heart and 5, 5.5 and 7 kb in skeletal muscle.
Endo H treatment and Western blot analysis of isolated mouse cardiac sarcoplasmic reticulum
and in vitro translation products indicate that there are three distinct mouse cardiac triadin
isoforms having molecular weights of 35, 35.5 and 40 kDa. We termed those three isoforms as
mouse cardiac triadin 1, mouse cardiac triadin 2 and mouse cardiac triadin 3.


http://www.sciencedirect.com/science/article/B6T39-43HT1F4-
R/2/4cf1c7903b32bd0d6535e5597cd03f

Platelet-derived growth factor (PDGF) affects cell proliferation and differentiation during
mammalian embryogenesis. In a number of avian species, PDGF-[alpha] receptors and PDGF-A
chain (PDGF-A) are present during chicken limb and lens development. However, little is
understood about the chicken PDGF-A gene. The present study identified short form type 1 (S1),
long form (L) and short form type 2 (S2) cDNA clones encoding chicken PDGF-A chain (PDGF-
A). These clones were isolated from a chicken hepatoma cell line (LMH) mRNA by reverse
transcriptase-polymerase chain reaction (RT-PCR) and cDNA library cloning. Genomic
sequencing and Southern blotting revealed that these forms were generated by alternative
splicing. The mRNAs of S1 and L contained two transcription start sites on one exon. At the
amino acid level, the mature protein encoded by the L clone showed 90 and 85% homology with
the processed coding regions of the long form of human and Xenopus PDGF-A, respectively. The
putative mature peptides of all forms of chicken PDGF-A encompassed the eight cysteine
residues conserved in all known forms of PDGF. We examined the expression of the three forms
in chicken tissues and cells using RT-PCR. Expression of these forms varied among tissues and
cells. Levels of PDGF mRNAs were very low in chicken thrombocytes, which are analogous to mammalian platelets. However, the level of PDGF-A chain mRNA expression in chicken thrombocytes peaked 4 h after exposure to type 1 collagen or thrombin, and then decreased gradually with continued incubation. These results suggest that chicken PDGF in thrombocytes plays an important role in the vascular system and in healing damaged tissue.


http://www.sciencedirect.com/science/article/B6T39-429XV3N-10/2/36a423e4eae972461bfa934540965af0

The Sox gene family (ry like HMG b gene) is characterised by a conserved DNA sequence encoding a domain of approximately 80 amino acids which is responsible for sequence specific DNA binding. We initially published the identification and partial cDNA sequence of murine Sox18, a new member of this gene family, isolated from a cardiac cDNA library. This sequence allowed us to classify Sox18 into the F sub-group of Sox proteins, along with Sox7 and Sox17. Recently, we demonstrated that mutations in the Sox18 activation domain underlie cardiovascular and hair follicle defects in the mouse mutation, ragged (Ra) (Pennisi et al., 2000. Mutations in Sox18 underlie cardiovascular and hair follicle defects in ragged mice. Nat. Genet. 24, 434-437). Ra homozygotes lack vibrissae and coat hairs, have generalised oedema and an accumulation of chyle in the peritoneum. Here we have investigated the genomic sequences encoding Sox18. Screening of a mouse genomic phage library identified four overlapping clones, we sequenced a 3.25 kb Xbal fragment that defined the entire coding region and approximately 1.5 kb of 5' flanking sequences. This identified (i) an additional 91 amino acids upstream of the previously designated methionine start codon in the original cDNA, and (ii) an intron encoded within the HMG box/DNA binding domain in exactly the same position as that found in the Sox5, -13 and -17 genes. The Sox18 gene encodes a protein of 468 aa. We present evidence that suggests HAF-2, the human HMG-box activating factor -2 protein, is the orthologue of murine Sox18. HAF-2 has been implicated in the regulation of the Human IgH enhancer in a B cell context. Random mutagenesis coupled with GAL4 hybrid analysis in the activation domain between amino acids 252 and 346, of Sox18, implicated the phosphorylation motif, SARS, and the region between amino acid residues 313 and 346 as critical components of Sox18 mediated transactivation. Finally, we examined the expression of Sox18 in multiple adult mouse tissues using RT-PCR. Low-moderate expression was observed in spleen, stomach, kidney, intestine, skeletal muscle and heart. Very abundant expression was detected in lung tissue.


Prostate apoptosis response factor-4 (Par-4) is critical to cell growth and apoptosis. Induction of Par-4 expression has been shown to be required for apoptosis in a diversity of cellular systems, including neurons. Neuronal populations in individuals with degenerative disorders show elevated levels of Par-4 protein in advance of cellular and functional loss. To understand the regulation of par-4 expression, we isolated and characterized 5.7 kb of the human par-4 promoter. We demonstrated that the isolated promoter was functional. Similar to the endogenous par-4 gene, par-4 expression could be induced upon apoptotic insult with thapsigargin following introduction of the promoter DNA into human A375 cells. Also, increased levels of the atypical protein kinase
C, [zeta]PKC, was shown to negatively regulate expression from the ectopic par-4 promoter. A 550 bp sequence immediately upstream to the 5'-untranslated region of the gene was found to be responsible for par-4 promoter induction to apoptosis by thapsigargin.


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http://www.sciencedirect.com/science/article/B6T39-3WRB6HH-V/2/f0d2ce151e389e8efc12d94f3f861670


http://www.sciencedirect.com/science/article/B6T39-3V7RVWT-K/2/d19ac92747c56f78ba5d2b75ebcb0ee5

Genomic cloning and sequencing of a human homologue (the gene name, endonuclease III-like 1; gene symbol, NTHL1 or NTH1) for Escherichia coli endonuclease III, that is involved in pyrimidine base excision repair, were performed. The sequence covered the entire NTHL1 gene consisting of six exons and five introns spanning 8 kb with 5' flanking (8 kb) and 3' flanking (3.8 kb) regions. Southern blot analysis suggested that the NTHL1 gene exists as a single copy in a haploid genome. The sequenced 5' flanking region lacks typical TATA and CAAT boxes, but contains a CpG island having putative binding sites for several transcription factors such as Ets1 and Sp1. The NTHL1 gene lies immediately adjacent to the tuberous sclerosis 2 (TSC2) gene on chromosome 16p13.3 in a 5'-to-5' orientation. Transcription initiation sites of both NTHL1 and TSC2 genes were suggested to be multiple by 5' RACE experiments. The northern hybridization experiment suggested that both genes are expressed in all tissues, but at different levels. Downstream of the NTHL1 gene, the gene for the regulatory factor 2 (SLC9A3R2/E3KARP; also called OCTS2, TKA-1 and SIP-1) of the solute carrier family 9 (sodium/hydrogen exchanger), isoform A3, lies in a 3'-to-3' orientation. This paper demonstrates for the first time the spatial relationship of these three genes (TSC2, NTHL1 and SLC9A3R2) at the nucleotide level, and the presence of multiple transcription initiation sites of the NTHL1 and TSC2 genes.


http://www.sciencedirect.com/science/article/B6T39-3VNR70Y-
Genomic DNA segments (approximately 17 kb) containing three DFR genes in the Japanese and common morning glories were sequenced. The three DFR genes in both plants were found to be arranged in a tandem array, and all of them comprised six exons with identical intron positions. Their DFR-B genes carrying longer introns than the DFR-A and DFR-C genes were expressed extensively in the young buds of pigmented flowers, and the transcription starting site for the DFR-B mRNA of the Japanese morning glory was determined. The DFR-B gene of the common morning glory was expressed considerably in stems, moderately in sepals and leaves, whereas the DFR-A and DFR-C genes of the same plant were expressed scarcely but significantly in the young flower buds and stems. Several novel mobile element-like sequences of around 200 bp were found in the genomic DFR regions. A phylogenetic tree indicated that each DFR gene in the Japanese morning glory is most closely related to the corresponding DFR gene in the common morning glory, and that the DFR-B gene is the most diversified gene among the three DFR genes. These structural and functional features of the DFR genes and their evolutionary implications are discussed.


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AIE-75 is a protein identified as an autoantigen in patients with autoimmune enteropathy and as a colon cancer-related antigen. It has recently been assigned to be a causative gene for Usher type 1C congenital syndromic hearing loss. The novel protein has three PSD-95/Dlg/ZO-1 (PDZ) protein-protein interaction domains and is therefore implicated to function as a molecular anchor or sorter. We have identified a novel protein that binds to AIE-75 by yeast two-hybrid screening. The protein has a high homology to the tumor suppressor MCC (mutated in colon cancer; or MCC1 hereafter) and was named MCC2. MCC2 protein binds the first PDZ domain of AIE-75 with its C-terminal amino acids -DTFL. Since the MCC1 does not bind to AIE-75 and the MCC2 displays different expression patterns in various organs compared to MCC1, they appear to play distinct roles in cells. The MCC2 gene is located on chromosome 19p13 in the vicinity of APCL gene, while MCC1 maps near to APC tumor suppressor gene. Because of negative expression of MCC2 in a panel of cancer cell-lines compared to the corresponding normal tissues, we suggest that further study is necessary to investigate a possible role of MCC2 as a tumor suppressor.


PQBP-1 has been identified as a protein that binds to huntingtin, androgen receptor and transcription factor Brain-2 through their homopolymeric glutamine repeats. We here report the genomic organization of the human PQBP-1 gene and its multiple alternative transcripts. The coding region of PQBP-1 comprises six exons and five introns, and four types of alternative transcript, designated PQBP-1a to PQBP-1d, were found in addition to the PQBP-1 transcript reported originally. All of the PQBP-1 transcripts retain the WW domain in the N-terminal region, a potent transactivator domain. On the other hand, there is a wide variation in their C-terminal regions. Importantly, PQBP-1a and PQBP-1d lack the domain responsible for the interaction with
homopolymeric glutamine repeats and a nuclear localization signal.


Damask roses are one group of old rose varieties and a key material in old European rose improvement in the 19th century. To clarify the origin of Damask roses, we selected four varieties as the oldest Damask varieties and examined the relationship between the Damask varieties and their putative ancestors at the molecular level. Randomly amplified polymorphic DNA analysis of the Damask varieties proved that they had an identical profile, indicating they were established from a common ancestor. They have never been allowed to reproduce sexually; their reproduction depends entirely on vegetative propagation. We identified three Rosa species, *R. moschata*, *R. gallica* and *R. fedschenkoana*, as parental species of the original hybridization that contributed to forming the four oldest Damask varieties by sequencing the internal transcribed spacer of ribosomal DNA. We also found that all the four oldest Damask varieties had chloroplasts derived only from *R. moschata*, as judged from *psbA-trnH* spacer sequences. This triparental origin of the four oldest Damask varieties can explain some morphological characteristics of the four oldest Damask varieties, like fruit shape, leaf color and the 'Moss' character.


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A novel approach in molecular design is presented, where in vivo formed complementarity determining regions (CDR) from antibody genes were shuffled into a specific framework region. A synthetic gene library of soluble VH-fragments was created and the complexity of the library was determined by sequencing. The synthetic genes were diverse and contained random combinations of CDR from different germlines. All CDR were randomised in one step and by using in vivo formed CDR, the length, sequence and combination were varied simultaneously.


http://www.sciencedirect.com/science/article/B6T39-4BVNYC4-1/2/66dd933ca8e2309db15a47102c977576
Large-scale single-pass sequencing of randomly selected cDNA clones from cell type specific libraries has proven to be a powerful approach for the discovery of novel gene functions, identification of novel gene family members, and definition of gene expression profiles. HCS-2/8 chondrocyte has been used as a cell culture model to study chondrocyte differentiation. Here we performed 3350 single-pass sequencing reactions obtained from the 5' ends of cDNAs from HCS-2/8 cells. To define the expression profiles of HCS-2/8 chondrocytes, we analyzed the identity of these representative cDNA sequences using database searches (BLAST). The sequences represent 1927 unique genes with known function (i.e., unigene clusters), 38 transcripts that are similar to genes with known function, 739 expressed genes with unknown function (i.e., expressed sequence tags), and 18 cDNAs which have not previously been sequenced. Interestingly, many transcripts were expressed from chromosome 12 compared with total genes, while the fewer numbers of cDNAs were derived from genes on chromosomes 14, 18 and Y. The chondrocytic phenotype of HCS-2/8 cells is reflected by abundant expression of genes related to cell structure and motility and the 20 most frequently expressed unigenes reflect a chondrocyte-related gene expression signature. Thus, our data establish a representative set of more than 2000 genes expressed in a chondrocytic cell line. This finding provides a framework for understanding cell growth and differentiation of chondrocytes and their metabolic function in the formation and remodeling of cartilage.

http://www.sciencedirect.com/science/article/B6T39-3YXJ0JD-8/2/e4dc301b1ddca13ec0279a2df43a250d


Dengue virus type 2 (DEN-2), a member of the Flaviviridae family, has a positive-strand RNA genome, 10 723 nucleotides (nt) in length and encoding a single polyprotein precursor consisting of 3391 amino acids (aa). In order to construct a full-length cDNA clone, the viral genome was cloned into 5' (nt 1-2203 under the control of the T7 promoter (pT7)) and 3' (nt 2203-10723) constructs. A full-length DEN-2 cDNA under pT7 control was assembled in vitro after excising the two cDNA inserts from the 5' and 3' constructs, and joining them with T4 DNA ligase. The RNA produced by in vitro transcription of the cDNA using T7 RNA polymerase was infectious, as shown by transfection of permissive BHK-21 and Vero cells, and propagation of the virus particles released into the culture media. The virus particles stably maintained the conservative mutation introduced into the 5' construct, and the cells infected with the infectious RNA-derived virus synthesized virus-specific DEN-2 antigens, as shown by immunofluorescence and immunoprecipitations. The full-length infectious clone for DEN-2 should be useful for the study of molecular mechanisms involved in viral RNA replication and virus assembly.

http://www.sciencedirect.com/science/article/B6T39-49RCM3-
The ligand-gated ion channel family includes receptors for serotonin (5-hydroxytryptamine, 5-HT), acetylcholine, GABA, and glutamate. Drugs targeting subtypes of these receptors have proven useful for the treatment of various neuropsychiatric and neurological disorders. To identify new ligand-gated ion channels as potential therapeutic targets, drafts of human genome sequence were interrogated. Portions of four novel genes homologous to 5-HT3A and 5-HT3B receptors were identified within human sequence databases. We named the genes 5-HT3C1-5-HT3C4. Radiation hybrid (RH) mapping localized these genes to chromosome 3q27-28. All four genes shared similar intron-exon organizations and predicted protein secondary structure with 5-HT3A and 5-HT3B. Orthologous genes were detected by Southern blotting in several species including dog, cow, and chicken, but not in rodents, suggesting that these novel genes are not present in rodents or are very poorly conserved. Two of the novel genes are predicted to be pseudogenes, but two other genes are transcribed and spliced to form appropriate open reading frames. The 5-HT3C1 transcript is expressed almost exclusively in small intestine and colon, suggesting a possible role in the serotonin-responsiveness of the gut.


[http://www.sciencedirect.com/science/article/B6T39-4FC449B-3/2/6aed8791ffeed54dda5b46ba8e0e8b00](http://www.sciencedirect.com/science/article/B6T39-4FC449B-3/2/6aed8791ffeed54dda5b46ba8e0e8b00)

About 20 ethnic groups reside in Mongolia. On the basis of genetic and anthropological studies, it is believed that Mongolians have played a pivotal role in the peopling of Central and East Asia. However, the genetic relationships among these ethnic groups have remained obscure, as have their detailed relationships with adjacent populations. We analyzed 16 binary and 17 STR polymorphisms of human Y chromosome in 669 individuals from nine populations, including four indigenous ethnic groups in Mongolia (Khalkh, Uriankhai, Zakhchin, and Khoton). Among these four Mongolian populations, the Khalkh, Uriankhai, and Zakhchin populations showed relatively close genetic affinities to each other and to Siberian populations, while the Khoton population showed a closer relationship to Central Asian populations than to even the other Mongolian populations. These findings suggest that the major Mongolian ethnic groups have a close genetic affinity to populations in northern East Asia, although the genetic link between Mongolia and Central Asia is not negligible.


We have cloned a Saccharomyces cerevisiae gene (COF1) encoding a low-Mr actin-binding protein of 143 amino acid (aa) residues (yeast cofilin; Cof); its aa sequence is 35% identical to porcine Cof. The yeast recombinant Cof produced in Escherichia coli exhibited in vitro activities on actin filaments similar to those of mammalian and avian Cof. Gene disruption and tetrad analysis showed that gene COF1 is essential for yeast cell growth. Expression of the cDNA of porcine Cof or destrin (Des), the latter a Cof-related protein, complemented the cof1 null allele in yeast cells.
IS605, an insertion sequence (IS) that is unusual in containing homologs of genes for the single putative transposases of two other unrelated IS elements (IS200 and IS1341), was found in nearly one-third of a set of 238 independent isolates of the gastric pathogen Helicobacter pylori. Hybridization and PCR tests indicated that any strain carrying one of these ORFs also carried the other, which implies that both ORFs are in the same unit of transposition. The IS605 ends and target sites for insertion were identified by sequencing eight preexisting insertions in strain NCTC11638, corresponding empty sites in other strains, and new transpositions in E. coli of an IS605 derivative marked with a selectable chloramphenicol-resistance gene. These tests showed that IS605 is also unusual in: (1) having unique, not inverted repeat, ends; (2) not duplicating (or deleting) target sequences during transposition; and (3) inserting with its left (IS200-homolog) end next to 5'-TTTAA or 5'-TTTAAC. IS605 was implicated in at least two genome rearrangements in strain NCTC11638. A second member of the IS605 family, called IS606 (25% amino acid identity to IS605 in inferred proteins) was found in one-third of 38 H. pylori strains tested, many of which did not carry IS605. The features of these two chimaeric IS elements are discussed in terms of possible transposition mechanisms, IS element evolution, and effects of IS elements on genome organization and evolution in the microbes that they inhabit.

Transposition of mini-transposon Tn5supF to phage [lambda] can be selected in two ways: (i) by plaque formation on a dnaB amber strain of Escherichia coli, which requires expression of the transposon-borne suppressor tRNA gene (supF) during lytic phage growth, or (ii) by lysogenization of a strain with amber mutations in tet and amp resistance genes, and selection of TcRApR (Sup+) transductant colonies. Tn5supF insertions in several [lambda] clones were isolated and mapped using a polymerase chain reaction (PCR) amplification method. Among insertions selected during lytic growth, more than 90% were oriented such that supF could be transcribed from an upstream [lambda] promoter. In contrast, half of those selected by transduction were in each orientation. These results indicate that Tn5supF insertion occurs with equal frequency in each orientation. However, Tn5supF insertion phages in which transcription from the [lambda] and supF promoters would collide tend to be lost when supF is selected during lytic growth. The tendency to recover Tn5supF insertions in only one orientation is useful in a transposon- and crossover-PCR-based method for preparing templates for DNA sequencing.

Four isoforms of the catalytic [alpha] subunit of the Na,K-ATPase have been previously identified. We characterized and mapped a genomic copy of the human ATP1A4 isoform between D1S2707.
and WI-9524, telomeric to a nearby isoform ATP1A2, and within a candidate region at 1q23 for familial hemiplegic migraine (FHM). Human ATP1A4 gene shares 84% identity with the mouse Atp1a4 gene, and both consist of 22 exons and 21 introns. The predicted polypeptide is 1029 amino acids and shares 82 and 79.8% identity, respectively, with human ATP1A2 and ATP1A1. ATP1A4 is larger than other isoforms and most divergent at the N-terminus. ATP1A4 and ATP1A2 are paralogous genes with the same number and organization of putative H-transmembrane domains, conserved exon-intron boundaries, and are found approximately 8.5 kb apart. Expression analysis of the ATP1A4 gene revealed a new major ~7.5 kb transcript in human skeletal muscle, with expression also shown in mouse muscle. Predictive analysis of promoter regions identified muscle specific regulatory elements for ATP1A4 and Atp1a4. Mutation analysis among eight affected individuals from a single large, highly penetrant FHM family was negative in ATP1A4 and ATP1A2 although multiple polymorphisms were identified.


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To understand the molecular mechanisms underlying alterations in the pathophysiologic status of dietary obesity, we examined hepatic genes differentially expressed in a long-term high-fat intake-induced obesity mouse model. C57BL/6J male mice were fed with two kinds of diets for 12 weeks; a low-fat diet (LFD), a high-fat diet (HFD; n=8), and the expression levels of ~10,000 transcripts in liver tissues from the two groups were assessed using cDNA microarray analysis. Twelve-week feeding with the HFD resulted in significant increase in body weight, visceral fat accumulation and circulating cholesterol concentration, compared with the LFD group. The cDNA microarray analysis revealed marked differences in the expressions of 97 hepatic genes. These genes were categorized into seven groups:1. metabolism; 2. defense, stress, and inflammation responses; 3. signal transduction, apoptosis, and cell cycle; 4. transcription regulation; 5. protein synthesis and modification; 6. transport; and 7. cellular adhesion, cytoskeleton and trafficking. The expression of genes involved in fatty acid catabolism and ketone body synthesis, such as acyl-CoA oxidase1 (Acox1) and HMG-CoA lyase (Hmgcl), was significantly increased, and expression of genes involved in lipogenesis and cholesterol synthesis, such as acetyl-CoA synthetase2 (Acs2), fatty acid synthase (Fasn), and squalene epoxidase (Sqle), was drastically decreased in the HFD group. Interestingly, the genes implicated in defense and stress responses, such as glutathione S-transferases (GSTs) and heat shock proteins (Hsps), were also highly represented in the HFD group. Besides, a number of previously unappreciated regulatory molecules were changed by the HFD. These results revealed a transcriptional adaptation to long-term HFD and provided interesting information about the molecules involved in the development and maintenance of the obesity phenotype in vivo.

Using a gene trap technique, we identified a murine homologue of the yeast LUC7-like gene (Luc7l), which is a serine-arginine-rich protein (SR protein) that localizes in the nucleus through its arginine-serine-rich domain (RS domain) at the C-terminus and shows a speckled distribution pattern. Although its transcripts are widely expressed in embryos and adults, they are rarely detected in adult skeletal muscle, and Luc7l expression was found to be negatively regulated during the course of development of limb skeletal muscle, as well as during in vitro differentiation of the myoblast cell lines Sol8 and C2C12. We also demonstrated that forced expression of Luc7l protein inhibited myogenesis in vitro. Based on our results, Luc7l is thought to play an important role in the regulation of muscle differentiation.


We have isolated 20 different human endogenous retroviruses (ERV) related to ERV3, Hsirt and Humer 4-1. Phylogeny and the presence of these ERV among different primates were determined by computer and Southern blot analyses. Preferential localization of ERV to the human, chimpanzee and orangutan Y chromosomes among the low-copy-number ERV is demonstrated. The reason for this accumulation of ERV on the strongly heterochromatic Y chromosome is probably mediated by (i) the absence of recombination of the Y chromosome that makes it more difficult for sequences to be lost, and (ii) integration of retroviruses in heterochromatic regions is less harmful to the organism. If ERV located on the Y chromosome are transcribed and translated to peptides, such peptides could be potential HY-antigens.


Animal and plant globin-encoding genes (Glo) contain two introns in strictly conserved positions. Plant Glo genes possess an additional, centrally located intron. We have determined the cDNA sequence and gene structure of a putative Glo gene from the free-living nematode, Caenorhabditis elegans. The gene encodes a one-domain globin with a single intron, corresponding to the central intron of plant Glo genes. The two introns common to virtually all animal and plant Glo genes are missing. Comparison with the related organisms Trichostrongylus colubriformis, Ascaris suum and Pseudoterranova decipiens, provides evidence of gene duplication, intron loss, and functional divergence within the Glo genes of the nematode phylum. It is now apparent that differential intron loss during evolution has generated Glo genes with a panoply of exon/intron permutations.

The complete coding deoxyribonucleic acid for a novel tyrosine kinase (TK) of the human parasite Schistosoma mansoni has been cloned and characterized. The molecule was designated TK4. The sequence predicts a translation product of about 140 kDa containing two Src homology 2 domains and a tyrosine kinase domain. Data base analyses indicate that TK4 belongs to the Syk family of TKs which has not been identified in schistosomes or other Acoelomata yet. The presence of a member of the Syk family in this phylum supports previous findings demonstrating that TK subclasses were established early in evolution. Although Northern blot and reverse transcription polymerase chain reaction analyses show transcription of TK4 in larval stages and adult schistosomes of both genders, TK4 is more abundantly transcribed in males. In situ hybridization data demonstrate the gender-independent occurrence of TK4 transcripts in parenchymatic cells. Significant signals were detected in the oocytes of the female and in the spermatocytes of the male suggesting that TK4, among other functions, may play a role in germ cell development. This is an unexpected finding considering that Syk-family TKs of invertebrates and vertebrates described so far are not involved in the differentiation of the gonads.


The human REIC gene is a recently found mortality-related gene and a candidate tumor suppressor gene expression of which is largely attenuated in many immortalized and tumor-derived cell lines (Biochem. Biophys. Res. Commun. 268 (2000) 20-24). To gain insight into the mechanisms of the down-regulation, we investigated the genomic structure and promoter activity of the human REIC gene. The gene, identical with the DKK-3 gene, resides on chromosome 11p15.1, consists of nine exons, and has two promoters. Methylation in the main promoter region was detected in 11 out of 21 cell lines tested (52%) derived from a variety of human tumors, in which the expression of the REIC gene was decreased. In ten of these 11 cell lines the minor promoter was also methylated. Similarly, the REIC gene expression was decreased in 14 of 24 fresh non-small cell lung cancer specimens (58%) compared to that in corresponding non-cancerous tissue, though allelic loss and tumor-specific mutation were rare. Of these 14 tumors, at least five tumors exhibited heavy methylation of the REIC promoter region. These results indicate that the down-regulation of the REIC gene expression is ascribed to the aberrant promoter hyper-methylation at least in a subset of human tumors. The expression was restored upon treatment of SQ5 cells with 5-aza-deoxycytidine, confirming DNA methylation as the mode of downregulation. A notable single nucleotide polymorphism in the coding region (cSNP) with an amino acid substitution of glycine (GGG) to arginine (AGG) was found at codon 335 of the REIC gene. However, the distribution of the cSNP showed no significant difference between lung cancer patients and healthy population.


We have characterized the apolipoprotein multigene family of the pufferfish Takifugu rubripes.
The pufferfish mainly contains 28-kDa, 27-kDa, and 14-kDa apolipoproteins in its plasma and was designated apo-28 kDa, apo-27 kDa, and apo-14 kDa, respectively. N-terminal amino acid sequencing revealed that pufferfish apo-28 kDa and apo-27 kDa have an identical amino acid sequence except an additional propeptide in the former; and both are homologues of apoA-I from other animals. The sequence of pufferfish apo-14 kDa is homologous to that of eel apo-14 kDa previously reported, both being apparently specific to fish. In silico screening, using the publicly available Fugu genome database confirmed the pufferfish apoA-I and apo-14 kDa genes. The database further contained the genes encoding four types of apoA-IV, one apoC-II and two types of apoE. Thus, pufferfish contains nine genes encoding apolipoprotein multigene family. Two apoA-IV and one apoE genes were tandemly arrayed and located on one scaffold. Thus two sets of these genes formed two gene clusters. The apoC-II and apo-14 kDa genes are also located on a single scaffold. apoA-I and apo-14 kDa gene transcripts were mainly expressed in liver and less abundantly in brain. The transcripts of the former gene were also observed in intestine. In contrast, the transcripts encoding four apoA-IVs, one apoC-II, and two apoEs were mainly expressed in intestine. These structural details of pufferfish apolipoproteins and tissue distribution of their gene transcripts provide a novel evidence for better understanding of evolutionary relationships of apolipoprotein multigene family.


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A novel caspase recruitment domain protein (CARD) was isolated from common carp Cyprinus carpio L. by expressed sequence tag analysis. This gene consist of a 2016 bp open reading frame and untranslated regions, which is putatively translated to a protein of 535 amino acid residues. The gene harbors domains (CARD and Coiled-coil domain), which are conserved in proteins of CARD family. The CARD domain have carp was similar to human CARD9 with 72.4% identity. Expression analysis revealed that CARD gene of carp (carp-CARD) expressed in normal tissues of head kidney, spleen, liver, heart and brain. Here we demonstrated that the expression of carp-CARD increased by cortisol treatment in all the tissues and had a high and long lasting expression in cortisol treated spleen.


http://www.sciencedirect.com/science/article/B6T39-3VXNK10-4/2/a2752673af9335c6220d196b875e45a8

The 58-kDa inhibitor of the interferon-induced double-stranded RNA-activated protein kinase (PKR) is a cellular protein that is activated during influenza virus infection to down-regulate the activity of PKR. This study was initiated to further our understanding of the inhibitor which, when overproduced, has the capacity to malignantly transform cells. We report here the isolation and characterization of cDNA clones encoding the inhibitor, designated p58, from human HeLa and mouse NIH 3T3 cells. The human and mouse p58 cDNAs were 6.5 and 1.6 kb in length, respectively. Surprisingly, the deduced amino acid sequences of the human and mouse p58 were 96% identical, indicating a remarkably high degree of conservation between species. An examination of p58 mRNA expression in human tissues revealed a 6.5-kb transcript in all tissues examined, with a particularly high level of expression present in the pancreas and liver, and also in certain leukemic cell lines. Similarly, p58 expression was detected in all mouse tissues examined, with the highest level of expression found in liver. In contrast to human tissues, three
p58 transcripts of approximately 1.7, 3.3 and 5.4 kb were observed in mouse tissues, suggesting that p58 expression may be regulated differently in human and mouse cells. Western blot analysis of subcellular fractions and indirect immunofluorescence analysis of intact cells revealed that p58 was found predominantly in the cytoplasm, consistent with its function as an inhibitor of PKR, which is also a predominantly cytoplasmic protein.


http://www.sciencedirect.com/science/article/B6T39-47PNYB9-XP/2/9c58f127c8a155d1c2a0c52245cec83a

Full-length cDNAs encoding bovine urokinase-type plasminogen activator (u-PA) and urokinase receptor (u-PAR) were cloned from an aortic endothelial cell cDNA library using PCR-amplified cDNA fragments as probes. Bovine u-PA amino acid identity ranges from 79.5 to 70.9% when compared to its pig, human, baboon and mouse analogues, while bovine u-PAR is 61.8 and 59.6% identical to its human and mouse counterparts, respectively. All Cys residues previously found in mature u-PA and u-PAR from these different species are also conserved in the bovine molecules. Bovine u-PA and its cell-surface receptor display one and six potential sites of N-linked glycosylation, respectively. Northern blot hybridization demonstrated a moderate induction of u-PA and u-PAR mRNA in bovine aortic endothelial cells after treatment with 10 nM and 1 [mu]M retinoic acid for 8 hours.


http://www.sciencedirect.com/science/article/B6T39-47PH5MW-W0/2/edf8c1fa996a861ccd05dfda94a39b5f

We present a simple and convenient protocol for the direct sequencing of bacteriophage T4 genomic DNA. The method utilizes the thermostable DNA polymerase from Thermus aquaticus (Taq) and 32P-end-labeled oligodeoxyribonucleotide primers to produce extension products that allow the analysis of at least 200 nucleotides (nt) on a single sequencing gel. Single-nt changes in the template were easily detectable following an overnight exposure of the autoradiograms. Comparison of sequences from fully modified T4 DNA containing glucosylated hydroxymethyldeoxycytosine or from templates containing cytosine showed little difference in sequence clarity. These techniques considerably simplify the molecular analysis of T-even bacteriophages and should be compatible with automated sequencing methods which employ 5'-end-labeled primers.


http://www.sciencedirect.com/science/article/B6T39-47P8FWJ-5X/2/0ff1f62a2b93e5c4c617d7bb121c14596

We have developed a cassette for the integration of cloned DNA sequences at multiple sites in the Saccharomyces cerevisiae genome, taking advantage of the naturally repeated sigma sequences. This cassette contains one engineered sigma element which allows the targeting of
an embedded gene at different genomic sigma elements by gene replacement. Two yeast genes, ARG4 and URA3, were thus integrated in the absence of any bacterial sequences, individually or sequentially on twelve chromosomes. Consequently, these studies led to the genetical tagging of individual members of the sigma family.


http://www.sciencedirect.com/science/article/B6T39-3WBG1Y1-M/2/f66b3a566d41b5c158a6c691e66b68104a

A gene cluster comprising the alanine racemase gene alr was identified 5' to the sigB operon in Staphylococcus aureus. It is flanked upstream by four ORFs of which one shows similarity to the dpj gene of Escherichia coli, and downstream by two ORFs of which the last shows similarity to the E. coli pemK gene. Preliminary data suggest that the seven ORFs orf1-orf2-orf3-dpj-alr-orf6-pemK may form an operon. Disruption of the proposed operon by insertional mutagenesis leads to a drastic loss in the -alanine (-Ala) substitution of lipoteichoic acid and to delayed autolysis, without affecting the -Ala substitution of the wall teichoic acid.


http://www.sciencedirect.com/science/article/B6T39-43KGNS7-6/2/a4e2bef4c7f8156d927fcd256a40bde4

One of the evolutionary mechanisms for acquisition of novel functional sequences can be domestication of exogenous retroviruses that have been integrated into the germ line. The whole genome mapping of such elements in various species could reveal differences in positions of the retroviral integration and suggest possible roles of these differences in speciation. Here, we describe the number, locations and sequence features of the human endogenous retrovirus HERV-K (HML-2) long terminal repeat (LTR) sequences on human chromosome 21. We show that their distribution along the chromosome is not only non-random but also roughly correlated with the gene density. Amplification of orthologous LTR sites from a number of primate genomes produced patterns of presence and absence for each LTR sequence and allowed determination of the phylogenetic ages and evolutionary order of appearance of individual LTRs. The identity level and phylogenetic age of the LTRs did not correlate with their map locations. Thus, despite the non-random distribution of LTRs, they have apparently been inserted randomly into the chromosome relative to each other. As evidenced in previous studies of chromosomes 19 and 22, this is a characteristic of HERV-K integration.


http://www.sciencedirect.com/science/article/B6T39-44CNR71-1/2/29d28ef57b9185f13b5a9652ffe454a8

NR-binding SET-domain-containing protein (NSD1) is a mouse nuclear protein containing
su(var)3-9, enhancer-of-zeste, trithorax (SET), proline-tryptophan-tryptophan-proline (PWWP) and plant homeodomain protein (PHD)-finger domains (Huang et al., EMBO J. 17 (1998) 3398). This protein also has two other distinct nuclear receptor (NR)-interaction domains, called NID-L and NID+L, and acts as both a NR corepressor and a coactivator by interacting directly with the ligand-binding domain of several NRs. Thus, NSD1 is a bifunctional, transcriptional, intermediary factor. We isolated the human homologue (NSD1) of the mouse NSD1 gene (Nsd1), mapped it to human chromosome 5q35, and characterized its genomic structure. NSD1 consists of at least 23 exons. Its cDNA is 8552 bp long, has an 8088 bp open reading frame, contains at least six functional domains (SET, PWWP-I, PWWP-II, PHD-I, PHD-II, and PHD-III) and ten putative nuclear localization signals, and encodes 2696 amino acids. NSD1 shows 86% identity with the mouse Nsd1 at the nucleotide level, and 83% at the amino acid level. NSD1 is expressed in the fetal/adult brain, kidney, skeletal muscle, spleen, and the thymus, and faintly in the lung. Two different transcripts (9.0 and 10.0 kb) were consistently observed in various fetal and adult tissues examined. These findings favor the character of NSD1 as a nucleus-localized, basic transcriptional factor and also a bifunctional transcriptional regulator, such as that of the mouse Nsd1. It remains to be investigated whether mutations of NSD1 lead to a specific phenotype in man.


http://www.sciencedirect.com/science/article/B6T39-3W2XNW0-20/2/831205fde5b35982452049c8787d61d0

A short tandem repeat (STR) in the D12S391 locus was sequenced in more than 40 individuals. Twenty different alleles were found and these could be grouped into 12 allelic classes in accordance with the total number of repeats. This is a compound STR consisting of blocks of (AGAT) and (AGAC) repeats with basic sequence structure (AGAT)8-17(AGAC)6-10(AGAT)0-1. Whereas smaller alleles (15-18) have variation limited to the (AGAT) unit, in larger alleles the complexity is greater with variation in the number of tandem arrays in the two motifs (AGAT) and (AGAC). Population data showed that this is a highly polymorphic STR with a heterozygosity of more than 0.9. This fact, together with its simple structure, makes this STR an interesting DNA polymorphism for forensic and genetic purposes.


http://www.sciencedirect.com/science/article/B6T39-3Y6HKBK-8K/2/5885fc6228d0f1ddd8f60f9465c432e7

Recently, a mammalian kinase cascade was discovered that is triggered by stress and heat shock, and leads to the stimulation of mitogen-activated protein kinase (MAPK)-activated protein kinase-2 (MAPKAPK-2). Surprisingly, this process turns out to be independent of the classical MAPK. The stress-induced activation of MAPKAPK-2, in turn, results in the phosphorylation of small heat-shock proteins (Hsp). We have isolated a Drosophila melanogaster (Dm) cDNA encoding a polypeptide that has extensive sequence similarity to the mammalian MAPKAPK-2. As in mammalian MAPKAPK-2, the Dm MAPKAPK-2 possesses a MAPK phosphorylation site and a nuclear targeting sequence located C-terminal to the catalytic domain. However, in contrast to its mammalian counterpart, it lacks the Pro-rich N-terminal region proposed to form Src-homology domain 3 (SH3) binding domains. A 2.4-kb MAPKAPK-2 message is expressed throughout development, while two shorter transcripts of 2.3 and 1.8 kb appear to be specifically expressed in the germline.
specific polyadenylation signal (AATATA) located early within the 3' untranslated region. Dm MAPKAPK-2 is located at cytological position 5D in the Dm genome.


Gm776 is a 776-bp subregion of a member of an interspersed family of relatively homogeneous repetitive DNA elements from soybean (Glycine max). The fragment was originally amplified from soybean DNA by the polymerase chain reaction using a single 22-nucleotide primer, and consequently terminates in an inverted repeat. The elements defined by Gm776 are at least 10.6 kb in length and constitute a family of 500-800 members per haploid genome. The family has been designated SIRE-1 (soybean interspersed repetitive element 1). Overlapping regions of Gm776 exhibit suggestive DNA sequence similarity to Tal and Tyl, copia-like retrotransposons from Arabidopsis thaliana and Saccharomyces cerevisiae, respectively. However, there are no similarities at the amino acid level, and the regions of similarity are not functionally related.


http://www.sciencedirect.com/science/article/B6T39-492090F-6/2/5685e47e18d303cd5e8bd15c39c01f32

A human myosin heavy chain gene was identified in chromosome 19q13 by computational sequence analysis, RT-PCR and DNA sequencing of the cDNA. The complete cDNA has a length of 6786 bp and comprises 41 exons (40 coding) included in 108 kb of genomic sequence. Alternative splicing variants were also identified. The gene is expressed in a multitude of tissues, but mainly in small intestine, colon and skeletal muscle. The putative protein (228 kDa) carries the common myosin domains and presents high homology with the non-muscle myosin heavy chains (MYH9 and MYH10) as well as the smooth muscle myosin heavy chain MYH11. Nevertheless, phylogenetic analysis indicated that these homologous proteins are more related among themselves than to MYH14, suggesting that possibly this myosin heavy chain should be classified in a new myosin-subfamily.


http://www.sciencedirect.com/science/article/B6T39-402KBCD-Y/2/c15a78cdd4f2999d3a8f374f8c8f7d69

We isolated *Rivulus marmoratus* mitochondrial DNA by long-polymerase chain reaction with conserved primers, and sequenced it with 36 sets of internal conserved primers, which were designed from the extensive sequence similarities of mitochondrial DNA from several fish species. The *R. marmoratus* mitochondrial DNA has 17,329 bp with a conserved structural organization compared to those of other fish. *Rivulus marmoratus* mitochondrial DNA also has two nearly identical control regions. The basic characteristics of the *R. marmoratus* mitochondrial genome are discussed.


Upon exposure to infection with digenetic trematodes such as *Echinostoma paraensei*, the freshwater snail *Biomphalaria glabrata* produces increased quantities of hemolymph lectins, some of which are unique polypeptides containing both immunoglobulin superfamily (IgSF) and fibrinogen domains. These unusual lectins have been termed fibrinogen-related proteins (FREPs), and recognize and precipitate digenean antigens. We here report 11 distinct FREP-encoding sequences from *B. glabrata*, and provide the complete genomic sequence for two of the most frequently recovered FREPs. The unique juxtaposition of IgSF and fibrinogen domains, previously known only from incomplete cDNAs, is confirmed. Sequences corresponding to known peptides derived from FREPs from hemolymph were found in one of these genes. Both genes contain four exons, the first encodes a putative signal peptide, the second and third a portion of an IgSF-type loop, and the fourth a fibrinogen domain. Cysteines, postulated to form an intrachain loop, are present in the IgSF domain and are separated from one another by 78 or 79 residues. The IgSF sequences most closely resemble V (variable)-type Ig domains, based on canonical and hydrophobic residues and predicted secondary structure. Some minor differences in genomic fragments isolated for each of the two sequences were noted and may represent allelic variants. The results may be of relevance in understanding the role of *B. glabrata* in transmission of *Schistosoma mansoni*, a digenean parasite that infects nearly 100 million people in the tropics.


Tripeptidyl-peptidase II (TPP II) is a ubiquitously expressed exopeptidase. The expression of this enzyme is increased, e.g. in some tumor cells, but the regulation of the expression of the gene has not been investigated previously. The gene encoding human TPP II (TPP2) is 82 kb and consists of 30 exons. An 8 kb NcoI fragment covering the 5'-flanking region of the TPP2 gene, including the initiation codon, was cloned into a luciferase-containing reporter vector. Human embryonic kidney cells (HEK-293 cells) and murine fibroblasts (NIH3T3 cells) were transiently transfected with the construct. Through sequential deletions and analysis of short PCR-fragments, the promoter could be localized to a 215 bp fragment upstream of the initiation codon. This region is GC-rich, lacks a TATA-box and contains two inverted CCAAT-boxes and a GC-box. Electrophoretic mobility shift assays showed that nuclear proteins bind to the promoter fragment. The 85 bp 5'-end of the promoter fragment is essential for transcriptional activation. Out of this a 44 bp fragment suffices to compete with binding of nuclear proteins to the 215 bp fragment. Supershift assays demonstrated that the CCAAT-binding factor (CBF; NF-Y) is involved in the formation of a complex with the 215 bp fragment. Although Sp1 binds to the promoter fragment in vitro, it was found to bind to the 3'-end of the 215 bp fragment which is not essential for transcription. The potential role of Sp1 in transcription of TPP2 therefore remains to be established.


The human gene encoding the Na+/Ca2+ exchanger family member 3 (NCX3) undergoes extensive alternative splicing, with four variants previously identified. In this study, we report two novel alternative transcripts encoding two N-terminally truncated NCX3 proteins specifically expressed in human fetal brain. The identified transcripts, designated NCX3-tN.1 and NCX3-tN.2, are approximately 2.8 kb and 2.9 kb, respectively. The open reading frames (ORFs) are predicted to encode separately a 284 and a 298 amino acid (aa) polypeptide. Sequence analysis and bioinformatics reveal that NCX3-tN.1 and NCX3-tN.2 are the result of alternative splicing of the NCX3 gene. They have their own potential start codons and unique 5' untranslated regions (UTRs) that are different from those of the known NCX3 variants. The variants include a part of intron 2 of the original gene organization as their first exon (exon "a") at the 5' end of the novel transcripts. NCX3-tN.2 consists of six exons including exon "a" and exons 4, 6, 7, 8 and 9 of NCX3, while NCX3-tN.1 lacks exon 4, but is otherwise similar to NCX3-tN.2. Expression studies show that both variants can be translated into protein and NCX3-tN.1 seems more efficiently translated. Based on their structural features, NCX3-tN.1 and NCX3-tN.2 proteins are potentially involved in regulation of Na+/Ca2+ homeostasis.
http://www.sciencedirect.com/science/article/B6T39-3WWDH80-J/2/b147081af8a90edab0e4c713bbb51e76


In mammals, the response to nephrotoxicant-induced renal injury is limited to repair of the proximal tubule by surviving epithelial cells. In contrast, bony fish are capable of both repair, and de novo production of nephrons in response to renal damage. Importantly, toxicant-induced nephron neogenesis in goldfish (Carassius auratus) parallels nephron development in the mammalian embryo, providing a vertebrate model for kidney development. We utilized this model system to identify genes induced by the renal toxicant, gentamicin, that may function in nephron neogenesis. A novel ubiquitin-like (UBL) gene, 40.1, was identified by differential display analysis of control and gentamicin-treated goldfish kidney. 40.1 was induced dramatically 3-7 days following a sublethal dose of gentamicin, and returned to basal level by 14 days post-treatment. The induction of 40.1 coincided with early renal injury in the proximal tubules of gentamicin-injected fish; however, expression was not restricted to the kidney, suggesting that 40.1 induction may be a more general response to cell injury. Sequence analysis revealed that 40.1 contains tandem UBL domains, and shares homology with ISG15, a 15 kD interferon-(IFN) stimulated UBL found in mammals. Analysis of the genome database for the pufferfish, Fugu rubrides, identified a goldfish ISG15 (gfISG15) homologue with an IFN-stimulated response element in the promoter region, providing further evidence that gfISG15 is the true teleost ISG15 orthologue. Zebrafish and catfish ISG15 genes were subsequently identified by sequence analysis. Consistent with its predicted function as a UBL, gfISG15 formed conjugates with cellular proteins in vitro and in transient transfections. Similar to the induction of mammalian ISG15 by microbial challenge, gfISG15 was induced in the spleen of mycobacteria-infected fish. These studies identified the first teleost ISG15 orthologue. The induction of gfISG15 as an early genetic event in response to a renal toxicant, and its conserved, stress-associated, expression in higher vertebrates suggests that ISG15 is an important component of the host response to diverse stress stimuli.

We identified and partially characterized another member of the uncoupling protein termed UCP3. Human and mouse UCP3 protein sequences are 86% identical to each other, and 73% and 59% identical to UCP2 and UCP1, respectively. Expression of human UCP3 in yeast resulted in a drastic decrease of mitochondria membrane potential. Northern analysis showed that UCP3 was highly expressed in skeletal muscle in human, rat, and mouse. Mapping of UCP3 placed it to the same chromosomal region of UCP2 in both human and mouse, a region that is linked to obesity and hyperinsulinemia. Furthermore, adenovirus-mediated leptin expression in obese ob/ob mice led to increased expression of UCP3 in skeletal muscle. The data indicate that UCP3 encodes a muscle-specific uncoupling protein that may play an important role in the regulation of energy expenditure and development of obesity.
We have characterized a new ankyrin (ANK) repeat-containing Saccharomyces cerevisiae gene, YAR1, located between the HSP82 and SUI3 genes on chromosome XVI. YAR1 encodes a 200-amino-acid (aa) protein with two ANK repeat motifs and an acidic C terminus rich in PEST-like sequences. The Yarl ANK repeats are most similar to the conserved ANK repeats in the yeast cell cycle transcription factor, Swi6. We show that YAR1 is transcribed as an 800-nucleotide (nt) poly(A)+mRNA from a promoter lacking a consensus TATA sequence. YAR1 is transcribed in both haploid and diploid cells, and in haploid cells arrested in G1 with a-factor or in S phase with hydroxurea. YAR1 shares an intergenic region with HSP82, and while HSP82 transcription is induced 15-fold by heat shock, transcription of YAR1 is transiently repressed by heat shock. We show that YAR1 is not an essential gene, but that haploid cells bearing a yarl deletion grow significantly more slowly than do isogenic wild-type cells, especially at low temperature.


Rat Ndrg4 is a member of the NDRG gene family and has been suggested to relate to brain development. The structure of the rat Ndrg4 gene was studied to understand the mechanism for the expression of multiple forms of Ndrg4 protein, which were revealed in the brain. Subcloning and DNA sequencing analysis of a bacterial artificial chromosome (BAC) clone, together with analysis of a transcriptional start site by a cap-site hunting, indicated that the Ndrg4 gene spans about 39 kilobases (kb) and consists of 19 exons, in which the first and second exons were first found in rat. An alternative promoter usage at different transcriptional start sites may produce three types of messages, Ndrg4-A, Ndrg4-B, and Ndrg4-C, and there is a variant that lacks exon 18 for each type of transcript. Thereby, Ndrg4-A1, Ndrg4-A2, Ndrg4-B1, Ndrg4-B2, Ndrg4-C1, and Ndrg4-C2 were identified to be expressed. These six variants might explain the heterogeneity of the Ndrg4 protein in the brain. The variants without exon 18 were revealed in the embryonic and early postnatal brains while those with exon 18 were detected in the maturing and adult brains. Radiation hybrid mapping suggests that the rat Ndrg4 gene is located on chromosome 19 at 90.6 centirays (cR) from the top. Comparison of the noncoding sequence of the rat Ndrg4 gene to those of the orthologous mouse and human genes suggests that the AP-1 binding site is a candidate regulatory element.


(SES) is described. SES-PCR is simple and efficient. Optimal utilization of nucleotides, ability to use only partially purified oligodeoxyribonucleotides, and elimination of kination and ligation of intermediates make SES-PCR-mediated gene synthesis more economical in terms of time, labour and money. Site-directed mutagenesis and/or gene fusion by SES-PCR is not limited by the prior availability of the gene(s) in question. The potentials of this novel method in gene synthesis, mutagenesis at multiple loci of DNA and gene fusion have been demonstrated using a chimeric gene encoding fusion between OmpA signal peptide and hirudin, as an example. The SES-PCR product was cloned and sequencing of positive clones demonstrated the presence of genes with expected sequence and bearing only the desired mutations. A nearly 100% efficiency of mutation was easily achieved by the design of the method.


http://www.sciencedirect.com/science/article/B6T39-47P8201-2C/2/aac666a38b9f1e91a9a83f59f3bff81e

A universal approach for improving the efficiency of cloning through a selective enrichment of recombinants has been developed. This was achieved by using novel polylinkers or multiple cloning sites (MCS) termed zero-background linkers (ZBL). These MCS have short-cutter site(s) incorporated in the wobble portion of the recognition sequence of the long-cutter site(s) in such a fashion that cloning at the short-cutter site disrupts the continuity of the long-cutter-specific sequence. Consequently, digestion of the ligation mixture with the long-cutter, prior to transformation, essentially eliminates the insert negative clones from transformants. The usefulness of such background removal has been illustrated through the design and synthesis of a model ZBL molecule and then its incorporation into various popular cloning and expression vectors. We demonstrate that replacement of the residing MCS of a vector with ZBL leads to enhanced cloning efficiency, as evidenced by the marked increase in the ratio of recombinants to non-recombinants. Such vector improvement is nearly universal as zero backgrounding is an add-on feature and can be adapted to practically any vector system, without having to alter the essential features of the parent vectors.


http://www.sciencedirect.com/science/article/B6T39-47PGBTF-3/2/781a6464a63c3a984cf35a90fd58f3fe

Mrjp1 gene belongs to the honeybee mrjp gene family encoding the major royal jelly proteins (MRJPs), secreted by nurse bees into the royal jelly. In this study, we have isolated the genomic clone containing the entire mrjp1 gene and determined its sequence. The mrjp1 gene sequence spans over 3038 bp and contains six exons separated by five introns. Seven mismatches between the mrjp1 gene sequence and two previously independently published cDNA sequences were found, but these differences do not lead to any change in the deduced amino acid sequence of MRJP1. With the aid of inverse polymerase chain reaction we obtained sequences flanking the 5' ends of other mrjp genes (mrjp2, mrjp3, mrjp4 and mrjp5). Putative promoters were predicted upstream of all mrjp genes (including mrjp1). The predicted promoters contain the TATA motif (TATATATT), highly conserved both in sequence and position. Ultraspiracle (USP) transcription factor (TF) binding sites in putative promoter regions and clusters of dead ringer TF binding sites upstream of these promoters were predicted computationally. We propose that USP, as a juvenile
hormone (JH) binding TF, might possibly act as a mediator of mrjp expression in response to JH. Mrjp1’s genomic locus is predicted to encode an antisense transcript, partially overlapping with five mrjp1 exons and entirely overlapping with the putative promoter and predicted transcriptional start point of mrjp1. This finding may shed light on the mechanisms of regulation of mrjps expression. Southern blot analysis of genomic DNA revealed that all so far known members of mrjp gene family (mrjp1, mrjp2, mrjp3, mrjp4 and mrjp5) are present as single-copy genes per haploid honeybee genome. Although MRJPs and the yellow protein of Drosophila melanogaster share a certain degree of similarity in aa sequence and although it has been shown that they share a common evolutionary origin, neither structural similarities in the gene organization, nor significant similarities between intron sequences of mrjp1 gene and fourteen yellow-like genes of D. melanogaster were found.


http://www.sciencedirect.com/science/article/B6T39-3W2T6C3-V/2/02941c925004da7b13d2b92513a03103

Alu repeat sequences and other multiple copy repetitive elements are present throughout the human genome and are active in promoting recombination. It is believed that reverse transcription of transcribed Alu repeats followed by chromosomal integration has been responsible for the wide dispersion and high copy number of these sequences. During studies on the hMSH2 gene we have used RT-PCR to amplify from peripheral blood lymphocytes a cDNA species in which 553 base pairs of hMSH2 cDNA have been deleted to be replaced by a short 36 base pair Alu sequence as a result of a genomic insertion/deletion event. The 36 base pair Alu insert is homologous to a 26 base pair Alu sequence previously implicated in the promotion of recombination and contains the GCTGG motif which is part of the prokaryotic chi sequence. A second chi-like sequence is also located within the deleted hMSH2 region. Both chi-like sequences are located within 4 bp of the two 4-bp regions of cross over containing the insertion/deletion breakpoints. This suggests that a double recombination event has occurred, providing direct evidence for the recombinogenic activity of this Alu element. Furthermore, it suggests that chi-like sequences may define recombination hotspots as in prokaryotes.


http://www.sciencedirect.com/science/article/B6T39-44CMXX2-5/2/8140b9eb29f317eb2ddb400b4e9b7f0

Macrophage migration inhibitory factor (MIF) from vertebrate species is a molecule that exerts a wide-range of effects in inflammatory responses, cell activation and cell differentiation. Several species of parasitic nematodes have been shown to express genes encoding orthologues of the mammalian MIF that appear to play a key role in immune evasion by modifying the activity of host cells. In addition, MIF accumulates in nematode somatic cells where its role has not yet been defined. In order to identify the role that MIF plays in the cell biology of nematodes, we have characterized the members of the mif gene family in the free-living species Caenorhabditis elegans. Unlike the single mif gene found in humans and mice, C. elegans expresses four distinct mif genes: Ce-mif-1, Ce-mif-2, Ce-mif-3 and Ce-mif-4. The Ce-MIF proteins are between 15-30% identical to each other, 34-38% identical to the MIFs from the parasitic nematode Brugia malayi, and 22-35% identical to mammalian MIFs. The transcription of Ce-mif-2 and Ce-mif-3, but not Ce-mif-1, was upregulated >100-fold compared to L2 levels when the worms entered the dauer stage. The transcription levels of Ce-mif-2 and Ce-mif-3 fell to near baseline a few hours after exit
from dauer. Ce-MIF/GFP transgenic animals and immunostaining were used to demonstrate that
the main sites of MIF production are in the hypodermis, body wall muscles and in the nuclei of
developing embryos. The results suggest a role for C. elegans MIF in cellular maintenance during
periods of adverse conditions that lead to developmental arrest.


http://www.sciencedirect.com/science/article/B6T39-3Y6HGSRT2/2/b52e0df50cace03d9ba2d198e7dc259

A 2288-bp cDNA sequence encoding dihydrolipoamide dehydrogenase (DLDH; dihydrolipoamide:
NAD+ oxidoreductase; EC 1.8.1.4) was obtained by isolating a 1762-bp cDNA clone from a
canine skeletal muscle library in the vector, [lambda]UNIZAP, combined with PCR amplification of
the 5' end of the mRNA. The DLDH cDNA sequence contains a 49-bp G+C-rich 5'-untranslated
region (UTR), followed by 1527 bp of coding region, and 695 bp of 3'-UTR preceding a 17-bp
poly(A) tail. The single open reading frame encodes a precursor DLDH of 509 amino acids (aa)
that begins with a 35-aa leader sequence. The 3'-UTR includes six possible polyadenylation
signals (three AATAAA, one TATAAA and two AATGAA) and one potential stem-loop region
extending from bp 1969-1991. Alignment studies of the canine and human DLDH demonstrate
homology within the coding region of 98% at the aa level and 94% at the nt level. Northern blot
analysis using the cDNA clone as probe showed wide tissue distribution of the mRNA, with
differences in the level of expression among tissues and possible utilization of different
polyadenylation sites.


http://www.sciencedirect.com/science/article/B6T39-4CX728C3/2/add293f73a2a1008a853798270f22543

Copper and iron act at different levels on gene expression. Due to their chemical reactivity, both
metals could play a role in the regulation of the protein machinery involved in their metabolism,
and/or of the metabolic function they are involved in. Experimental and clinical evidences raise
also the hypothesis of the existence of genes commonly regulated by both metals. Purpose of
this work was to find genes modulated by copper and iron in the rat intestine. A panel of 24
animals was randomly divided into three nutritional treatments including a control, a copper-
deficient and an iron-deficient diet. The positive regulation of iron responsive element (IRE)-
DMT1 gene was found, with different extent, in both experimental groups. A differential display
reverse transcription (DDRT)-polymerase chain reaction (PCR) analysis carried out on the rat
intestinal mRNAs demonstrated the differential expression of five cDNA fragments. Among these,
the Cytochrome c oxidase (COX) subunit II mitochondrial gene resulted to be regulated by both
metals, the Serum and Glucocorticoids-regulated Kinase (SGK) gene mainly by iron, and an
Ebnerin-like 2 kb mRNA dramatically down-regulated by copper. Two residual clones showed low
identity scores with sequences present in data bank. Finally, we observed that both iron and
copper are able to modulate the expression of the three characterized genes in some tissues,
other than intestine.

sequence similarity with somatostatin and angiotensin receptors." Gene 248(1-2): 183.

http://www.sciencedirect.com/science/article/B6T39-4067BC2-N/2/55044220843402c52f26d0ad37696538


http://www.sciencedirect.com/science/article/B6T39-4DM2G9T-1/2/c5598acc45ee1ba43a89fd50ce53a23a

We describe the isolation and characterization of a full-length cDNA encoded by a gene that was significantly down-regulated in the affected skin of patients with psoriasis vulgaris. The cDNA was isolated from a keratinocyte cDNA library and its sequence was found to correspond to a hypothetical locus recorded in GenBank with the accession number LOC146206. The nucleotide sequence of the full-length cDNA was found to have an open reading frame of 1365 amino acids and to span approximately 12 kb of genomic DNA with 39 exons on chromosome 16q22. The deduced amino acid sequence contains four distinct structural regions, an RGD motif, a leucine-rich repeat (LRR) region, a tropomodulin domain, and a proline-rich domain. The gene was consequently designated as RLTPR (RGD, leucine-rich repeat, tropomodulin and proline-rich containing protein). The RLTPR hypothetical protein has a functional domain organization similar to Acan125, a myosin-binding protein expressed by Acanthamoeba castellanni. RT-PCR with RLTPR PCR primers amplified products from cDNAs prepared from all of the 30 different tissues that we examined including thymus, spleen, colon, skin, skin keratinocytes, skin fibroblasts and fetal skin. During the course of screening the human keratinocyte cDNA library, some alternative splicing was also detected in three regions of the RLTPR gene. In addition, sequence analysis of the RLTPR genes from eight psoriasis patients and eight healthy controls revealed a number of synonymous and nonsynonymous SNPs that may be useful markers for future disease association studies.


http://www.sciencedirect.com/science/article/B6T39-47FW95F-5/2/fb759257a0e9c384ef0e0549915c32c7

The [beta]A1- and [beta]A3-crystallins are major polypeptides in the lenses of vertebrates. We present evidence that a single [beta]A3/A1 gene encodes these two proteins in the chicken. The [beta]A3/A1 gene has been sequenced and its functional promoter identified in transfection experiments. The chicken [beta]A3/A1 gene has the same structure as the human orthologue: six exons with standard splice sites and two alternative start codons from which the protein products are apparently translated. Northern analysis revealed an abundant 0.9-kb transcript in the lenses of 1-2-day-old chickens and no detectable transcripts in the rest of the eye, brain, heart, kidney, liver or skeletal muscle. The 5'-flanking sequence of the chicken [beta]A3/A1 gene is very similar to that of the human and mouse genes, suggesting conservation of important putative regulatory sequences in addition to the TATA box. A thymidine-rich element (bp -218 to -163) and a potential AP-1-binding site (bp -264 to -258) are present within the chicken 5'-flanking region. A DNA fragment from -382 to +22 of the chicken [beta]A3/A1 gene is sufficient to promote expression of the bacterial cat gene in transfected chicken primary lens epithelial cells, but not in transfected dermal fibroblasts. Moreover, the sequence from positions -382 to -143 of the chicken
[\text{\beta}]A3/A1 promoter appears to be critical for proper transcription and expression in the transfected lens cells.


http://www.sciencedirect.com/science/article/B6T39-45G023J-2/2/1043b932d8d188d4561db82644846e02

Importin [alpha]1 (also referred to as NPI1 or importin [alpha]S1) gene encodes a member of the cytosolic receptor protein superfamily that recognizes classical monopartite and bipartite basic type nuclear localization signals and mediates nuclear protein import via an importin [\text{\beta}]-dependent pathway. Here we report on the organization of the importin [alpha]1 locus in the mouse genome. The gene is approximately 40 kb in length from the translation initiation codon to the poly(A) additional site. The translated region of the gene is comprised of 13 coding exons and the exon-intron boundaries conform to the GT/AG rule. Importin [alpha]1 was mapped to a middle region of mouse chromosome 16 by fluorescence in situ hybridization analysis. Moreover, it was found by reverse transcriptase polymerase chain reaction analysis that importin [alpha]1 is widely expressed in various tissues in adult mice and at various stages during embyogenesis. This study is the first example that provides detailed genomic information on nuclear transport factors such as importins and exportins and provides a basis for further studies such as the generation of mutants in mice for purposes of investigating the role of importin [alpha]1 in development and differentiation.


http://www.sciencedirect.com/science/article/B6T39-47P8H5B-J6/2/07cf2f6b0780c517b1fe78b4b18bd69

An overlapping set of 21 yeast artificial chromosomes (YACs) spanning the RET proto-oncogene [Takahashi et al., Oncogene 3 (1988) 571-578] and D10S102 markers on human chromosome 10 was isolated in a series of hybridization-based chromosomal walks in a YAC library. Genetic linkage analyses implicate this chromosomal region as the location of the gene (MEN2A) responsible for multiple endocrine neoplasia type 2A. Four YACs carrying a RET sequence-tagged site (STS) and two YACs carrying a D10S102 STS were used to initiate chromosome walks. These were based on hybridization of Alu element-mediated polymerase chain reaction (Alu-PCR) products from YACs to dot blots of Alu-PCR products from complex pools of YAC clones. The hybridization anchor content of YACs identified in the walks was confirmed by probing blots of Alu-PCR products from individual YACs and by comparing Alu-PCR fingerprints of each YAC. Ten hybridization-based Alu-PCR anchors and three STS anchors were ordered within eleven intervals created by the 21 overlapping YACs. The order of anchors requiring the fewest gaps in the YACs is consistent with the walking results and establishes the STS anchor order as D10S102-D10S94-RET. The overlapping set of YACs represents about 1.55 Mb of the human genome according to restriction mapping of four representative YACs in the contig. These results demonstrate the power of Alu-PCR hybridization for chromosomal walking and provide a rich source of overlapping YACs which can be used to identify candidate MEN2A genes.

http://www.sciencedirect.com/science/article/B6T39-47PNWHM-1Y/2/367a1c2ef3e8695bc37fe6e8e12fc050

Northern blot analysis of rat testicular (Te) poly(A)+ RNA reveals that a transcript homologous to the major form of the asialoglycoprotein receptor (ASGP-R), designated RHL-1, is expressed as early as one week postnatally and that steady-state levels are approx. 8-times higher in the Te of an 8-week-old rat (sexually mature) as compared to an 84-week-old rat (aged). Partial cDNAs encoding RHL-1 and the minor form of the ASGP-R, designated RHL-2/3, have been cloned from two rat Te/epididymal (Ep) cDNA libraries and rat Te poly(A)+RNA. Sequence analysis of the Te/Ep RHL-1 cDNA and the Te/Ep RHL-2/3 cDNA indicates that these cDNAs are identical to the forms expressed in rat liver. Western blot analysis demonstrates the presence of a 49-kDa Te/Ep RHL-1-related protein band and a 54-kDa Te/Ep RHL-2/3-related protein band in both rat Te membrane fractions (MF) and rat Ep sperm MF. The RHL-1-related protein has been localized to late-stage Te spermatids at the time of release from the seminiferous tubules and to Ep sperm in the region of the sperm tail, referred to as the middle piece. Taken collectively, these data indicate that the authentic RHL-1 and RHL-2/3 genes of the ASGP-R are expressed in late-stage spermatids; however, the Te/Ep RHL-1-related protein differs in size from the hepatic RHL-1 polypeptide, possibly indicating a specific function of the RHL-1-related protein in spermatogenesis.


http://www.sciencedirect.com/science/article/B6T39-47PNWHM-1V/2/99525dc060e99a1e955c07b7e478d7ef

Northern blot analysis of poly(A)+ RNAs isolated from mouse liver or mouse testis (Te)/epididymis (Ep) reveals that both tissues express 1.5- and 7.5-kb transcripts which have extensive homology to the major form of the rat asialoglycoprotein receptor (ASGP-R). In situ hybridization studies have localized the expression of this ASGP-R-like transcript to late-stage sperm from Te and Ep of several different strains of mice. Swiss Webster mice express this ASGP-R-like transcript in late-stage spermatids at the time of release into the seminiferous tubule and in Ep sperm, while Balb/C, NIH Swiss and C57Bl/6 mice express this ASGP-R-like transcript predominantly in Ep sperm. cDNAs containing the entire coding region for this ASGP-R-like transcript have been cloned from mouse liver and mouse Te/Ep. These cDNAs are 100% identical in the coding region and 3' untranslated region (UTR), but differ in the 5'UTR. The gene encoding these cDNAs is called MHL-1, designating the major form of the mouse ASGP-R. The deduced amino acid (aa) sequence of MHL-1 shares 88% homology to the rat hepatic (He) lectin form 1 (RHL-1) and 78% homology to the human asialoglycoprotein receptor form I (H1). The three sites for N-linked glycosylation in the RHL-1 sequence are all conserved in the deduced MHL-1 sequence. Taken collectively, these data describe the cloning and sequencing of the MHL-1 cDNA and illustrate its deduced aa homology to RHL-1 and H1. Most importantly, these data show that mouse late-stage spermatids and Ep sperm express the authentic MHL-1 gene, suggesting this receptor may have an important role in spermatogenesis, in addition to its He function.
Homeodomain (HD)-containing proteins have been shown to regulate cellular commitment and differentiation in fungal, invertebrate and vertebrate systems. Bone marrow cells synthesizing the CD34 antigen are a complex mix of early, stem and progenitor cells at various stages of commitment to the many haemopoietic lineages. Here, we report the cloning and sequencing of 31 homeobox (HB) sequences, identified using degenerate oligodeoxyribonucleotide primers, in a polymerase chain reaction with cDNA derived from a purified CD34+ population of human haemopoietic cells. Of these sequences, 16 correspond to previously identified genes, and 13 are located within the HOXA, B and C clusters. Ten of the clones most likely represent human homologues of genes identified previously in other species. Five of the clones reported here represent novel HD sequences. The identification of five new genes using a subclass-specific 5' primer, designed from the engrailed and Xanfl sequences, suggests that there still remain several uncharacterised HB genes in the human genome. Haemopoietic cells purified on the basis of CD34 antigen synthesis are a rich source of regulatory genes consistent with their ability to differentiate into diverse haemopoietic cell types.


Cockayne syndrome (CS) is a human genetic disorder characterized by several neurological and developmental abnormalities. Two genetic complementation groups, CS-A and CS-B, have been identified. The CSB protein belongs to helicase superfamily 2, and to the SWI/SNF family of proteins. The CSB protein is implicated in transcription-coupled repair (TCR), basal transcription and chromatin remodeling. In addition, CS cells undergo UV-induced apoptosis at much lower doses than normal cells. However, the molecular function of the CSB protein in these biological pathways has remained unclear. Evidence indicates that the integrity of the Walker A and B boxes (motifs I and II) are important for CSB function, but the functional significance of the helicase motifs Ia, III-IV has not been previously examined. In this study, single amino acid changes in highly conserved residues of helicase motifs Ia, III, V, VI and a second putative nucleotide-binding motif (NTB) of the CSB protein were generated by site-directed mutagenesis to analyze the genetic function of the CSB protein in survival, RNA synthesis recovery and apoptosis after UV treatment. The survival analysis of these CS-B mutant cell lines was also performed after treatment with the chemical carcinogen, 4-nitroquinoline-1-oxide (4-NQO). The lesions induced by UV light, cyclobutane pyrimidine dimers, are known to be repaired by TCR whereas the lesions induced by 4-NQO are repaired by global genome repair. The results of this study demonstrate that the point mutations in highly conserved residues of helicase motifs Ia, III, V and VI abolished the genetic function of the CSB protein in survival, RNA synthesis recovery and apoptosis after UV treatment. Similarly, the same mutants failed to complement the sensitivity toward 4-NQO. Thus, the integrity of these helicase motifs is important for the biological function of the CSB protein. On the contrary, a point mutation in a C-terminal, second, NTB motif of the CSB protein showed full complementation in the ability to repair damage induced by UV light or 4-NQO, suggesting that this motif is not important for the CSB repair function.

We have identified a novel putative protein kinase-encoding gene from Schizosaccharomyces pombe (Sp), designated psk1+, by using a highly conserved amino acid (aa) sequence motif to design amplification of DNA fragments using PCR. The putative translation product of psk1+ contains 436 aa, with a molecular mass of 49 317 Da. A single psk1+ was identified by genomic Southern blot analysis, and the genomic mapping indicated that psk1+ was localized in Sp chromosome III. Growth of wild-type Sp cells was inhibited by 0.5 [mu]M phenylarsine oxide, a protein tyrosine phosphatase inhibitor, but psk1- cells were relatively resistant to this drug.


transcription and/or splicing, as does the rat Zis.


http://www.sciencedirect.com/science/article/B6T39-4778G3N-5/2/ee91d70d64a3279056370a6a42f480f

In our search for the disease gene underlying autosomal recessively inherited infantile onset spinocerebellar ataxia (IOSCA), we identified an expressed sequence tag cluster representing a previously uncharacterized transcript in the restricted genomic sequence covering the IOSCA locus on chromosome 10q24, and for mutation analyses in IOSCA patients isolated the corresponding novel human cDNA, C10orf6. Multiple tissue cDNA and Northern analyses showed that this gene is ubiquitously expressed, with expression levels highest in the skeletal muscle and less abundant in the brain, liver, and heart than in other tissues examined. C10orf6 consists of 20 exons forming a 7.3 kb cDNA which is capable of encoding a 1173 amino acid polypeptide and possesses orthologues in other mammals. Sequencing of RT and genomic PCR products of the gene revealed no alterations in IOSCA patients when compared to control subjects, and neither could differences be detected in expression levels between patient and control brain RNA samples, thus excluding mutation(s) in this novel gene as causative for IOSCA. However, this study facilitates future investigations on both the role of C10orf6 gene product in human cells as well as its possible involvement in the pathogenesis of other hereditary diseases mapped to chromosome 10q24.


http://www.sciencedirect.com/science/article/B6T39-3YWC1C-D/2/9e7602d47c08b7e042c2f915d7bace53


http://www.sciencedirect.com/science/article/B6T39-4B1X6XH-4/2/580c5cbeb2921d80ef5808e2ff686607

We have identified a novel human gene designated as IDH3GL (isocitrate dehydrogenase 3 [gamma]-like) that is expressed specifically in human testis. The gene corresponds in sequence to an EST (expressed sequence tag) A1476435 that was first detected by differential expression analysis using a microarray assay. The full-length cDNA sequence (1037 bp) was isolated from the human testis 5'-3'-RACE cDNA libraries and found to have 83% nucleotide sequence identity with part of the IDH3G (isocitrate dehydrogenase 3 [gamma]). The IDH3GL gene consists of 3 exons spanning approximately 220 kb within the region of the NELL1 gene on chromosome 11p15.1. Sequence analysis of the IDH3GL cDNA revealed the presence of a premature stop codon at nucleotide positions 337-339 that results in a truncated peptide with 112 amino acids. This stop codon is conserved in various human ethnic populations and in the chimpanzee (Pan troglodytes). In order to assess the functional status of IDH3GL, especially in relation to the
presence of the putative premature stop codon, single nucleotide polymorphisms (SNPs) were screened in the upstream, coding and non-coding regions of the IDH3GL gene in a Japanese population. As a result, a total of 10 SNPs were identified, seven were novel and one of them was a non-synonymous amino acid substitution from Leu to Val. We conclude that the IDH3GL gene sequence is a splice variant of the NELL1 gene and that it probably evolved from a transposed pseudogene of the IDH3 gene.


http://www.sciencedirect.com/science/article/B6T39-47PNXC3-G8/2/09086d5155052a0ebe3025e08ab614c8

The full-length cDNA encoding Rab23, a novel Ras-related small GTPase, was isolated using the sequence of a previously described [Chavrier et al., Gene 112 (1992) 261-264] short cDNA fragment and the rapid amplification of cDNA ends (RACE) PCR techniques. The deduced amino acid sequence was not very closely related to any previously described small GTPase, but was within the Rab subfamily. A Northern analysis revealed that the rab23 mRNA is predominantly expressed in the brain, which places the protein, together with Rab3a and Rab 15, in the group of small GTPases characteristic of the nervous system.


http://www.sciencedirect.com/science/article/B6T39-3R3GCDP-H/2/b4a0b2c4d55d4d61e34e8ebf93af5f9

Laccases are oxidoreductase enzymes involved in the oxidation of various phenolic compounds. They may play a role in the biodegradation of lignin and in the dechlorination of chlorophenols. The cDNAs encoding laccase Lcll and a putative laccase LccIV and the gene for Lcll from the white-rot basidiomycete Trametes versicolor were cloned, sequenced and characterized. The genomic DNA of lcll consists of 2128 bp, with the coding region interrupted by 10 introns; the cDNA consists of a 1560 bp open reading frame (ORF). The cDNA of the putative lccIV gene consists of a 1581 bp ORF, with a 794 bp 5’ untranslated region. The size of the major transcript for both lcll and lccIV is approximately 2.3 kb. Transcription of lccIV was induced by 2,5-dimethylaniline, whereas the opposite effect was observed for lcll. Laccases I and IV contain highly conserved histidinyl and cysteinyl residues, believed to be involved in binding copper, and share extensive sequence similarity with other laccases produced by both ligninolytic and non-ligninolytic fungi.


http://www.sciencedirect.com/science/article/B6T39-47T30VW-7W/2/31469f53a3499def50e1b83f232fc7bd

The major histone-like bacterial protein (HU)-encoding genes (hup) from five different Bacilli have been cloned, sequenced and overexpressed in Escherichia coli. The five Bacilli selected are closely related, but have different optimum growth temperatures: > 70[deg]C for Bacillus
caldolyticus and B. aldotenax; 60-65[deg]C for B. stearothermophilus (Bst); 37[deg]C for B. subtilis and 30[deg]C for B. globigii. The deduced amino acid (aa) sequences from the three thermophiles are identical. Those from the two mesophiles are also identical and differ from those of the thermophiles at eleven aa positions. The mesophilic proteins have an extra two aa at the C terminus. Cells harbouring plasmids containing the hup genes can produce HU. An efficient purification scheme using cation-exchange chromatography and fast protein liquid chromatography is presented. This gives approx. 30-40 mg of more than 95% pure Bst HU per litre of E. coli culture.


Lithium sensitive myo-inositol monophosphatase (IMPase) is a pivotal enzyme which controls the levels of brain inositol within the inositol-based signaling system. Its capacity to release free myo-inositol from inositol monophosphates generated from receptor-linked and de novo pathways is crucial to the maintenance of appropriate amounts of intracellular myo-inositol, which is essential for both inositol-based cell signaling and cell volume control. We present here the full length cDNA encompassing the coding and untranslated regions (5'- and 3'-UTRs) of rat brain IMPase. This cDNA was derived from rat cortex mRNA by the RT-PCR technique. Analysis of this cDNA revealed several interesting features which include a short 5'-untranslated region (5'-UTR) of 68 nucleotides followed by coding region of approximately 0.8 kb and a long 3'-untranslated region (3'-UTR) of 1.2 kb. Both 5'-rapid amplification of cDNA ends (5'-RACE) and 3'-RACE techniques were carried out to isolate both UTRs and double stranded sequencing was carried out to its entirety (~2.1 kb) by 'gene walking' using several oligonucleotide primers. All nucleotides were sequenced unambiguously using the sense and antisense strands of DNA. PCR analysis for the coding region and the deduced amino acid sequence demonstrated a DNA fragment of 831 bp and 277 amino acids, respectively, which are strikingly similar to human hippocampal IMPase. The 5'-UTR demonstrated distinct CpG doublets, characteristic of 'housekeeping' genes. The sequence around the initiator methionine, AAGATGG, conforms well to the Kozak consensus sequence for mammalian protein biosynthesis and the 3'-UTR demonstrated three canonical and one unusual poly-adenylation signals followed by a 31 base poly(A) tail. The presence of a CCTGTG in the 3'-UTR (putative carbohydrate response element) links IMPase mRNA to brain carbohydrate metabolic pathways. Computer analyses demonstrated several unique features of this mRNA, including the potential formation of hairpin loops which might be important in its intracellular regulation and turn-over. In summary, this lithium-sensitive brain IMPase mRNA has the following characteristics: a 5'-CpG-rich short untranslated segment, a highly conserved coding region, and a long 3'-untranslated region with several polyadenylation signals.(c) 1997 Elsevier Science B.V. All rights reserved.


http://www.sciencedirect.com/science/article/B6T39-3YGDG07-X/2/bbc0e9069af1beee45deb7c18def9f91

http://www.sciencedirect.com/science/article/B6T39-3YGDG07-5/2/5fed520f76f84c48a8db0cdddc74ee84


http://www.sciencedirect.com/science/article/B6T39-3Y6HK2X-2C/2/0df61a0bf275e7d8cddcfef56b995866b

A linear 5.2-kb HS2/β-globin construct with an upstream KpnI terminus (4-nucleotide (nt) 3' protruding single strand, PSS) and a downstream SalI terminus (4-nt 5' PSS) was microinjected into fertilized mouse eggs. The injected DNA fragments integrated into the mouse genome primarily as a head-to-tail tandem array. Chromosome/transgene junctions were obtained from seven of eight transgenic animals. All of the junctions occurred in the proximity of a transgene KpnI end; a maximum loss of 8 nt from the transgene terminus was observed. Two of these junctions completely preserved the 4-nt KpnI 3' PSS. Transgene/transgene junctions from two animals were analyzed. SalI/KpnI junctions that completely preserved both the SalI 5' PSS and the KpnI 3' PSS were found in each animal. These are the first examples of complete nt preservation at junctions formed between a 5' PSS terminus and a 3' PSS terminus in transgenic mice. The data are consistent with the fill-in model of Thode et al. [Cell 60 (1990) 921-928] in which alignment proteins juxtapose 5' PSS and 3' PSS termini; DNA polymerase then utilizes the recessed 3'-OH of the 5' PSS terminus as a primer to synthesize DNA across the gap. This mechanism results in the formation of junctions with no loss of sequence. The results described in the present paper suggest that this mechanism may be involved in the formation of junctions in transgenic mice.


http://www.sciencedirect.com/science/article/B6T39-3VWFYVS-1/2/127b969cc4feaa515561eab22b8bb0d5

We define intracellular immunization as the inhibition or inactivation of the function of a molecule by the ectopic intracellular expression of antibody binding domains which recognise the molecule. Such recombinant antibodies can be directed to different compartments of eukaryotic cells by means of previously defined targeting signals, thus permitting the study of any molecule in any cellular compartment for which an antibody is available. For this purpose, we have created a set of vectors based on the VHExpress vector described [Persic, L., Roberts, A., Wilton, J., Cattaneo, A., Bradbury, A. and Hoogenboom, H.R. (1997) An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from phage display libraries. *Gene* 187, 000-000], which has been modified to express scFvs (single chain fragments) linked to specific targeting signals. These permit the localisation of scFvs to different intracellular compartments: the endoplasmic reticulum (scFvE-er), the nucleus (scFvE-nuclear), the mitochondria (scFvE-mit), the cytoplasm (scFvE-cyto), and as secreted proteins (scFvE-sec). The function of these vectors has been assessed by the immunofluorescence of COS cells transiently transfected with constructs containing the [alpha]D11 scFv.
Phage display is now an established method to select antibody fragments specific for a wide range of diverse antigens. In particular, isolation of human monoclonal antibodies has become a reality and for most purposes bacterial expression of the selected recombinant antibody fragments is sufficient. However, there are some cases where the expression of complete human immunoglobulin in mammalian cells is, if not essential, at least desirable. For this reason we have designed and constructed a set of mammalian expression vectors which permit facile and rapid cloning of antibody genes for both transient and stable expression in mammalian cells. Immunoglobulin genes may be cloned into these expression vectors as V regions or as Fab fragments, using restriction sites which are rare in human V genes. All the important elements in the vectors - promoter, leader sequence, constant domains and selectable markers - are flanked by unique restriction sites, allowing simple substitution of elements. The vectors have been evaluated using the variable regions from the neutralizing anti-nerve growth factor (NGF) antibody, [alpha]D11, and the V regions from 2E10, a scFv selected from a scFv phagemid library.

Bone Gla protein (BGP, Osteocalcin) is a bone-specific vitamin K-dependent protein which has been intensively studied in mammals. Although BGP is the most abundant non-collagenous protein of bone, its mode of action at the molecular level remains unclear. From an evolutionary point of view, the appearance of BGP seems to parallel the appearance of hydroxyapatite-containing bone structures since it has never been found in elasmobranchs, whose skeleton is composed of calcified cartilage. Accordingly, recent work indicates that, in mammalian bone, BGP is required for adequate maturation of the hydroxyapatite crystal. Taken together, these data suggest that teleost fishes, presumably the first vertebrates to develop a BGP-containing skeleton, may be a useful model to further investigate BGP function. In addition, fish offer several advantages over mammalian models, due to a large progeny, external embryonic development and transparency of larvae. In the present work, the BGP cDNA and gene were cloned from a teleost fish, Sparus aurata, and its tissue distribution, pattern of developmental expression and evolutionary pathways analyzed. The molecular organization of the Sparus BGP (spBGP) gene is similar to mammalian BGP genes, and its expression throughout development follows the onset of calcification. The spBGP gene encodes a pre-propeptide of 97 amino acid residues, expressed only in bone and showing extensive homology to its mammalian homologs. Phylogenetic analysis of the available BGP sequences supports the hypothesis that all BGPs have a single origin and
share a common ancestor with a related vitamin K-dependent protein (Matrix Gla protein).


http://www.sciencedirect.com/science/article/B6T39-3VGR2GV-22/2/20ffcc9019471865e649ab5be3c8dea37

The aim of this study was to determine the causes of the high heterogeneity, in the number and the length, of the thyrotropin (TSH) [beta] mRNA in the European eel. Northern blot analysis showed that removal of the poly(A) tail did not affect this heterogeneity. PCR amplification on reverse-transcribed pituitary RNAs (RT) showed the main source of heterogeneity to be a highly variable region in the 3' untranslated region (UTR). PCR amplification of the 3' UTR from RTs and genomic DNAs demonstrated that the high variability reflected polymorphism within the eel TSH [beta] gene. Isolation and sequencing of 3' UTR amplification fragments showed that the variable region comprised more or less exact repetitions of a 26-42-bp fragment. The number of repetitions varied from one allele to another. This variable region could be characterized as a minisatellite. In conclusion, instability of a minisatellite in the 3' UTR of the TSH [beta] gene generated the multiple and widely differing TSH [beta] mRNAs.


http://www.sciencedirect.com/science/article/B6T39-47PNWW7-68/2/08947e64fd5417c7800e3b8aa0639aa8

We have constructed two plasmid vectors which allow selection for in vivo deletions within cloned DNA fragments. The plasmids are derivatives of pBR322 which carry the Escherichia coli rpsL (strA) gene, known to confer a dominant streptomycin (Sm)-sensitivity phenotype to the host cell, and a copy of the IS1 transposable element. Sm-resistant strains that harbor these plasmids display sensitivity to Sm. Spontaneous IS1-promoted deletions across the rpsL gene can be isolated simply by selection for Sm resistance. Hence, nested sets of deletions of a cloned DNA can be obtained and sequenced with an IS1-specific primer. Using this approach, we have determined the complete nucleotide sequence of the [Omega] interposon [Prentki and Krisch, Gene 29 (1984) 303-313].


DEAD-box genes are found throughout evolution and encode RNA-binding proteins. Such proteins include eukaryotic initiation factor-4A, which is essential for protein translation, Vasa, which is essential for germ line development, and a number of nuclear and mitochondrial RNA splicing factors. Transcription of a human DEAD-box gene, DDX1, is elevated in two retinoblastoma cell lines as a result of amplification of the immediate chromosomal region surrounding it, suggesting an important role for this gene in control of cell growth and division. We have isolated a Drosophila melanogaster (Dm) homologue (Ddxl) of DDX1 which is strikingly
similar to the human gene. The similarity (58.3% amino acid (aa) identity over 720 aa) extends beyond regions conserved in all DEAD-box proteins and covers the entire lengths of the proteins. The 2.7-kb Dm Ddxl RNA is expressed throughout development, but its levels are elevated in early embryos. Ddxl maps to polytene chromosome band 79D4 on the left arm of Dm chromosome 3.


Centromere protein A (CENP-A) is a centromere-specific histone H3 variant conserved amongst all eukaryotes. We have isolated the chicken gene for CENP-A (GgCENP-A). It encodes a 131-amino-acid polypeptide that possesses an average identity of 54% with human CENP-A, reaching 69% in the histone-fold domain. The gene spans 1.7 kb of genomic DNA and contains four exons that range in size from 78 to 186 bp. The exon/intron organisation of the chicken gene is conserved with its mammalian counterparts in the carboxy-terminal histone-fold domain (exons 2 to 4), consistent with the strong conservation of this domain at the amino acid level. Sequence analysis of the chicken CENP-A locus revealed that the gene is located within the class III genes of the major histocompatibility complex (MHC), and extended the previously defined limit of the compact chicken MHC complex. We compared the sequences of CENP-A from mammals, chicken and fishes and thereby identified conserved motifs in the otherwise variable amino-terminal tail that may be important for functional reasons. We also identified evolutionarily variable regions within the conserved histone-fold domain. We found that loop 1 between the first and second [alpha]-helix is the region that diverged most widely. This finding is in agreement with evolutionary studies in Drosophila species, and suggests that this domain could play a role in species-specific centromere targeting of CENP-A. In addition, protein sequence comparison of several vertebrate species revealed that the RT-PCR strategy we have developed for isolating the chicken centromeric histone H3 variant gene should be applicable to the isolation of CENP-A from a wide range of vertebrates.


http://www.sciencedirect.com/science/article/B6T39-3Y5MNJ3-3B/2/9d3b3693d545136b8dec65ba491c6a4b

One of two similar genes in the unicellular eukaryote Naegleria gruberi is shown to encode calcineurin B (CnB), the regulatory subunit of calcium-calmodulin-regulated protein phosphatase 2B. Over a span of 156 amino acids, excluding divergent N-termini, the encoded sequence shows 62% identity with vertebrate CnB, and also shows sequence elements specific, among calcium-binding proteins, to CnB. In contrast, the sequence shows only 23% identity with N. gruberi flagellar calmodulin. CNB mRNA is readily detected in amoebae; its abundance increases fourfold during differentiation to flagellates, reaches a peak at 50-70 min, when flagella are forming, and then declines. A genomic clone matches an expressed cDNA, except that it is interrupted by two phase I introns. The position of one intron, which separates the divergent N-terminal domain from the four calcium-binding domains (EF hands), is shared with a yeast CNB gene; the other is located in the central helix between the two pairs of calcium-binding loops; features that support an ancient origin. These introns, the first found in protein-coding genes of Naegleria, are flanked by characteristic splice junction sequences. N. gruberi CnB also shares similarities with recoveries. The finding in a protist of a CNB gene that contains two introns
separating functional domains, shares similarities to recoveries and shows increased expression
during differentiation is provocative. If the phylogeny of major groups derived from ribosomal RNA
is accepted, Naegleria is among the earliest branching eukaryotes known to contain canonical
pre-mRNA introns.


http://www.sciencedirect.com/science/article/B6T39-3W846W4-7/2/67c76ba02ae52358b5ba2a46221eaf1c

Rie, T., B. Anderson, et al. (2003). "Rapid and efficient transposon mutagenesis of Bartonella henselae
by transposome technology." Gene 313: 103.

http://www.sciencedirect.com/science/article/B6T39-493HSN6-4/2/6c6d9a80b7df176c7359dcb41abc4b93

Molecular genetics are difficult to perform in Bartonella henselae, the causative agent of cat
scratch disease and the vasculoproliferative disorders bacillary angiomatosis and bacillary
peliosis. To elucidate the underlying bacterial pathogenic mechanisms, genetic manipulation of B.
henselae is the method of choice. We describe how to perform transposon mutagenesis in B.
henselae using transposome technology. B. henselae mutants revealed by this technique showed
random transpositional insertion into the chromosome. In contrast to transposon mutagenesis by
conjugational transfer, transposome technology allows transposon mutagenesis of early
passaged Bartonella spp. with approximately 100-fold higher efficiency. The results show that
transposome technique is a rapid, efficient and simple method to generate transposon mutants of
B. henselae.


http://www.sciencedirect.com/science/article/B6T39-3Y6HGWH-3S/2/5d2fcb1334ca20b4043bb80df09631e

Fibrinogen, the major blood-clotting protein, is made up of three chains, A[alpha], B[beta] and
[gamma], which are synthesized and secreted by the liver. In this communication, we describe
the complete cDNA sequence, deduced amino acid (aa) sequence and organization of the gene
encoding the B[beta] subunit of fibrinogen from Xenopus laevis (Xl). The cDNA representing the
predominant form of the B[beta] mRNA comprises 2390 nucleotides (nt), with an open reading
frame of 1467 nt coding for a 488-aa protein. The percent identity between Xl B[beta] and that of
other animals ranges from 50% for lamprey to 66% for human. The Xl B[beta] gene consists of
nine exons, one more than found in the human gene. The exon/intron boundaries in the frog and human B[beta] genes are in exactly conserved positions, except for junctions in the highly variable fibrinopeptide-encoding regions. Three of the exon/intron boundaries in the XI B[beta] gene are also analogous to ones in A[alpha] and [gamma] genes of other species, supporting the notion of a close evolutionary relationship between the genes for all three subunits. This analysis of B[beta] from an amphibian provides the first complete description of the arrangement of exons and introns in any fibrinogen subunit gene from a non mammal and gives insight into the most highly conserved aspects of fibrinogen protein structure and gene organization.


http://www.sciencedirect.com/science/article/B6T39-3VBSRC2-1/2/87f887f85ef657a00f6e07e3522b62f4

Quantitative reverse transcriptase-polymerase chain reaction (QRT-PCR) and the ion-pair reverse-phase (IP-RP)-HPLC product purification and detection system were developed to facilitate the isolation and proportional quantification of alternatively spliced RUSH mRNAs. RUSH isoforms result from alternative splicing of a 57-bp exon and encode SNF/SWI-related proteins that bind to the uteroglobin promoter. QRT-PCR was performed using total RNA, and a pair of primers designed to flank the 57-bp exon. When more than one splice variant was expressed, IP-RP-HPLC identified the specific homoduplex products, as well as the heteroduplexes formed as a consequence of partial sequence complementarity between the products. Data analysis included the correct re-allocation of heteroduplex components to achieve accurate quantitation of changes in the relative levels of RUSH message isoforms. The preferential expression of the RUSH-1[alpha] isoform by all the tissues except estrous uterine endometrium and lactating mammary gland indicates RUSH pre-mRNAs are alternatively spliced in a tissue-specific manner. A 61-fold difference in the relative rate of RUSH pre-mRNA splicing is indicated by the difference in the ratios of RUSH mRNA isoforms from uterine endometrium and testis. Clearly, QRT-PCR and IP-RP-HPLC are powerful and versatile tools for the detection and quantitation of mRNA splice variants.


http://www.sciencedirect.com/science/article/B6T39-3W07P0SN-2/2/f56ba3e117f2517ded0fd115007b08a6


http://www.sciencedirect.com/science/article/B6T39-3Y6HGRC-2/2/16ab33ff25caeeba200f6d7fb1fa74a

In the pond snail Lymnaea stagnalis (Ls), growth and associated processes are likely to be controlled by a family of molluscan insulin-related peptides (MIP). Here we report on the cloning of a cDNA encoding a putative receptor for these MIP. This cDNA was isolated from Ls via PCR with degenerate oligodeoxynucleotides corresponding to conserved parts of the tyrosine kinase
domain of the human insulin receptor and its Drosophila homologue. Many of the typical insulin-
receptor features, including a cysteine-rich domain, a single transmembrane domain and a
tyrosine-kinase domain are conserved in the predicted, 1607-amino acid (aa) protein.
Comparison of the aa sequence of the molluscan receptor to other insulin-receptor sequences
revealed strong variations in the percentage of sequence identity for the different domains,
ranging from 70% sequence identity in the tyrosine-kinase domain to virtually no sequence
identity in the C-terminal sequence. Striking differences are the absence of a clear tetrabasic
cleavage site, and the extremely long C-terminus of 308 aa that contains seven Tyr residues.
Southern blot analyses at varying stringencies, extensive screening of cDNA- and genomic
libraries, and PCR experiments indicate the presence of a single putative MIP receptor. This
suggests that the four different MIP may exert their functional role in Ls by binding to the same
receptor.

http://www.sciencedirect.com/science/article/B6T39-3WBG1Y1-
P/2/7b9ecb30633b0c11d299f3dc8f31ed90

The Tat protein of equine infectious anemia virus, EIAV, was shown to augment viral gene
expression, presumably through interaction with the Tat responsive element, TAR. Recently, cell-
free polyadenylation assays suggested that perturbation of the EIAV TAR secondary structure
diminished polyadenylation efficiency. The present study indicates that the EIAV TAR regulates
the efficiency of the 3'-end processing of viral RNA also in transfected cells. Moreover, our data
suggest that the provision of the EIAV Tat protein in trans potentiates read-through transcription
through the 3' viral long terminal repeat (3' LTR), thus suggesting activation of downstream-
located cellular genes.

submaxillary gland of mice show extensive evolutionary divergence in the protein coding region." Gene 142(2): 175.
http://www.sciencedirect.com/science/article/B6T39-47P8HFC-
MP/2/91447eb635fb4c397586a0c801830ef4

We have previously characterized an abundant male-specific mRNA from the submaxillary gland
(SMG) of rats, encoding the SMR1 (androgen-regulated) protein, which has the structure of a
prohormone and is processed by maturation enzymes to release a small peptide in the blood and
saliva. We have now characterized three SMR1 -related cDNAs in the SMG of Balb/c mice.
These cDNAs encode three novel proteins, designated MSG1, MSG2 and MSG3. They are 639,
662 and 471 nucleotides (nt) long, respectively, and the corresponding mRNAs appear to be
expressed only in the SMG. The putative polypeptides they encode carry an N-terminal secretory
peptide sequence and are, therefore, presumably secreted into saliva. Although closely related,
the three mRNAs show striking differences: a particularly different expression pattern and an
extremely high degree of variability observed in the central part of the molecules. The MSG1 and
MSG3 cDNAs are identical, except for a 173-bp insert found only in MSG1. This insert contains
three Pro-rich repeats (GPGIGRPPPPPPP), reminiscent of the most abundant multigenic family of
the SMG, the Pro-rich proteins (PRP). Although MSG1 shares several common features with
PRP, it is structurally related to SMR1. The unusually high ratio of replacement/silent nt changes
provides a basis to address complex aspects concerning the molecular events leading to the
emergence of new proteins in the SMG.


http://www.sciencedirect.com/science/article/B6T39-3SBNJXV-N/2/8989c92fe7fb3d165190d37afa8910d

Haemophilus ducreyi is the etiologic agent of the sexually transmitted disease chancroid, an ulcerative condition implicated in increased HIV transmission. There is increasing evidence for the roles of oxidative stress proteins including superoxide dismutase enzymes in the survival and persistence of pathogenic organisms within the host. The sodA gene of Haemophilus ducreyi was isolated from a genomic plasmid library on the basis of its ability to rescue the hydrogen peroxide hypersensitivity of an *Escherichia coli* sodA-sodB- strain. The *H*. ducreyi SodA protein also complemented the aerobic growth defect of the *E*. coli sodA-sodB- strain in minimal medium. The deduced amino-acid sequence of the *H*. ducreyi sodA gene product is 74 and 70% identical to the Mn-SODs of *Haemophilus influenzae* and *E*. coli, respectively. However, unlike Mn-SODs, the *H*. ducreyi SodA protein was inhibited by hydrogen peroxide in native gels stained for SOD activity.


http://www.sciencedirect.com/science/article/B6T39-3TJ452W-D/2/38f24a572fa315da5c5c4708959fd4b4

To identify *Cryptosporidium parvum* genes expressed during intracellular development, differential mRNA display was used to detect differences in gene expression between mock-infected and *C*. parvum-infected human epithelial cells. A reproducible band present only in *C*. parvum-infected cells, ddHC-23, was isolated and cloned. Southern blot analysis demonstrated that ddHC-23 represented a *C*. parvum gene. RT-PCR revealed that HC-23 mRNA levels decreased from 6 to 12 h post-infection (pi), were maximally expressed at 24 h pi, and returned to low levels at 48 and 72 h pi. Northern blot analysis determined that the approx. 3.6 kb transcript is expressed by sporozoites prior to invasion of epithelial cells. Screening of a *C*. parvum genomic library with ddHC-23 isolated a genomic subclone which contained a 2790 bp ORF, uninterrupted by introns. Sequence analysis indicated that the encoded protein, which displayed no similarity to any sequences in the public databases, contained a high proportion of polar amino acids, with the most abundant being Asp (17.3%), Ser (15.8%) and Gly (8.1%). Numerous potential sites for posttranslational modification were present including: casein kinase II and protein kinase C phosphorylation sites, N-myristolation sites and N-glycosylation sites. These findings demonstrate the usefulness of differential mRNA display for identifying developmentally regulated *C*. parvum genes within the background of genes expressed by the host cell.


A novel family of genes expressed in human brain has recently been identified. Gene 239FB, transcribed extensively in fetal brain, was isolated from the chromosome 11p13 region associated with mental retardation component of the WAGR (Wilms tumor, aniridia, genitourinary anomalies, mental retardation) syndrome. This report presents a cDNA sequence and expression profile of a related gene, 239AB, isolated from adult brain library, that was mapped to chromosome 22. While similar in structure, the two genes differ in their expression pattern and may have different roles in central nervous system development and function. In contrast to the 239FB, which is expressed predominantly in fetal brain, the 239AB gene is transcribed in adult tissues. Both human genes encode novel proteins of unknown function that are highly conserved from Caenorhabditis elegans to birds and mammals. Phylogenetic analysis suggested that the two lineages of the ancient gene family represented by 239FB and 239AB have been in existence prior to the emergence of modern animals.


http://www.sciencedirect.com/science/article/B6T39-3RM6VM7-G/2/6a30b0dd311ee7f8239b8769cc4b049a

Lysenin, which causes contraction of rat vascular smooth muscle, is a protein that was isolated from the earthworm Eisenia foetida. A cDNA encoding lysenin was isolated by use of a partial cDNA probe that had been generated by the PCR with a primer designed by reference to an internal peptide sequence of lysenin. This clone had an ORF encoding 297 amino acid residues. The amino acid sequence deduced from the cDNA revealed the absence of any significant homology to those of previously characterized vasoactive substances. The recombinant lysenin was produced in Escherichia coli. This protein and native lysenin isolated from the earthworm had similar contractive activities when tested on rat aorta. Northern blot analysis of the RNA from various tissues of the earthworm indicated that lysenin is produced by the coelomocytes.


http://www.sciencedirect.com/science/article/B6T39-47P8MDG-1NJ/2/1ff84c05be523f6fa696fe7d6617880c

A novel human cDNA encoding a putative nucleotide-binding protein (NBP) was obtained by screening a human SHSY5Y neuroblastoma library. The deduced protein contains 320 amino acids (aa) with a Mr of 34 540. NBP displays sequence similarity with the product of the minD gene from Escherichia coli. MinD is involved in the proper placement of the division septum, and has ATPase activity. NBP and MinD contain consensus nucleotide (nt)-binding domains. The NBP mRNA is approx. 1500 nt in length and is expressed in several human cell lines and in all rat tissues examined, with the highest levels in lung and testis.

http://www.sciencedirect.com/science/article/B6T39-3W25BP2-33/2/87a95b6deftb316f4e922108dee91ccbe

DNA sequencing of intron 4 of the p53 gene from seven cutaneous melanoma cell lines showed an absence of mutations. However, both control and melanoma cell lines sequences were different from the reference source obtained from GenBank databank (accession No. X54156). Base pairs 101 and 689 were determined to be T (instead of A) and C (instead of G). Also, an additional C was not detected at position 371. Comparative analysis with p53 DNA-binding sequences, a sequence recognized by a p53 intron 4-binding protein and consensus sequences recognized by transcription factors demonstrated that intron 4 contains putative sequences for NF-κB, SP1, AP1 and TFIID binding. Binding of transcription factors could be one of the mechanisms by which intron 4 modulates human p53 expression.


http://www.sciencedirect.com/science/article/B6T39-3RD1R45-3/2/fd4a00418e86919e37f9a6bf42e3d381

As a step toward understanding the transcriptional regulation of the adrenocorticotropin receptor (ACTH-R) gene, we examined the full length cDNA sequence of the mouse ACTH-R by rapid amplification of cDNA ends, and the organization of the gene. Mouse ACTH-R mRNA consists of 374 bp in the 5'-untranslated region (UTR), 888 bp in the coding sequence, and 445 bp in the 3'-UTR, the 1707 bp being fairly compatible with the 1.8-kb adrenal mRNA detected by Northern analysis. The mouse ACTH-R gene consists of at least four exons; the first three exons encode 5'-UTR and the fourth exon encodes part of 5'-UTR, the entire coding region, and the whole of 3'-UTR. We also defined two mRNA species, one with and one without the 57-bp exon 2, produced by alternative splicing.


http://www.sciencedirect.com/science/article/B6T39-44KPS90-3/2/f7e2f214a6e631c3594ad7024ae480f9

Ameloblastin (AMBN) is one of the enamel sheath proteins which presumably has a role in determining the prismatic structure of growing enamel crystals. There may therefore be a relationship between the molecular evolution of the AMBN gene and the development of enamel prismatic structures. To investigate whether such a relationship exists, it was necessary to identify the homologues of the AMBN gene in a reptile whose teeth lack an enamel prismatic structure. To this end, several clones containing AMBN cDNA were isolated from caiman jaws using the reverse transcription-polymerase chain reaction (RT-PCR) method. Sequence analysis of the AMBN cDNA revealed an open reading frame of 1221 bp encoding a 407-amino-acid protein. Translation of the caiman cDNA starts at the methionine corresponding to the second of two putative start codons conserved in mammalian AMBN genes. The N-terminal part of the caiman AMBN shows high amino acid sequence similarities to human, pig, cattle, rat and mouse AMBN sequences, as well as several other features that have been conserved throughout the evolution of reptiles and mammals. Unexpectedly, the nucleotide sequences of the 3'
untranslated region (UTR) are also conserved, not only within mammalian genes but also between reptilian and mammalian genes. The caiman AMBN gene is a single-copy gene, transcribed only in the jaws, presumably in teeth.


http://www.sciencedirect.com/science/article/B6T39-49M0W0P-4/2/9928ddc99ee56172baf8eeded30768eb

Ameloblastin (AMBN) is an enamel sheath protein that presumably has a role in determining the prismatic structure of growing enamel crystals. To investigate the relationship between the molecular evolution of the AMBN gene and development of enamel prismatic structures, it is considered to be of great significance in the identification of homologues of the AMBN genes in nonmammals whose teeth lack an enamel prismatic structure. Several clones containing AMBN cDNA were isolated from an African clawed toad tooth cDNA library by screening with a polymerase chain reaction (PCR) method. Sequence analysis of the clones revealed that they were derived from different genes (toad-A and toad-B), which were found to contain ORFs encoding 408- and 352-amino-acid proteins, respectively. The N-terminal part of the toad AMBN proteins and the phosphorylation motif for casein kinase II, as well as several features, were found to be highly conserved throughout the evolution of tetrapods. Exon-intron boundaries were shared by toad and caiman genes with the exception of exons 6, 7 and 10 while human and caiman genes shared them exclusive of exons 8 and 9 which have been found only in the human. As for exon 7, it was absent in both toad genes. Moreover, the AMBN genes were transcribed only in the upper jaw, presumably in teeth. These results may provide useful information for investigation of the evolution of enamel.


http://www.sciencedirect.com/science/article/B6T39-47PNWHM-1S/2/2bfb5112ef4b12e7f09762a80e5d6a99

Receptor protein tyrosine kinases (RPTK) are critical components of signal transduction pathways in multicellular organisms. Identification of new RPTK constitutes an initial step in understanding the variety of signalling pathways in which these proteins participate. In this study, a cDNA containing a complete coding sequence for Cek7 (chicken RPTK) has been cloned from a chicken embryo expression library using anti-phosphotyrosine antibodies (Ab). Cek7 is a member of the EPH (human RPTK) subfamily of RPTK; this subfamily is characterized by extracellular domains containing an immunoglobulin-like motif, a Cys-rich region and two fibronectin type-III repeats. Analysis of additional cDNAs revealed that two positions of alternative splicing in primary transcripts may produce several isoforms of this RPTK; cDNAs corresponding to three isoforms of this receptor are reported. These isoforms are predicted to have altered extracellular ligand-binding domains and/or altered cytoplasmic juxtamembrane regions. The nucleotide sequence of cek7 cDNAs identified in this study diverges at the 3' end from the sequence found in a recently described partial cek7 cDNA [Sajjadi and Pasquale, Oncogene 8 (1993) 1807-1813]. Therefore, a third position of alternative splicing may produce Cek7 RPTK with divergent C-terminal tails. RNA blot analysis revealed expression of this receptor at highest levels in the central nervous system and eyes of 10-day-old chicken embryos.

http://www.sciencedirect.com/science/article/B6T39-42D2CGD-R/2/110029785d424fc0aa5092ce91b1bf1

Four clones encoding homologous protein(ase)s were isolated from the Vipera lebetina (snake) venom gland cDNA library. One of them represented DNA encoding factor V activating enzyme (Siigur et al., 1999), the other is homologous to VLFVA but has two principal discrepancies in the translated protein sequence in comparison with snake venom serine proteinase structures: in the active site triad Ser195 is replaced by Asn195 and His57 by Arg57. The third and the fourth clone represent combinations of the first two clones. The possibilities of generation of such clones via trans-splicing of the primary gene transcript, by exon shuffling or by unequal crossing-over on the genome level are discussed.


http://www.sciencedirect.com/science/article/B6T39-433NSPG-4/2/2c6e6b8d2b3a4724958c447a13d88770

Analysis of genetic instability in breast cancer tissues compared to uninvolved breast tissues from the same individuals by RAPD (random amplified polymorphic DNA)/AP-PCR (arbitrarily primed PCR) fingerprinting using 30 arbitrary primers revealed 190 amplified DNA fragments. Presumably, each of these represents a gene locus in a different region of the genome of breast cancer tissues. Among these amplified DNA fragments, 65 (34.2%) exhibited presence and absence or reductions and enhancements in the intensity in breast cancer tissues compared to uninvolved breast tissues from the same individuals, and 11 amplified DNA fragments (5.7%) represented polymorphisms in the uninvolved human breast tissues. Reductions and enhancements in the intensity of some of the amplified fragments were observed indicating allelic gains or losses in the breast tumor genome compared to the matched uninvolved tissue genome. The presence or absence of some of the amplified DNA fragments were observed in this study indicating homozygous deletions or insertions in the breast tumor DNA compared to the matched uninvolved tissue DNA. Notably, an insertion of a 1270 bp amplified fragment was observed in 81% (17 of 21) of the tumor samples using the primer, OPC04. This amplified fragment resolved into two, 1200 and 1300 bp, single-stranded amplified fragments on the denaturing sequencing gel. This separation into single-stranded fragments suggests that the amplified fragment contains a conformation that is semistable. The 1270 bp amplified fragment localizes to the q11.2 region of chromosome 17. Sequence analysis of this fragment showed a significant DNA base sequence similarity (93%) with one of the breast tumor-specific human EST. The similarity with EST sequences and RT-PCR analysis showed that a part of this amplified fragment is from the coding region of the genome. Any one of the events observed in this study could play an important role in the development of breast cancer or could occur during the clonal expansion of the genetically unstable breast cells.


ERV9 is a low repeated family of human endogenous retroviral elements, which has close to 50 members, in addition to at least 4000 solitary LTRs. Previous work has shown that randomly selected LTRs can promote transcription of reporter genes, raising the possibility that these sequences may affect the expression of adjacent cellular genes. We performed Northern blot experiments using sequences from ERV9-LTR, and we observed a different pattern of expression in several different hemopoietic tumor cell lines. It is possible that by the result of a somatic integration event, or by virtue of their original dispersal in the genome, ERV9-LTRs may specifically induce the expression of different cellular sequences in different cell lineages. Here, we describe the identification and analysis of four chimeric cDNA clones isolated from the T-lymphoma Peer cell line, having a structure consistent with transcription initiation from an ERV9-LTR. All the cDNA clones represent transcripts derived from unique cellular sequences. We also report the genomic localization of these cDNA clones.

Transforming Growth Factors-[beta] (TGF-[beta]s) have been described in many vertebrate species of amphibians, aves and mammals. In this report we demonstrate the presence of TGF-[beta]2 in pisces. TGF-[beta]2 has been cloned from a fish, Cyprinus carpio, by RT-PCR using degenerate oligonucleotide primers. Sequence analysis of the amplified product and alignment of the deduced amino acid sequence with the human TGF-[beta]2 amino acid sequence revealed 81% and 93% identity in the precursor and the mature regions, respectively. The northern blot analysis of fish heart RNA shows a major messenger RNA species of about 8.0 kb and two messages of very low abundance of about 5.0 kb and 4.0 kb. The identification of TGF-[beta]2 isoform in Pisces and it's high degree of homology with the mammalian isoform suggests that among all TGF-[beta] isoforms, TGF-[beta]2 is the most conserved during evolution.

Human and non-human primate ABO blood group genes show relatively large numbers of nucleotide differences. In this study, we determined exon 7 sequences for 10 individuals of common chimpanzee and for four individuals of bonobo to estimate nucleotide diversities among them. Sequence data showed the existence of chimpanzee specific 9-base deletion in the beginning of the exon 7 coding region. From a phylogenetic network of exon 7 sequences of ABO blood group genes for human, common chimpanzee, bonobo and gorilla, effects of parallel substitutions and/or some kinds of convergent events are inferred in the chimpanzee lineage. We
also estimated nucleotide diversities for common chimpanzee and bonobo ABO blood group genes, and these values were 0.4% and 0.2%, respectively. These values are higher than that of most human genes.


Phosphoenolpyruvate carboxykinase (PEPCK) is one of the key regulatory enzymes in gluconeogenesis. In human liver, PEPCK is about equally distributed in both cytosol (PEPCK-1) and mitochondria (PEPCK-2). The human pepck2 gene and cDNA have been reported, but the cloning of the promoter region of the pepck2 gene has not been elucidated yet. We isolated and characterized human genomic P1-artificial chromosome (PAC) clones carrying the human pepck2 gene promoter. The oligocapping method revealed that the transcriptional start point (tsp) of the human pepck2 gene is located at 97 bp upstream of the first adenine residue of the translation start site. We also determined the nucleotide sequence to 1819 bp upstream of tsp. Sequence analysis of this region revealed that it contained several potential regulatory elements, including five GC boxes and three CCAAT boxes. Reporter analysis using transient transfection with firefly luciferase synthetic gene indicated 5’ flanking region up to 822 bp, and 317 bp upstream of tsp had transcriptional activity. These results suggest that these regions of the human pepck2 gene play an important role for its expression.


http://www.sciencedirect.com/science/article/B6T39-3TJ452W-4/2/e88f724522884526fd3e575fbb3c4d7

Five clustered polyketide synthase (PKS) genes, rifA-rifE, involved in rifamycin (Rf) biosynthesis in Amycolatopsis mediterranei S699 have been cloned and sequenced (August, P.R. et al., 1998. *Chem. Biol.* 5, 69-79). The five multifunctional polypeptides constitute a type I modular PKS that contains ten modules, each responsible for a specific round of polyketide chain elongation. Sequence comparisons of the Rf PKS proteins with other prokaryotic modular PKSs elucidated the regions that have an important role in enzyme activity and specificity. The [beta]-ketoacyl:acyl carrier protein synthase (KS) domains show the highest degree of similarity between themselves (86-90%) and to other PKSs (78-85%) among all the constituent domains. Both malonyl-coenzyme A (MCoA) and methylmalonyl-coenzyme A (mMCoA) are substrates for chain elongation steps carried out by the Rf PKS. Since acyltransferase (AT) domains of modular PKSs can distinguish between these two substrates, comparison of the sequence of all ten AT domains of the Rf PKS with those found in the erythromycin (Er) (Donadio, S. and Katz, L., 1992. *Gene*
111, 51-60) and rapamycin (Rp) (Haydock, S. et al., 1995. FEBS Lett. 374, 246-248) PKSs revealed that the AT domains in module 2 of RifA and module 9 of RifE are specific for MCoA, whereas the other eight modules specify mMCoA. Dehydration of the [beta]-hydroxyacylthioester intermediates should occur during the reactions catalysed by module 4 of RifB and modules 9 and 10 of RifE, yet only the active site region of module 4 conforms closely to the dehydratase (DH) motifs in the Er and Rp PKSs. The DH domains of modules 9 and 10 diverge significantly from the consensus sequence defined by the Er and Rp PKSs, except for the active site His residues. Deletions in the DH active sites of module 1 in RifA and module 5 in RifB and in the N- and C-terminal regions of module 8 of RifD should inactivate these domains, and module 2 of RifA lacks a DH domain, all of which are consistent with the proposed biosynthesis of Rf. In contrast, module 6 of RifB and module 7 of RifC appear to contain intact DH domains even though DH activity is not apparently required in these modules. Module 2 of RifA lacks a [beta]-ketoacyl:acyl carrier protein reductase (KR) domain and the one in module 3 has an apparently inactive NADPH binding motif, similar to one found in the Er PKS, while the other eight KR domains of the Rf PKS should be functional. These observations are consistent with biosynthetic predictions. All the acyl carrier protein (ACP) domains, while clearly functional, nevertheless have active site signature sequences distinctive from those of the Er and Rp PKSs. Module 2 of RifA has only the core domains (KS, AT and ACP). The starter unit ligase (SUL) and ACP domains present in the N-terminus of RifA direct the selection and loading of the starter unit, 3-amino-5-hydroxybenzoic acid (AHBA), onto the PKS. AHBA is made by the products of several other genes in the Rf cluster through a variant of the shikimate pathway (August, P.R. et al., inter alia). RifF, produced by the gene immediately downstream of rifE, is thought to catalyse the intramolecular cyclization of the PKS product, thereby forming the ansamacrolide precursor of Rif B.

http://www.sciencedirect.com/science/article/B6T39-3YHWR69-2/2/40f600119a706601d2ae1e44060de742

http://www.sciencedirect.com/science/article/B6T39-3W07P0S-D/2/d1b4b1fbe6dd3e15683fe47128c38ac1

http://www.sciencedirect.com/science/article/B6T39-3Y9GDS5-P/2/f99635e80796eafe42d3ee4pee9c56689

We have mapped the human ORFX gene to chromosome 9q34 and determined its complete gene structure. Comparison with RING3, the human MHC-linked homologue on 6p21.3, shows the two gene structures to be highly conserved but with an approximate threefold expansion in the ORFX introns. RING3 and ORFX are found to be ubiquitously expressed in human adult and foetal tissues. Evidence suggests that the two genes may have arisen from an ancient duplication


A novel Isopeptidase T gene (ISOT-3) has been identified on human chromosome 3q26.2-q26.3. This gene shows 67.3% nucleotide identity and 54.8% amino acid identity to human Isopeptidase T-1 (ISOT-1). Northern blot analysis has shown that ISOT-3 is highly expressed in ovary and testes, with low-level expression in six other tissues tested. In contrast, ISOT-1 is expressed at high levels in the brain, and there is no detectable expression in ovary. The exonic organization of these two genes is highly conserved with only one variant intron position. Intron 15 in ISOT-3 is absent in ISOT-1, but there is an alternate splice site at the same location. Although the exon-intron structure has been conserved between the two genes, ISOT-3 has significantly larger intronic regions, and the overall size of this gene is at least 90 kb compared to 15 kb for ISOT-1. These data suggest that both ISOT-1 and ISOT-3 have descended from a common ancestor. In addition, the low overall sequence identity and different expression patterns may reflect differences in substrate specificity.


http://www.sciencedirect.com/science/article/B6T39-3YJB847-M/2/79221ac0df8a97510d6fb0c5afa17f7d

The organization and structure of the human RFC-1 gene encoding a folate transporter were determined. The RFC-1 gene spans 22.5 kb and was found to be distributed in eight exons, including five primary exons and three alternatives of exon 1. Most splice junctions conform to consensus sequences for such junctions. The human RFC-1 gene differs from the mouse and hamster genes both in terms of the total number of exons and in regard to alternatives of exon 1 which encode 5' end heterogeneity. Previously described cDNA variants (GenBank/EMBL accession no. U19720) are now shown to incorporate one of two alternatives (exons 1a and 1b) to exon 1 and exons 2-6 as a result of RNA splicing. Another variant also described may not be full length in that it incorporates a probable alternative (exon 1c) to exon 1 along with exon 2 and a truncated exon 3. A relatively GC-rich region of the genome 5' of the alternatives to exon 1 appears to be distinctly promoter like and incorporates a number of putative cis-acting elements, including multiple SP1 sites, involved in the regulation of transcription. Primer extension analysis of this upstream region in two human cell types revealed a similar pattern of multiple transcription start sites (tsp) proximal to the 5' end of exon 1. However, there was a greater number of potential tsp within the region immediately upstream of exon 1b than within the regions upstream of exons 1a and 1c. The existence of true alternatives to exon 1 in this gene incorporating different 5' ends indicates that its transcription is under the control of multiple promoters. The identity of two such promoters was obtained by functional deletion analysis, showing that expression of a luciferase reporter gene was directed separately by discrete stretches of nucleotide sequence proximal to exon 1a (promoter 1) or exon 1b (promoter 2) in transient transfection experiments. Promoter 1 appeared to have a three-fold lower basal activity than promoter 2, but was enhanced up to nine-fold in fusion constructs containing an SV40 enhancer element. Also, promoter 2 partly consists of a highly GC-rich direct repeat element containing at least three putative SP-1 and 3 putative MZF1 sites. Finally, the activity of these promoters
relative to each other was consistent with the results of primer extension analysis showing a greater multiple and usage of tsp within promoter 2 (exon 1b) than within promoter 1 (exons 1a and 1c), suggesting that the variant incorporating exon 1b was the most abundant.


http://www.sciencedirect.com/science/article/B6T39-4BYR95B-1/2/1005179671831b74eab27d37b3e26951

The Japanese pufferfish, torafugu (Fugu rubripes), has a haploid genome of about 400 Mb in size, which has been sequenced to approximately 90% coverage. Here we identified six Fugu tropomyosin (TPM) gene sequences by using the BLASTN program and the sequence of the white croaker TPM1 gene in our collection against the draft assembly of the Fugu genomic sequence database. TPM2, TPM3 and TPM4 genes were identified together with a set of two potentially duplicated genes of TPM1 (TPM1-1 and TPM1-2) as described in our previous report and TPM4 (TPM4-1 and TPM4-2) newly found in this study. The expression patterns of these Fugu TPM genes were determined by reverse transcription polymerase chain reaction (RT-PCR). A phylogenetic tree was constructed using the deduced amino acid sequences, which were encoded by the exons common to all vertebrate TPM genes. This indicated that the Fugu TPM1 and TPM4 genes had resulted from a gene duplication in the fish evolutionary lineage.


http://www.sciencedirect.com/science/article/B6T39-4F9N6YJ-1/2/14b62b360ea2e592adc5f7efaf8b4d6db

Linkage disequilibrium (LD) mapping is often used in searches for genes governing economically significant traits and diseases. The D' coefficient is a commonly used measure of the extent of LD between all possible pairs of alleles at two markers. This study aimed to test the utility of the D' coefficient for LD mapping of a trait in a thoroughbred population. Microsatellite genotype data and grey coat colour as a trait model in a thoroughbred population were used to assess the extent of LD. We demonstrated that LD mapping was a reasonable approach for initial genome-wide scans in a thoroughbred population. Significant LD was demonstrated at approximately 7 cM, implying that roughly 430 appropriately spaced microsatellites were needed for systematic whole-genome LD mapping in this model. LD mapping methods using D' in a thoroughbred population were useful for identifying the chromosomal regions for diseases and economic trait loci (ETL). It was suggested that a thoroughbred population represented a population particularly suitable for LD mapping.


http://www.sciencedirect.com/science/article/B6T39-3RM6VMT-19/2/3a77499018895f4fd7ed7e1c5f00d666

We describe here the cloning of the human BAPX1 gene, a homologue of the Drosophila bagpipe.
gene which has 87% aa identity within the homeodomain relative to the fly gene. We recently
have identified the murine bagpipe homolog. The predicted aa sequence of the human gene has
85% overall identity to the murine gene, with 100% identity in the homeodomain. In mouse, this
gene maps to the proximal portion of chromosome 5. We show that the human gene maps to
4p16.1, the human region syntenic with mouse chromosome 5. Expression of BAPX1 was
evaluated during human embryonic development by RT-PCR analysis and by RNA in situ
hybridization. RT-PCR analysis showed that BAPX1 is expressed in embryo tissues, particularly
the limb, and at a lower level in an embryonic lung cell line. RNA in situ hybridization revealed
that BAPX1 is predominately expressed in mesenchymal condensations of the fetal limb and axial
skeleton, and in lateral plate mesoderm giving rise to visceral muscle. The expression pattern of
BAPX1 combined with the chromosomal localization to 4p16.1, where several human genetic
diseases involving dysmorphology of the skeleton have been assigned, raises the potential of it
being a candidate gene for one of these disorders.

Trueman, L. J., A. Richardson, et al. (1996). "Molecular cloning of higher plant homologues of the high-
affinity nitrate transporters of Chlamydomonas reinhardtii and Aspergillus nidulans." Gene 175(1-
2): 223.

http://www.sciencedirect.com/science/article/B6T39-3W25BFT-
18/2/3e44a525c22b98655965cf93a1652f17

The crnA nitrate transporter from Aspergillus nidulans was identified as belonging to the major
facilitator superfamily (MFS) of membrane transporters. Degenerate oligonucleotides
corresponding to the crnA sequences at the locations of two conserved sequence motifs were
designed and used in the polymerase chain reaction (PCR) to amplify related sequences from
barley root poly(A)+ RNA. A 130 bp cDNA fragment with sequence similarities to crnA was
amplified and used as a probe to screen a barley root cDNA library. Two full-length clones
(pBCH1 and pBCH2) were isolated. The nt sequences of pBCH1 and pBCH2 are closely related
(80% identical) and potentially encode hydrophobic polypeptides of 54.7 and 55.0 kDa
respectively, with twelve predicted transmembrane domains. The encoded polypeptides are 41-
43% identical to the A. nidulans CRNA protein and 56-57% identical to NAR-3, a high-affinity
nitrate transporter from the eukaryotic alga Chlamydomonas reinhardtii. Phylogenetic analysis
indicated that crnA, nar-3 and the barley homologues belong to a new family within the MFS, a
family that also includes narK, the gene for a nitrite efflux pump in Escherichia coli. In northern
blots, BCH1 hybridised to a mRNA species of 1.9 kb which is rapidly induced in barley roots by
NO3-, but not by NH4+, and genomic Southern blots indicated that there may be seven to ten
BCH1-related genes in the barley genome.

van den Berg, L., S. Imholz, et al. (2004). "Isolation and characterization of the canine serotonin receptor
1B gene (htr1B)." Gene 326: 131.

http://www.sciencedirect.com/science/article/B6T39-4B8BX05-
3/2/f474301cbf1d1f8838a88593e2fa85723

The serotonin receptor 1B gene (htr1B) has been suggested to be implicated in mental disorders
in both humans and other species. We have isolated a canine bacterial artificial chromosome
(BAC) clone containing htr1B, revealed the coding and surrounding DNA sequence of canine
htr1B and designed primer sets for genomic sequencing of the gene. A mutation scan in 10 dogs
revealed five single nucleotide polymorphisms in the htr1B coding sequence. By random
sequencing of subclones of the BAC a polymorphic microsatellite repeat was found. We found
evidence for at least four extended haplotypes in six dogs of the same breed. The chromosomal
localization of the gene was confirmed by fluorescence in situ hybridisation and radiation hybrid
mapping. This work provides a starting point for mutation scans and association studies on dogs with behavioural problems.


We describe conditions that improve the specificity of amplification of a G + C-rich (57% G + C) DNA by PCR. Under standard conditions a 368-bp segment of the approx. 2.1-kb repeat unit of a satellite DNA that accounts for approx. 3% of the genome of the Bermuda land crab, Gecarcinus lateralis, was not amplified specifically. To establish optimal conditions for amplification of the segment of the G + C-rich satellite, we used two genetically engineered enzymes, AmpliTaq DNA polymerase and AmpliTaq DNA polymerase. Stoffel fragment (SF), and a number of denaturants or co-solvents. In the absence of denaturants or co-solvents, amplified products of both enzymes contained non-specific bands upon gel electrophoresis. Addition of certain denaturants or co-solvents to PCR mixtures resulted in the production of the single specific band of the expected size. Reagents that improved specificity of the amplified product were formamide, glycerol, DMSO, Tween-20 and NP-40; on the other hand, urea, ethanol and 1-methyl-2-pyrrolidone (NMP) inhibited amplification. Of the two enzymes, SF was more specific and efficient. The products of AmpliTaq DNA polymerase included one or more extra bands, even in the presence of denaturants or co-solvents, except for glycerol or DMSO.


http://www.sciencedirect.com/science/article/B6T39-49D256V-3/2/8c77a4bb7ac0b349c1ca886f18e328ab

This study reports the characterization of a novel human gene, chromosome 3 open reading frame 6 (C3orf6), mapped to chromosome 3q28, within the critical region of hereditary spastic paraplegia SPG14 locus. Based on computational "spliced" EST alignment and RT-PCR, two C3orf6 transcript variants were identified. The longer C3orf6 transcript contains a 1449-nt ORF, encoding a protein of 482 aa, while the shorter variant contains a 921-nt ORF, encoding for a protein of 306 aa. C3orf6 gene is organised on 12 exons and the shorter transcript comes from an alternative splicing event skipping exon 6. The two mRNA are differentially expressed in brain and in several other human tissues with a predominant level for the shorter transcript. By database analysis, EST assembling and RT-PCR, we identified the transcripts of mouse and rat C3orf6 orthologous genes. The involvement of C3orf6 in the spastic paraplegia was investigated by sequencing all coding exons and flanking sequences in the SPG14 family, excluding the presence of causative mutations.


http://www.sciencedirect.com/science/article/B6T39-44357HF-F/2/67f92ffe7ca6abe10b4c4f8bda4b6d7b
The karyotypes of Eulemur species exhibit a high degree of variation, as a consequence of the Robertsonian fusion and/or centromere fission. Centromeric and pericentromeric heterochromatin of eulemurs is constituted by highly repeated DNA sequences (including some telomeric TTAGGG repeats) which have so far been investigated and used for the study of the systematic relationships of the different species of the genus Eulemur. In our study, we have cloned a set of repetitive pericentromeric sequences of five Eulemur species: E. fulvus fulvus (EFU), E. mongoz (EMO), E. macaco (EMA), E. rubriventer (ERU), and E. coronatus (ECO). We have characterized these clones by sequence comparison and by comparative fluorescence in situ hybridization analysis in EMA and EFU. Our results showed a high degree of sequence similarity among Eulemur species, indicating a strong conservation, within the five species, of these pericentromeric highly repeated DNA sequences.


Allantoicase is one of the enzymes involved in uricolyis. The enzymes of this catabolic pathway (i.e. allantoinase, allantoicase, ureidoglycolate lyase and urease) were lost during vertebrate evolution and the causes for this loss are still unclear. In mammals, as well as in birds and reptiles, the activity of allantoicase is absent; notwithstanding, we recently cloned human and mouse cDNA sequences with high similarity with previously characterized allantoicases. In the present paper, we report the genomic organization of the allantoicase gene in mouse and in man. Both genes are constituted by 11 exons that appear to be very conserved; introns are more variable in length while maintain the same phase but for intron 4. We have also detected a second transcript of the human allantoicase gene in which exon 1 is absent. Moreover, the mouse gene maps in chromosome 12 at 13.0 cM from the centromere.


http://www.sciencedirect.com/science/article/B6T39-3Y51FGV-N/2/b3d34d6e35c9de768ba4f85673ff879d


http://www.sciencedirect.com/science/article/B6T39-3RD1R45-14/2/61f22ac00538fcb89501d848b521f6f272

The [beta]3-adrenergic receptor (ADR[beta]3) is a seven-membrane spanning, G-protein linked receptor expressed in brown adipose tissue in rodents, and visceral adipose tissue in humans. Stimulation of the receptor by norepinephrine leads to lipolysis and thermogenesis. In rodent models of obesity and diabetes, administration of [beta]3-agonists results in weight loss and improved glucose tolerance. Studies indicate that the pharmacological properties of the ADR[beta]3 differ markedly between rodents and humans, making generalizations of rodent studies to humans difficult. We hypothesized that the obesity and diabetes prone rhesus monkey
(Macaca mulatta) would provide an excellent animal model to study the role of the ADR[beta]3 in the development of obesity and diabetes as well as for assessment of the therapeutic efficacy of [beta]3-agonists. We sequenced the entire coding region of the rhesus ADR[beta]3 gene. Like humans, the rhesus ADR[beta]3 has two exons. There is 89% amino acid (aa) identity between human and rhesus compared to 82% aa identity between human and mouse. A single base deletion results in divergence of the intracellular carboxy terminus accounting for 26 of the 45 aa changes and 10 additional aa. Of the 15 rhesus monkeys studied, all were homozygous for Arg64. In humans, Arg64 (rather than Trp) is associated with increased body mass index, insulin resistance, and an earlier onset of type II diabetes mellitus. We conclude that the rhesus ADR[beta]3 is more similar to the human ADR[beta]3 than to the rodent ADR[beta]3 suggesting that this primate model may be more appropriate for physiologic and therapeutic studies of the ADR[beta]3 axis, and that Arg64 may influence susceptibility in this species to obesity, insulin resistance, and type II diabetes.


http://www.sciencedirect.com/science/article/B6T39-3X64DJG-F/2/fc5d0c0ca155ffa50af07566a1877286


The nucleotide (nt) sequence of a chicken cDNA encoding a protein homologous to the human GRB2 (growth factor receptor-binding protein) was determined. Remarkably high identities were found on the nt (88%) and deduced amino acid sequence (96%) levels.


http://www.sciencedirect.com/science/article/B6T39-40RTKY5-J/2/fa00a703bd07a7e0694314e0c02ae606


http://www.sciencedirect.com/science/article/B6T39-3WHKRH2-4/2/e0ab8879760adecf2c3d753c9ed349fa

We describe a series of plasmid vectors for DNA cloning in staphylococci. pPSII is a promoter probe plasmid containing a promoterless lipase (Lip)-encoding gene (lip). Insertion of a promoter-bearing DNA fragment at the single BamHI site turns on lip expression. Lip activity can be easily determined to estimate the strength of the inserted promoter. pPSII served also as a basis for the construction of vectors which allow xylose-inducible gene expression in Staphylococcus carnosus (Sc). Using plasmid pCX15, we studied xylose-inducible lip expression in Sc. The lip expression is under transcriptional control of the repressor, XylR. The xylR gene, the XylR target sequence and the xylA promoter/operator sequence with the cis-acting catabolite-responsive element (cre) are derived from the xyl operon of S. xylosus. The single BamHI site in front of the lip ribosome-binding site (RBS) also makes it possible to put other promoterless genes under transcriptional control of XylR. To facilitate the controlled expression of genes which merely start with the start codon and have no RBS, or to insert genes with their own RBS, pCX26 and pCX26[Delta]lip were constructed. The influence of xylose and glucose on lip expression was studied both in a batch culture and in a fermentor under controlled pH conditions. With pCX15, the presence of xylose led to a 40-fold increase in extracellular Lip activity, while the presence of glucose caused a repression of lip expression. The results suggest that the xylA promoter is subject to two different regulatory mechanisms, one of which involves the repression of the xylA promoter by XylR in the absence of xylose, and the other involves a glucose-mediated catabolite repression which dominates over the xylose induction.


clones. Of the 16 actin-specific cDNA clones analyzed, 15 (93%) were full-length. This approach for cloning full-length cDNAs from available ESTs or partial cDNA sequences will facilitate a more rapid and efficient characterization of gene structure and function.


http://www.sciencedirect.com/science/article/B6T39-3WD5BHJ-6/2/e4132f6ed4c5c15896f75affee5a5450


http://www.sciencedirect.com/science/article/B6T39-44357HF-B/2/cf98395a21a92e0d1301b7c97b9be969

Periodontal ligament (PDL) is one of the most important tissues in maintaining the homeostasis of tooth and tooth-supporting tissue, periodontium. In this study, we investigated the expression profile of active genes in the human PDL obtained by collecting sequences with a 3'-directed cDNA library, which faithfully represents the composition of the mRNA population. We succeeded in obtaining a total of 1752 cDNA sequences by sequencing randomly selected clones and identified a total of 1318 different species as gene signatures (GS) by their sequence identity, 344 of which were known genes in the GenBank, and 974 of which were new genes. The resulting expression profile showed that collagen type I and type III were the most abundant genes and that osteogenesis-related proteins, such as SPARC/osteonectin and osteoblast specific factor 2, were highly expressed. By comparing the expression profile of PDL with 44 profiles similarly obtained with unrelated human cell/tissue, nine novel genes, which are probably expressed specifically in PDL, were discovered. Among them, we cloned a full-length cDNA of GS5096, which is frequently expressed in freshly-isolated periodontal tissue. We found that it encodes a novel protein, which is a new member of the class I small leucine-rich repeat proteoglycan family, and designated it PLAP-1 (periodontal ligament associated protein-1). PLAP-1 mRNA expression was confirmed in in vitro-maintained PDL cells and was enhanced during the course of the cytodifferentiation of the PDL cells into mineralized tissue-forming cells such as osteoblasts and cementoblasts. These findings suggest the involvement of PLAP-1 in the mineralized matrix formation in PDL tissues.


http://www.sciencedirect.com/science/article/B6T39-436W3KY-10/2/3a8462abb92cf2315d8430181be55f8d

We previously postulated that the single-minded 2 (SIM2) gene identified on the human chromosome 21q22.2 is a good candidate gene for the pathogenesis of mental retardation in Down syndrome because its mouse homolog exhibits preferential expression in the mouse diencephalon during early embryogenesis. We analyzed the genomic sequence of the entire SIM2 gene which consists of 11 exons and spans over 50 kb. As a step toward understanding the molecular mechanisms of SIM2 gene expression, we have analyzed the human SIM2 gene expression in nine established human cell lines. Three transcripts of 3.6, 4.4, and 6.0 kb were
detected in the glioblastoma cell line, T98G, neuroblastoma cell line, TGW, and transformed embryonic kidney cell line, 293. The RACE analysis using SIM2-expressing human cell line T98G provided evidence for the transcription start site at ~1.2 kb upstream of the translation initiation site. The transfection assay using various deletion constructs with reporter gene suggested the presence of a presumptive promoter region. Transient transfection assay in T98G cell line revealed a significant promoter activity located in the 60 bp sequence between nt -1385 and -1325 upstream region of the translation initiation site. This 60 bp sequence contains cis-elements for c-myb, E47 and E2F transcription factors. Moreover, the gel retardation assay using oligo-DNA of various cis-element sequences indicated the presence of protein factor(s) which bind to the cis-element for c-myb. These results suggested that binding of a protein transcription factor(s) such as c-myb or that alike regulates transcription of the SIM2 gene by binding to a small upstream region.


http://www.sciencedirect.com/science/article/B6T39-3RD1R45-7F/2/4cfff88a996bd976d5387764c1d11c4

Two Toxoplasma gondii genes were characterized that are differentially expressed during the parasite’s life cycle. The genes named LDH1 and LDH2, respectively, encode polypeptides similar to the enzyme lactate dehydrogenase (LDH; -lactate:NAD+ oxidoreductase, EC 1.1.1.27) from a variety of organisms. They show 64.0% nucleotide identity in the coding region and both have an intron at the same relative position. The deduced amino acid sequences of LDH1 and LDH2 share 71.1% identity. LDH1 and LDH2 are most similar to an LDH of Plasmodium falciparum (46.5% and 48.5% amino acid identities, respectively). The mRNA of LDH2 was only detected in the bradyzoite stage, while the mRNA of LDH1 was detected in both the bradyzoite and tachyzoite stages. However, by isoelectric focusing and immunoblot analysis, only one LDH isoform was found to be expressed in each stage. Furthermore, the expression of a reporter gene carrying chloramphenicol acetyltransferase (CAT) coding sequence and the putative LDH2 promoter sequence was significantly up-regulated by growing parasites in tissue culture in media with alkaline pH (pH 8.2, a condition known to induce the expression of bradyzoite-specific antigens), while the expression of a CAT reporter construct carrying the putative LDH1 promoter sequence was down-regulated by similar treatment. These results indicate that LDH expression is developmentally regulated in T. gondii and suggest a possible correlation between stage conversion and alteration in carbohydrate or energy metabolism in this parasite.


http://www.sciencedirect.com/science/article/B6T39-491J42W-3/2/5a93f6358ad740c284f7c891696f0633

The tuberactinomycins are a family of basic cyclic peptides that exhibit potent antitubercular activity. These peptides are characterized by the presence of an amino acid with a 6-membered cyclic guanidine side chain (capreomycidine) and two or more 2,3-diaminopropionate residues. Viomycin (tuberactinomycin B) is a well-studied member of the family, was once prescribed for the treatment of tuberculosis, and has been shown to block translocation during protein biosynthesis. The gene cluster encoding viomycin biosynthesis was identified and cloned from Streptomyces vinaceus. The cluster was identified by screening genomic libraries with the viomycin phosphotransferase self-resistance gene (vph) and non-ribosomal peptide synthetase (NRPS) gene probes amplified from S. vinaceus genomic DNA. The viomycin cluster was
localized to ca. 120 kb of contiguous DNA defined by four overlapping cosmid inserts. Each cosmid hybridized with one or more peptide synthetase gene probes and two also hybridized with vph. Confirmation that the cluster encoded viomycin biosynthesis was obtained from the disruption of two NRPS adenylation domains. Partial sequence analysis revealed an ORF (svox) predicted to encode a rare non-heme iron, [alpha]-ketoglutarate dependent oxygenase proposed to function in the oxidative cyclization of arginine to the capreomycidine residue. Insertional disruption of svox resulted in complete loss of viomycin production, confirming its involvement in the pathway.


http://www.sciencedirect.com/science/article/B6T39-3VGR20J-2/2/608448a61d65ac3bc5636ef48e60c62b

Neuropsin is a serine protease which is thought to function in a variety of tissues including the brain and skin. This protease has been shown to have important roles in neural plasticity in mice. Here we have cloned a cDNA and analyzed the gene for human neuropsin by polymerase chain reaction-based strategies. The cDNA had 72% identity to mouse neuropsin. The deduced amino acid sequence showed 72% identity to mouse neuropsin. Key amino acid residues for the enzyme activity and all cysteine residues were conserved between human and mouse neuropsin. The gene for human neuropsin had six exons and five introns, and the gene organization is similar to trypsin-type serine proteases. The mRNA was expressed in primary cultures of keratinocytes.


http://www.sciencedirect.com/science/article/B6T39-413KWGN-F/2/5be6ee63cfdc65bcb49213b9bd6801f


http://www.sciencedirect.com/science/article/B6T39-3SRBHWR-3/2/7b053fad8ea937b3192cfca2af4094f

Three age-related mtDNA deletions were identified, and the competitive polymerase chain reaction (PCR) was used to quantitate their levels in different Fisher 344 rat tissues. Deletions that removed 4834; 13273; or 13415 nt of the mitochondrial genome were shown to be associated with 16 (mtDNA4834), nine (mtDNA13273), or five (mtDNA13415) nt direct repeats, respectively. The mtDNA4834 deletion was detected in an age-related manner in all tissues screened; the mtDNA13415 deletion was detected in old heart, and in both young and old brain; and the mtDNA13273 deletion was only detected in old brain tissues. The mtDNA4834 deletion was found to be at its highest level in the liver (1.88 x 10-2%), followed by the brain (0.22 x 10-2%) and kidney (0.40 x 10-2%) of old animals. Much lower levels were observed in old heart (0.07 x 10-2%) and lung (0.04 x 10-2%). This distribution of mtDNA deletions in old rat tissues is in contrast to work done in humans where age-related deletions are present at the highest levels
in post-mitotic tissues with much lower levels in more mitotic tissues. An inverse relationship was observed between the level of mtDNA deletions and the size of the deleted region, since the mtDNA13415 deletion was present at about a 100-fold lower level (0.53 x 10^{-5}%) than the smaller mtDNA4834 deletion in old heart tissue.


http://www.sciencedirect.com/science/article/B6T39-47PNY80-X0/2/928a9526f380b837008e74dcd9636888

We developed a modified nonradioactive method for the detection of DNA. This method makes use of the polymerase chain reaction for preparation of probes; that is, a DNA fragment inserted in the polylinker region of an M13 or pUC vector is amplified with primers that have a modified cytosine tail at the 5' terminus (C-tailed primers). By this method, large amounts of labeled probes can be obtained easily. After hybridization, modified cytosine tails can be detected immunologically. DNA labeled by this method could be used in plaque hybridization. We could detect 0.05 pg of dot-blotted labeled DNA in 30 min with an enzyme-catalyzed chemiluminescence reaction.


http://www.sciencedirect.com/science/article/B6T39-3RD1R45-3N/2/be41d1df758d73615b71df6684703597

Proteasomes are large, multisubunit particles that act as the proteolytic machinery for most regulated intracellular protein breakdown in eukaryotic cells. The core proteinase of this complex, known as the 20S proteasome, is a hollow barrel-shaped structure made up of four stacked rings of seven subunits each, with the outer two rings each being made up of seven distinct [alpha]-type subunits, and the two inner rings composed of seven different [beta]-type subunits. Here we present the cloning, sequencing and genetic mapping of a Drosophila melanogaster gene, [alpha]5_dm, encoding one of the proteasome [alpha] subunits. This gene, which is homologous to the yeast PUP2 and the human Zeta genes, maps to chromosome 2 at position 54B3-5. The map positions of the previously cloned proteasome genes Pros25 and Pros29 were also determined, and found to lie at positions 87B and 57B, respectively. A search for other D. melanogaster [alpha]5_dm-like genes encoding potential isoforms of this subunit failed to identify any closely related genes.


http://www.sciencedirect.com/science/article/B6T39-4DWHJ4Y-2/2/3cd09ad7c7a2fac603835f367e07745b

WNK1 is one of WNK (With No K=Lysine) protein kinases which comprise a newly described subfamily. Our studies showed that expression of the mouse WNK1 gene was dramatically suppressed in a tumor cell line when its phenotype was altered by suppression of the GD3-synthase gene expression. The mouse WNK1 gene was expressed at a high level at early stage
of embryonic brain and its expression decreased as brain developed, similar to the expression pattern of the GD3-synthase gene. To study transcriptional regulation, we cloned a 5'-flanking 1239-bp fragment of the mouse WNK1 gene. This fragment contains a number of potential consensus binding sites for transcription factors, including Sp1, AP2, CCAAT, Est-1, Oct-1, CNBP, and NFkB, but lacks a TATA box. Primer extension identified multiple putative transcriptional initiation sites, including several sites downstream of the ATG codon. Activities of the promoter fragments were assessed in mouse breast Sa/R-MT cells by transient transfection and the results showed that the promoter elements between -700 and -977 is required for maintaining a high level of promoter activity of the TATA-less mouse WNK1 gene.


http://www.sciencedirect.com/science/article/B6T39-44JJHYF-2/2/5c19e2831f38a621af717b029ed7eaf

We have cloned a novel hematopoietic granulocyte colony-stimulating factor (G-CSF)-induced olfactomedin-related glycoprotein, termed hGC-1 (human G-CSF-stimulated clone-1). mRNA differential display was used in conjunction with a modified two-phase liquid culture system. Cultures were enriched for early precursors of erythroid, myeloid, and megakaryocytic lineages, which were isolated after induction with erythropoietin, G-CSF, and thrombopoietin, respectively. RNA from the enriched cells was subjected to differential display analysis to identify lineage-specific expressed genes. One clone specifically induced by G-CSF, hGC-1, was characterized. The 2861 bp cDNA clone of hGC-1 contained an open reading frame of 1530 nucleotides, translating into a protein of 510 amino acids with a signal peptide and six N-linked glycosylation motifs. The protein sequence of hGC-1 showed it to be a glycoprotein of the olfactomedin family, which includes olfactomedin, TIGR, Noelin-2 and latrophilin-1. Olfactomedin-like genes show characteristic tissue-restricted patterns of expression; the specific tissues expressing these genes differ among the family members. hGC-1 was strongly expressed in the prostate, small intestine, and colon, moderately expressed in the bone marrow and stomach, and not detectable in other tissues. In vitro translation and ex vivo expression showed hGC-1 to be an N-linked glycoprotein. The hGC-1 gene locus mapped to chromosome 13q14.3. Together, our findings indicate that hGC-1 is primarily expressed as an extracellular olfactomedin-related glycoprotein during normal myeloid-specific lineage differentiation, suggesting the possibility of a matrix-related function for hGC-1 in differentiation.


http://www.sciencedirect.com/science/article/B6T39-473HYTB-B/2/d217cb862551b806b0def9c5b2520c5e

The hSEP1 gene is the human homolog of yeast SEP1. Yeast SEP1 is a multifunctional gene that regulates a variety of nuclear and cytoplasmic functions including homologous recombination, meiosis, telomere maintenance, RNA metabolism and microtubule assembly. The function of hSEP1 is not known. We show loss or reduced expression of hSEP1 messenger RNA (mRNA) in three of four primary osteogenic sarcoma (OGS)-derived cell lines and in eight of nine OGS biopsy specimen. In addition, we find a heterozygous missence mutation (Valine1484>Alanine) at a conserved amino acid in the primary OGS-derived cell line U2OS. Importantly, we identified a homozygous missence mutation involving a CG-dinucleotide leading to a change in a conserved amino acid, aspartic acid1137 >asparagine, in the primary OGS-
derived cell line, TE85. hSEP1 mRNA expression was nearly undetectable in TE85 and low in U2OS cell lines. None of these mutations were identified in 20 normal samples consisting of bone, cartilage and fibroblast. The hSEP1 gene is located in chromosome 3 at 3q25-26.1 between markers D3S1309 and D3S1569. An adjacent locus defined by the polymorphic markers D3S1212 and D3S1245 has previously been reported to undergo loss of heterozygosity (LOH) at a >70% frequency in OGS and claimed to harbor an important tumor suppressor gene in osteosarcoma. The homozygous mutation in the hSEP1 mRNA in TE85 cell line suggest that this gene itself is subject to LOH. Taken together, these results suggest that hSEP1 acts as a tumor suppressor gene in OGS.


http://www.sciencedirect.com/science/article/B6T39-3W2698DF2/2/68ca013408f16ff937df2f60f668a962

A 4040-bp cDNA was cloned from a human placenta library by screening with a polymerase chain reaction-amplified fragment. The fragment was generated from the library using primers corresponding to conserved sequences encompassing the topa quinone (TPQ) cofactor sites of the copper-containing proteins, bovine serum amine oxidase (BSAO) and human kidney diamine oxidase (DAO). The cloned cDNA contains a coding sequence from positions 161 to 2449. Between bases 2901 and 2974, in a very long 1591-bp 3'-untranslated region, there is a G/A-rich region in the minus strand, which contains a (AGG)5 tandem repeat. The human placenta cDNA sequence and its translated amino acid sequence are 84% and 81% identical to the corresponding BSAO sequences, while the identities for the placenta sequences and those for human kidney DAO are 60% and 41%, respectively. The TPQ consensus nucleotide and protein sequences are identical for the placenta enzyme and BSAO, but the corresponding sequences for human kidney DAO are nonidentical. Three His residues that have been identified as Cu(II) ligands in other amine oxidases are conserved in the human placenta amine oxidase protein sequence. It was concluded that the placenta cDNA open-reading frame codes for a copper-containing, TPQ-containing monoamine oxidase. A putative 19-amino acid signal peptide was identified for human placenta amine oxidase. The resulting mature protein would be composed of 744 amino acids, and would have a Mr of 82 525. Comparison of the human placenta amine oxidase with DNA sequences found in GenBank suggests that the gene for this enzyme is located in the q21 region of human chromosome 17, near the BRCA1 gene.


http://www.sciencedirect.com/science/article/B6T39-3YS34MWK2/9d5d43554a86d69f17c475e7e64c99b5

Gene Expression Patterns (3)

New techniques are being applied to identify all the genes involved in mammalian gonad development and differentiation. As this list of genes increases, understanding the potential interactions between these genes will become increasingly difficult. We used a real time reverse transcription PCR (real time RTPCR) protocol to examine and compare the relative expression levels of 55 genes in individual mouse fetal gonads. Real time PCR analysis demonstrated that except for Sry, no differences in relative gene expression were detectable between XX and XY gonad/mesonephroi complexes at embryonic day (E)11.5. Following Sry peak expression at E11.5, a number of genes were expressed at significantly higher relative levels in E12-14 XY than XX gonads. Of six genes expressed at higher levels in E12.5-14 XX than XY gonads, three, Bmp2, Emx2, and Fgfr2, had not been reported previously. Our results caution that differential localization patterns observed with whole mount in situ hybridization techniques may not accurately reflect changes in transcript levels. We conclude that real time PCR is an efficient and powerful tool for studying multiple gene expression patterns during gonad development and differentiation, and can provide insight into gene interactions.


Frizzleds are transmembrane receptors that can transduce signals dependent upon binding of Wnts, a large family of secreted glycoproteins homologous to the Drosophila wingless (wg) gene product and critical for a wide variety of normal and pathological developmental processes. In the nervous system, Wnts and Frizzleds play an important role in anterior-posterior patterning, cell fate decisions, proliferation, and synaptogenesis. However, little is known about the role of Frizzled signaling in the developing eye. We isolated cDNAs for ten chick Frizzleds and analyzed the spatial and temporal expression patterns during eye development in the chick embryo. Frizzled-1 to -9 are specifically expressed in the eye at various stages of development and show a complex and partially overlapping pattern of expression.


The only molecular similarity shown so far for sexual regulatory genes among different phyla involves doublesex (dsx) of Drosophila, mb-3 and mb-23 of Caenorhabditis elegans, and Dmrt1 of vertebrates. These genes encode DM domain transcription factors (DM=dsx and mb-3) and are required for sexual differentiation. In the case of dsx and mb-3, the two genes control analogous aspects of sexual development, bind similar DNA sequences, and are capable of functional substitution in vivo. All three phyla have multiple DM domain genes, but it is unknown how many of these are involved in sexual development. Mammals, for example, have at least seven DM domain genes, but embryonic expression has only been examined in detail for Dmrt1(dsx- and mb-3 related transcription factor 1). We have identified additional murine DM
domain genes and have examined their expression in the mouse embryo, with emphasis on the developing gonad. At least three murine DM domain genes in addition to Dmrt1 are expressed in the embryonic gonad: Dmrt4 is expressed at similar levels in gonads of both sexes; Dmrt3 is more highly expressed in males; and Dmrt7 is more highly expressed in females. Expression of three other genes is low or absent in the embryonic gonad. Two of these, Dmrt5 and Dmrt6, are expressed primarily in the brain, and the third, Dmrt2, is expressed in presomitic mesoderm and developing somites. Our data suggest that multiple DM domain genes may be involved in mammalian sexual development, and that they may function in both testis and ovary development.

**General and Comparative Endocrinology** 13


http://www.sciencedirect.com/science/article/B6WG0-4BVP3YD-4/2/f43e453de5aface4ad0d86fac1d3c085

We analyzed the genes that exhibit transcriptional changes during sex differentiation in Xenopus, using fluorescent differential display (FDD). Search was then undertaken for sequences that were homologous to the differentially displayed DNA. In this report, trans-acting factors of activating transcription factor 4 (ATF 4) and heat shock proteins were selected, on the basis of homology, from candidate genes thought to be involved in the expression cascade of aromatase and estrogen receptor genes. The stage and tissue specificities and the effect of estradiol treatment on the expression of these genes were then examined using real-time quantitative polymerase chain reaction (RQ-RT-PCR). The expression of ATF 4, a member of the ATF/cAMP-responsive element-binding protein (CREB) family of genes, peaked in the gonads at stage 50 of development. Interestingly, expression of the genes encoding the heat shock cognate protein 70, Hsc70. II (Hsc70. II) and the heat shock protein 70 (Hsp70) binding protein was strongly activated at stages 50 and 48 of development, respectively. The three genes revealed a higher transcription activity in the gonads than in other tissues. Although the expression of all of the genes encoding ATF 4, aromatase, Hsc70. II, and Hsp70 binding protein was activated in vitro by estrogen treatment, that of Hsc70. II and Hsp70 binding protein was found to be transient.


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Effects of salmon gonadotropin-releasing hormone (sGnRH) and estradiol-17[beta] (E2) on gene expression and release of gonadotropins (GTHs) were examined in masu salmon (Oncorhynchus masou) using primary pituitary cell cultures at three reproductive stages, initiation of sexual maturation in May, pre-spawning in July, and spawning in September. Amounts of GTH subunit mRNAs were determined by real-time polymerase chain reaction, and levels of GTH released in
the medium were determined by RIA. In control cells, the amounts of three GTH subunit mRNAs ([alpha]2, FSH[beta], and LH[beta]) peaked in July prior to spawning. FSH release spontaneously increased with gonadal maturation and peaked in September, whereas LH release remained low until July and extensively increased in September. Addition of E2 to the culture extensively increased the amounts of LH[beta] mRNA in May and July in both sexes. It also increased the [alpha]2 mRNA in July in the females. In contrast, sGnRH alone did not have any significant effects on the amounts of three GTH subunit mRNAs at all stages, except for the elevation of [alpha]2 and FSH[beta] mRNAs in July in the females. Nevertheless, synergistic effects by sGnRH and E2 were evident for all three GTH subunit mRNAs. In May, sGnRH in combination with E2 synergistically increased the amounts of LH[beta] mRNA in the males and [alpha]2 mRNA in the females. However, in July the combination suppressed the amounts of [alpha]2 and FSH[beta] mRNAs in the females. sGnRH alone stimulated LH release at all stages in both sexes, and the release was synergistically enhanced by E2. Synergistic stimulation of FSH release was also observed in May and July in both sexes. These results indicate that a functional interaction of sGnRH with E2 is differently involved in synthesis and release of GTH. The synergistic interaction modulates GTH synthesis differentially, depending on subunit, stage, and gender, whereas it potentiates the activity of GnRH to release GTH in any situation.


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We developed a one-tube two-temperature real-time RT-PCR that allows to absolutely quantify the gene expression of hormones using the standard curve method. As our research focuses on the expression of the insulin-like growth factors (IGFs) in bony fish, we established the technique for IGF-I and IGF-II using the tilapia (Oreochromis niloticus) as model species. As approach, we used primer extension adding a T7 phage polymerase promoter (21 nt) to the 5' end of the antisense primers. This procedure avoids the disadvantages arising from plasmids. Total RNA extracted from liver was subjected to conventional RT-PCR to create templates for in vitro transcription of IGF-I and IGF-II cRNA. Correct template sizes including the T7 promoter were verified (IGF-I: 91 nt; IGF-II: 94 nt). The PCR products were used to create IGF-I and IGF-II cRNAs which were quantified in dot blot by comparison with defined amounts of standardised kanamycin mRNA. Standardised threshold cycle (Ct) values for IGF-I and IGF-II mRNA were achieved by real-time RT-PCR and used to create standard curves. To allow sample normalisation the standard curve was also established for [beta]-actin as internal calibrator (template: 86 nt), and validation experiments were performed demonstrating similar amplification efficiencies for target and reference genes. Based on the standard curves, the absolute amounts of IGF-I and IGF-II mRNA were determined for liver (IGF-I: 8.90 +/- 1.90 pg/[mu]g total RNA, IGF-II: 3.59 +/- 0.98 pg/[mu]g total RNA) and extrahepatic sites, such as heart, kidney, intestine, spleen, gills, gonad, and brain considering the different lengths of cRNAs and mRNAs by correction factors. The reliability of the method was confirmed in additional experiments. The amplification of descending dilutions of cRNA and total liver RNA resulted in parallel slopes of the amplification curves. Furthermore, amplification plots of the standard cRNA and the IGF-I and IGF-II mRNAs showed signals starting at the expected Ct values. Thus, the one-tube RT-PCR described here is highly sensitive (detection level ~2 pg/[mu]g total RNA) and allows precise absolute quantification. The method is rapid as there are neither separate reverse transcriptions nor post-amplification steps, and can be executed with low risk of contamination. Therefore, it will be helpful when investigating gene expression in any species and tissue whenever absolute levels are of concern.
Fish endure long periods of fasting and demonstrate an extensive capacity for rapid and complete recovery after refeeding. The underlying mechanisms through which nutrient intake activates an increase in somatic growth and especially in muscle growth is poorly understood. In this study we examined the expression profile of major muscle growth regulators in trout white muscle 4, 12, and 34 days after refeeding, using real-time quantitative RT-PCR. Mean insulin-like growth factor I (IGFI) mRNA level in muscle increased dramatically 8- and 15-fold, 4 and 12 days, respectively, after refeeding compared to fasted trout. This declined thereafter. Conversely, only a weak but gradual increase in mean insulin-like growth factor II (IGFII) mRNA level was observed during refeeding. Inversely to IGFI, mean IGF receptor Ia (IGFRIa) mRNA level declined after ingestion of food. In contrast, IGF receptor Ib (IGFRIb) mRNA level was not affected by refeeding. Mean fibroblast growth factor 2 (FGF2) mRNA level increased by 2.5-fold both 4 and 12 days after refeeding, whereas fibroblast growth factor 6 (FGF6) and myostatin mRNA levels were unchanged. Subsequent to IGFI and FGF2 gene activation, an increase in myogenin mRNA accumulation was observed at 12 days post-refeeding suggesting that an active differentiation of myogenic cells succeeds their proliferation. In conclusion, among the potential growth factors we examined in this study, IGFI and FGF2 were identified as candidate genes whose expression may contribute to muscle compensatory growth induced by refeeding.


Previous studies have demonstrated that FSH stimulates cell proliferation in the ovary and the testis of the chick embryo. This study analyzed the presence of FSH receptor and the cell subpopulations that proliferate in response to FSH in chick embryo gonads. FSH receptor mRNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) in the male and female gonads of the 6 to 14-day-old chick embryo. Somatic cells of the ovary expressed the FSH receptor in the 14-day-old chick embryo. Ovarian surface epithelium of the 14-day-old chick embryo increased the mitotic index 15-18 h after FSH treatment. Similarly, the mitotic index in oogonia was increased 24 h after receiving a pulse of FSH; this result was confirmed by an increase in the number of germ cells that incorporated bromodeoxyuridine (BrdU). Somatic cells of the medullary cords in the ovary displayed an increase in the mitotic index 15-21 h after the FSH injection. In the chick embryo testis, at the same stage of development, the treatment with FSH increased the mitotic index in cells of the seminiferous tubules and to a lesser extent in cells at a peritubular and interstitial location. Present results demonstrate that in the chick embryo,
FSH stimulates the proliferation of ovarian surface epithelium, oogonia in the cortex, and somatic cells of the medullary cords of the chick embryo ovary. In the chick embryo testis, FSH stimulates cell proliferation in seminiferous tubules and peritubular cells.


http://www.sciencedirect.com/science/article/B6WG0-47K2MV9-2/2/c0c9f343f10f13d3b cad1deed972f076

It has been hypothesized that estrogen production may play a pivotal role in the sex determination of reptiles with temperature-dependent sex determination (TSD). This hypothesis has been furthered by studies that have shown higher aromatase activity in the developing ovaries in some reptiles. However, other studies have not consistently supported this hypothesis. In the current study we addressed this issue by cloning P450 aromatase cDNA in the turtle, Trachemys scripta, and developing a quantitative competitive RT-PCR for aromatase. This assay was then used to quantify aromatase mRNA levels in adrenal-kidney-gonad complexes (AKG) during TSD. Aromatase mRNA was detected in the AKGs at both male- and female-producing temperatures from the earliest stage of development sampled (stage 15), through hatching (stage 26). However, levels remained relatively constant during the thermosensitive period of TSD. Further, no significant difference was detected between male- and female-producing temperatures during the thermosensitive period. After the thermosensitive period, aromatase mRNA levels increased in females (this coincides with the period during which the ovaries are differentiating). These results are consistent with those of several previous studies of certain reptiles with TSD. The current results suggest that the expression of aromatase may not be a pivotal regulatory step in the sex determination cascade of this turtle.


http://www.sciencedirect.com/science/article/B6WG0-48JK165-8/2/983f0b23c4815e2bb0855cafed7da5fc

In the present study we cloned, sequenced, and confirmed the presence of mRNAs of gonadotropins (FSH-[beta], LH-[beta] subunits) from the brain and pituitary of tilapia, Oreochromis niloticus. Further, we examined the spatio-temporal expression pattern of FSH-[beta] and LH-[beta] in the brain and pituitary of two species of teleost (tilapia, O. niloticus; sockeye salmon, Oncorhynchus nerka), using in situ hybridization and immunological methods. The expression of FSH and LH immunoreactivity appeared simultaneously in the brain and pituitary (tilapia, 14 days; sockeye, 51 days after fertilization). In the pituitary, FSH mRNA and peptide expressing cells were distinct from LH expressing cells located in the ventral proximal pars distalis. In the brain, FSH and LH immunoreactivity was co-localized in cells of the preoptic nucleus parvocellularis, magnocellularis, and gigantocellularis. Fibers immunoreactive to FSH and LH antisera were seen along the forebrain-hypothalamus and in the neurohypophysis of the pituitary. Double-label immunofluorescence revealed FSH and LH immunoreactivity co-localized in arginine vasotocin synthesizing preoptic neurons. Our results show that FSH and LH-producing cells have developmental origins in the brain as well as in the pituitary. In addition, we propose that the brain-derived gonadotropins may function as hypophysiotropic hormones that regulate pituitary cells and along with arginine vasotocin could act as neuromodulators of reproductive behaviors.
The hormone insulin-like growth factor-I (IGF-I) regulates vertebrate growth. The liver produces most circulating IGF-I, under the control of pituitary growth hormone (GH) and nutritional status. To study the regulation of liver IGF-I production in salmon, we established a primary hepatocyte culture system and developed a TaqMan quantitative real-time RT-PCR assay for salmon IGF-I gene expression. A portion of the coho salmon acidic ribosomal phosphoprotein P0 (ARP) cDNA was sequenced for use as a reference gene. A systematic bias across the 96 well PCR plate was discovered in an initial IGF-I assay, which was corrected when the assay was redesigned. IGF-I mRNA levels measured with the validated assay correlated well with levels measured with an RNase protection assay, and were highest in liver compared with other tissues. We examined the time course of hepatocyte IGF-I gene expression over 48 h in culture, the response to a range of GH concentrations in hepatocytes from fed and fasted fish, and potential effects of variation in IGF-I in the medium. IGF-I gene expression decreased over time in culture in hepatocytes in plain medium, and in cells treated with 5 nM GH with or without a combination of metabolic hormones (1 [mu]M insulin, 100 nM triiodothyronine, and 0.1 nM dexamethasone). GH stimulated IGF-I gene expression at all time points. In cells treated with GH plus metabolic hormones, IGF-I gene expression was intermediate between the controls and GH alone. Increasing concentrations of GH resulted in biphasic IGF-I gene expression response curves in cells from fed and fasted fish, with the threshold for stimulation from 0.5 to 2.5 nM GH, maximal response from 5 to 50 nM, and a reduced response at 500 nM. Medium IGF-I (5 nM) did not affect basal or GH stimulated IGF-I gene expression. This study shows that primary hepatocyte culture and the TaqMan IGF-I assay can be used to study the regulation of hepatic IGF-I gene expression in salmon, and provides the first evidence of a biphasic response to GH concentration in fish hepatocyte culture.

Melatonin and arylalkylamine N-acetyltransferase (AANAT), the rate-limiting enzyme in melatonin synthesis, have taken on special importance in vertebrate circadian biology. Recent identification of genes encoding two AANAT (AANAT1 and AANAT2) and two subtypes of melatonin receptor (Mel-R; Mel1a and Mel1b) in several fish species has led to rapid advances in characterizing the physiological roles of melatonin. In the present study, partial cDNAs encoding these four genes were cloned from the eye and brain of chum salmon (Oncorhynchus keta). Based on the nucleotide sequences, we developed highly sensitive real-time PCR systems for these four mRNAs. The development of daily rhythmicity in AANAT1, AANAT2, Mel1a, and Mel1b transcript levels was examined in the eye and brain of chum salmon during embryonic and post-embryonic stages (from day -9 to day +180). In a parallel experiment, ocular and brain melatonin levels were measured by radioimmunoassay. Parallelism in developmental changes and in circadian rhythms of AANAT mRNAs and melatonin levels in the eye and the brain supports a hypothesis that the developmental increases of nocturnal melatonin levels results partly from the elevated transcription of AANAT genes. Moreover, abundant expression of AANAT and Mel-R mRNAs in the optic tectum, thalamus, hypothalamus, cerebellum, and eye indicates possible roles of
melatonin in visual processing and neuroendocrine regulation, through which melatonin might be involved in migratory behavior of chum salmon.

Slagter, B. J., J. D. Kittilson, et al. (2004). "Somatostatin receptor subtype 1 and subtype 2 mRNA expression is regulated by nutritional state in rainbow trout (Oncorhynchus mykiss)." General and Comparative Endocrinology 139(3): 236.

http://www.sciencedirect.com/science/article/B6WG0-4DPGX17-2/2/22470cf38983b4aa9d630c0452728941

Somatostatin receptors (sst) mediate the numerous physiological actions (e.g., aspects of growth, development, and metabolism) of the somatostatin family of peptides. In this study, we used rainbow trout (Oncorhynchus mykiss) to establish the pattern of sst subtype 1A, 1B, and 2 mRNA expression in selected tissues (optic tectum of brain, endocrine pancreas, and liver) and to evaluate nutritional regulation of sst expression. Quantitative real-time reverse transcription-PCR, sensitive to less than 100 copies, revealed that sst1s and sst2 was differentially expressed, both in terms of distribution among the tissues of study and in terms of relative abundance within a particular tissue. Under normal physiological (fed) conditions, sst1B levels were two times greater than those of sst1A in all tissues examined and levels of sst2 were 2-5 times greater those of sst1B, except in optic tectum, in which sst1B and sst2 mRNA levels appeared equal. Nutritional state modulated the pattern of sst1 and sst2 mRNAs expression. Fasting for 2 or 6 weeks reduced the expression of sst mRNAs in optic tectum; whereas, fasting increased the expression of sst mRNAs in both pancreas and liver. Re-feeding animals for 2 weeks following a 4-week fast restored mRNA levels to near those in tissues from animals which were fed continuously. These findings indicate that the pattern of sst expression in optic tectum, pancreas, and liver is regulated by nutritional state.


http://www.sciencedirect.com/science/article/B6WG0-49R5F1V-1/2/888bf5dfb016686daad2c7e01eb37afc

Proopiomelanocortin (POMC) is a precursor for several pituitary hormones including adrenocorticotropic (ACTH), melanocyte-stimulating hormone (MSH) and endorphin (END). Fish POMCs in four taxonomic classes, Cephalaspidomorphi (lampreys), Chondrichthyes (cartilaginous fish), Sarcopterygii (lobe-finned fish), and Actinopterygii (ray-finned fish) have been identified. However, two essential species, ratfish in Chondrichthyes and hagfish in Agnatha, are still missing in the evolutionary image of this molecule. The present study reports analysis of POMC cDNA in the ratfish, Chimaera phantasma, which belongs to another subclass in the Chondrichthyes. Partial cDNA clones were amplified by PCR from single-strand cDNA prepared on total RNA from a complex of pituitary and hypothalamus, and subsequently overlapped to obtain a full-length sequence. Ratfish POMC cDNA consists of 1294 bp excluding the poly(A) tail. It encodes a signal peptide of 25 amino acids and POMC of 300 amino acids. [gamma]-MSH, ACTH, [alpha]-MSH, [delta]-MSH, [beta]-MSH, and [beta]-END are located at prePOMC (76-87), (120-158), (120-132), (212-227), (275-290), and (293-325), respectively. [delta]-MSH, originally found in elasmobranch POMCs, was also present in ratfish POMC, suggesting this structure might have appeared after the divergence of chondrichthians from the ancestral lineage. Thus, we demonstrated the common occurrence of four MSHs in chondrichthian POMC and established a clear understanding of the molecular evolution of POMC in gnathostomes.
Extrapituitary expression of the growth hormone (GH) gene has been reported for the immune system of various vertebrates. In the rainbow trout (Oncorhynchus mykiss), GH mRNA could be detected in several lymphoid organs and leucocytes by reverse transcriptase-polymerase chain reaction (RT-PCR). To understand the control of GH expression in the fish immune system, mRNA levels for two distinct GH genes (GH1 and GH2) in trout leucocytes isolated from peripheral blood were quantified using a real-time PCR method. Both GH mRNAs could be detected in trout leucocytes, although their levels were extremely low compared to those in pituitary cells. The levels of GH2 mRNA in leucocytes were several times higher than those of GH1, while no difference was observed between GH1 and GH2 mRNA levels in the pituitary. Administration of dibutyryl cyclic AMP and cortisol produced a significant elevation of GH mRNA levels in trout leucocytes, although the levels were unchanged by T3. GH1 and GH2 mRNA levels showed similarities in responses to those factors. The effect of cortisol on GH mRNA appears biphasic; a dose-depending elevation of GH gene expression was observed in leucocytes treated with cortisol at below 200 nM, however, cortisol had no effect at 2000 nM. Cortisol-treated leucocytes showed no significant change in the mRNA level of [beta]-actin or proliferative activity during the experiments. Our results thus show that, at the low levels, GH gene expression in trout leucocytes is regulated by cortisol, which has been known as a regulatory factor of GH gene expression in pituitary cells, and suggest a physiological significance of paracrine GH produced in the fish immune system.


Catecholamines induce direct vasoconstriction mediated by postsynaptic [alpha]-adrenergic receptors ([alpha]-ARs) of both the [alpha]1 and [alpha]2 type. To evaluate the contribution of each [alpha]2-AR subtype ([alpha]2A, [alpha]2B, and [alpha]2C) to this function, we used groups of genetically engineered mice deficient for the gene to each one of these subtypes and compared their blood pressure (BP) responses to their wild-type counterparts. Blood pressure responses to a bolus of norepinephrine (NE) were assessed before and after sequential blockade of [alpha]1-ARs with prazosin and [alpha]2-ARs with yohimbine. The first NE bolus elicited a brief 32 to 44 mm Hg BP rise (p 2A-AR gene knockouts differed, responding instead with a 20-mm Hg fall in BP, a significant change from baseline (p 2-vascular wall ARs. We conclude that the [alpha]2-AR-mediated vasoconstriction induced by catecholamines is attributable to the [alpha]2A-AR subtype because mice deficient in any one of the other subtypes retained the capacity for normal vasoconstrictive responses. However, the [alpha]1-ARs account for the major part (as much as 68%) of catecholamine-induced vasoconstriction.
Our earlier observations suggest that M3 muscarinic acetylcholine (ACh) receptors (mAChRs) are involved in Ca2+ signaling and regulation of c-fos gene expression in T lymphocytes. Here, we describe the effects of YM905, a novel M3 antagonist, on evoked Ca2+ signaling and c-fos gene expression in CEM human leukemic T cells. YM905 significantly inhibited increases in intracellular free Ca2+ evoked by 10 \(\mu\)M oxotremorine-M, an M1/M3 agonist (IC50=100 nM), and also inhibited 10 \(\mu\)M oxotremorine-M-induced upregulation of c-fos gene expression at 1 \(\mu\)M. These findings demonstrate that YM905 antagonizes the intracellular responses in T cells induced via mAChRs, possibly M3 receptors.


Nuclear export of mRNA is a central step in gene expression that shows extensive coupling to transcription and transcript processing. However, little is known about the fate of mRNA and its export under conditions that damage the DNA template and RNA itself. Here we report the discovery of four new factors required for mRNA export through a screen of all annotated nonessential Saccharomyces cerevisiae genes. Two of these factors, mRNA surveillance factor Rrp6 and DNA repair protein Lrp1, are nuclear exosome components that physically interact with one another. We find that Lrp1 mediates specific mRNA degradation upon DNA-damaging UV irradiation as well as general mRNA degradation. Lrp1 requires Rrp6 for genomic localization to genes encoding its mRNA targets, and Rrp6 genomic localization in turn correlates with transcription. Further, Rrp6 and Lrp1 are both required for repair of UV-induced DNA damage. These results demonstrate coupling of mRNA surveillance to mRNA export and suggest specificity of the RNA surveillance machinery for different transcript populations. Broadly, these findings link DNA and RNA surveillance to mRNA export.
To investigate the molecular basis of [beta]-globin gene activation, we analyzed factor recruitment and histone modification at the adult [beta]-globin gene in wild-type (WT)/locus control region knockout ([Delta]LCR) heterozygous mice and in murine erythroleukemia (MEL) cells. Although histone acetylation and methylation (Lys 4) are high before and after MEL differentiation, recruitment of the erythroid-specific activator NF-E2 to the promoter and preinitiation complex (PIC) assembly occur only after differentiation. We reported previously that targeted deletion of the LCR reduces [beta]-globin gene expression to 1%-4% of WT without affecting promoter histone acetylation. Here, we report that NF-E2 is recruited equally efficiently to the adult [beta]-globin promoters of the [Delta]LCR and WT alleles. Moreover, the LCR deletion reduces PIC assembly only twofold, but has a dramatic effect on Ser 5 phosphorylation of RNA polymerase II and transcriptional elongation. Our results suggest at least three distinct stages in [beta]-globin gene activation: (1) an LCR-independent chromatin opening stage prior to NF-E2 recruitment to the promoter and PIC assembly; (2) an intermediate stage in which NF-E2 binding (LCR-independent) and PIC assembly (partially LCR-dependent) occur; and (3) an LCR-dependent fully active stage characterized by efficient pol II elongation. Thus, in its native location the LCR functions primarily downstream of activator recruitment and PIC assembly.

**Genes Cells**  (5)


http://www.genestocellsonline.org/cgi/content/abstract/10/3/207

The general transcription factor TFIIIE plays essential roles in transcription by RNA polymerase II (PolII). Despite recent progress, the elucidation of its precise mechanisms including biological functions awaits further characterization. We report the isolation and characterization of Schizosaccharomyces pombe TFIIIE (spTFIIIE). Like human and other eukaryotic TFIIIE proteins, spTFIIIE consists of {alpha} and {beta} subunits and the genes encoding both subunits are essential for viability. Chromatin immunoprecipitation assays demonstrated that spTFIIIE localizes to promoters in vivo. Mutational analysis of the C-terminal basic helix-loop region of TFIIIE{beta}, which is involved in the transition from transcription initiation to elongation, revealed that transcription-defective mutants affected in this region are also cold sensitive. The spTFIIIE{beta} subunit binds both spTFIIIE{beta} and spTFIIIE{alpha} but spTFIIIE{alpha} binds only spTFIIIE{beta}. These results indicate that TFIIIE forms an {alpha}{beta}{alpha}{beta} heterotetramer in which two {alpha}{beta} heterodimers are connected via {beta} subunits. Further analysis of binding specificities showed that spTFIIIE{beta} binds the Rpb2 and Rpb12 subunits of PolII, whereas spTFIIIE{alpha} predominantly binds Rpb5, which is located at the clamp region and changes conformation upon transcription initiation.

We previously reported that DNA topoisomerase II (topo II) is required for the G0-to-S phase transition in mammalian cells [Hossain et al. (2002) ICRF-193, a catalytic inhibitor of DNA topoisomerase II, inhibits re-entry into the cell division cycle from quiescent state in mammalian cells. Genes Cells 7, 285-294]. In this study, we examined whether the requirement for topo II is evolutionarily conserved in Drosophila and yeast. ICRF-193, a catalytic inhibitor of topo II, inhibited DNA synthesis in Drosophila Schneider cells released from the G0 (stationary) phase, whereas the drug did not inhibit DNA synthesis in Schneider cells released from the M phase. Depletion of topo II mRNA by RNA-interference (RNAi) in G0-phase Schneider cells resulted in significant inhibition of DNA synthesis after release from G0-arrest. In the yeast topo II temperature-sensitive (ts) mutant, the initial cycle of DNA synthesis occurred at a restrictive temperature after release from starvation-induced G0 phase and doubling of the DNA content in the cells was confirmed by both flow cytometry and fluorescence spectrophotometry. DNA synthesis in yeast cells after release from the G0 phase was also observed in the presence of ICRF-193. Doubling of the DNA content was observed during spore germination of topo II ts mutant yeast at a restrictive temperature as determined by fluorescence spectrophotometry. These results indicate that topo II is required for the G0-to-S phase transition in Drosophila Schneider cells, but not in yeast.


DNA methylation controls various developmental processes by silencing, switching and stabilizing genes as well as remodeling chromatin. Among various symptoms in cloned animals, placental hypertrophy is commonly observed. We identified the Spalt-like gene3 (Sall3) locus as a hypermethylated region in the placental genome of cloned mice. The Sall3 locus has a CpG island containing a tissue-dependent differentially methylated region (T-DMR) specific to the trophoblast cell lineage. The T-DMR sequence is also conserved in the human genome at the SALL3 locus of chromosome 18q23, which has been suggested to be involved in the 18q deletion syndrome. Intriguingly, larger placentas were more heavily methylated at the Sall3 locus in cloned mice. This epigenetic error was found in all cloned mice examined regardless of sex, mouse strain and the type of donor cells. In contrast, the placentas of in vitro fertilized (IVF) and intracytoplasmic sperm injected (ICSI) mice did not show such hypermethylation, suggesting that aberrant hypermethylation at the Sall3 locus is associated with abnormal placental development caused by nuclear transfer of somatic cells. We concluded that the Sall3 locus is the area with frequent epigenetic errors in cloned mice. These data suggest that there exists at least genetic locus that is highly susceptible to epigenetic error caused by nuclear transfer.


BACKGROUND: The sex hormone 17beta-oestradiol (E2) has profound effects on many aspects of reproduction, development, as well as behaviour. Although the oestrogen receptor is well characterized on a molecular level, relatively few genes affected by E2 have been identified, and the mechanisms underlying the physiological changes caused by E2 are largely unknown. In order to identify oestrogen-regulated genes in vivo, early uterine gene expression profiles were
developed using DNA microarrays. RESULTS: Ovariectomized mice were exposed to 17beta-oestradiol for 6 h, and mRNA expression analysis for 9977 genes was performed. Although a large number of genes was affected by oestrogen administration, the genes that showed higher reproducibility in repetitive experiments were selected and further examined. For most of the selected genes, expression was induced in a dose-dependent manner, and gene expression was not altered following oestrogen treatment in oestrogen receptor-alpha (ER(alpha))-deficient mice. In combination with the estimation of gene expression levels using quantitative PCR, it was revealed that multiple genes related to sterol biosynthesis, tRNA synthesis, RNA processing, and growth signalling were activated. Based on the microarray data, we selected additional genes related to sterol biosynthesis and tRNA synthesis and confirmed that these genes are also activated by oestrogen. CONCLUSION: Genes suggesting a basis for the drastic uterotrophic effect observed several days following oestrogen administration were identified. These findings not only reveal the diverse effect of oestrogen signalling on transcript levels in vivo but also demonstrate the ability of DNA microarrays to identify cellular pathways affected by oestrogen.


http://www.genestocellsonline.org/cgi/content/abstract/8/4/403

**BACKGROUND:** BAT1 belongs to the DEAD-box family of proteins, and is encoded in the central region of the MHC, a region containing genes affecting immunopathological disorders including Type 1 diabetes. We showed that BAT1 can reduce inflammatory cytokine production, supporting its candidacy as a disease gene. Here we examined the proximal promoter region of BAT1.

**RESULTS:** Ten single nucleotide polymorphisms were identified in approximately 1.4 kb of sequence, defining at least seven alleles. Sections of the BAT1 promoter region were amplified from cells homozygous for the MHC haplotypes associated with susceptibility (HLA-A1, B8, DR3; 8.1 haplotype) and resistance (HLA-A3, B7, DR15; 7.1 haplotype) to diabetes and cloned into a promoter-less luciferase-encoding plasmid. Jurkat cells transiently transfected with fragments from the 8.1 haplotype exhibited a lower luciferase activity than those transfected with fragments from the 7.1 haplotype, indicating reduced transcription. The effect was clearest with the 520 bp immediately upstream of the transcriptional start site. Electrophoretic mobility shift assays using oligonucleotides spanning polymorphic sites within the 520 bp (proximal) promoter fragment showed haplotype-specific binding of nuclear proteins.

**CONCLUSIONS:** In view of the anti-inflammatory role of BAT1, reduced production on a disease-associated haplotype constitutes a novel and self-consistent model for the effect of central MHC genes on disease.
We report a reliable method for PCR (polymerase chain reaction) amplification of genomic DNA from PET. This method uses DNA extraction with the QIAquick kit and amplification with AmpliTaq Gold. Amplification of up to 959 bp from PET was achieved with this combination which exceeds the current reported upper limit of 800 bp. In summary, the gradual activation of the AmpliTaq Gold during thermal cycling allows both for higher-fidelity and higher-throughput PCR amplification from PET. The use of the QIAquick kit for DNA purification of PET is sensitive, reproducible and suitable for management of a high number of samples.


http://www.sciencedirect.com/science/article/B6T72-4840M2G-2N/2/4b5e1ef5c1593ebe95a2d01ac40679f9

Beginning with 103-105 molecules of a purified HIV-1 target sequence as a starting template, we have examined the effects of starting template concentration and cycle number on the amplification efficiency of the polymerase chain reaction. An external standard DNA sequence has been designed that when added to a DNA sample enables a determination of the starting concentration of HIV-1 target sequence in that sample of DNA. Varying ratios of external standard and target DNA sequences were amplified for 22 cycles. When the starting concentration of the external standard was within 50-fold of the starting concentration of the target, the amplifications of both sequences were proportional. These same results were obtained when the two templates were amplified in the presence of an excess of heterogeneous genomic DNA. Using this quantitative method, the number of starting target molecules in a DNA sample can be calculated to within a two-fold range of accuracy.


http://www.sciencedirect.com/science/article/B6T72-4840KWT-T/2/1a951e2b6a250763b3a5e93e5bad9688

Physical mapping of the human genome involves a variety of complex hybridization-based procedures, some of which rely upon the ability to separate human clones derived from human-rodent hybrid cell lines from those that contain background rodent-derived DNA sequences. The ability to block the repetitive element (Alu repeat) portion of inter-Alu PCR products derived from a variety of complex sources is also crucial for the isolation of unique DNA sequences. Here we report the construction and characterization of a new consensus Alu repeat probe (pPD39) designed for these purposes.


http://www.sciencedirect.com/science/article/B6T72-3VW821K-4/2/9327b4d3b07a553d158e493490a8ced5

We previously described a targeted genomic differential display method (TGDD: Broude NE, Chandra A, Smith CL. Differential display of genomic subsets containing specific interspersed repeats. Proc. Natl. Acad. Sci. USA 1997;94:4548-53). In that method, presently characterized as
method I, targeting was accomplished by capturing DNA fragments containing specific a sequence by hybridization with complementary single-stranded DNA. The captured fragments were amplified by PCR. Here, we describe method II where targeting is accomplished by PCR using primers specific to the target sequence. Method II takes advantage of PCR suppression to eliminate fragments not containing the target sequence (Siebert PDA, Chenchik A, Kellogg DE, Lukyanov KA and Lukyanov SA. An improved PCR method for walking in uncloned genomic DNA. Nucleic Acids Res 1995;23:1087-1088). Targeting focuses analysis on and around interesting areas and additionally serves to reduce the complexity of the amplified subset. These approaches are useful to amplify genome subsets containing a variety of targets including various conserved sequences coding for cis-acting elements or protein motifs.


http://www.sciencedirect.com/science/article/B6T72-3VS7DNK-B/2/bfdc93a59f24f39d70b802f9b4c2123d

A homogeneous detection mechanism based on fluorescence resonance energy transfer (FRET) has been developed for two DNA diagnostic tests. In the template-directed dye-terminator incorporation (TDI) assay, a donor dye-labeled primer is extended by DNA polymerase using allele-specific, acceptor dye-labeled ddNTPs. In the dye-labeled oligonucleotide ligation (DOL) assay, a donor dye-labeled common probe is joined to an allele-specific, acceptor dye-labeled probe by DNA ligase. Once the donor and acceptor dyes become part of a new molecule, intramolecular FRET is observed over background intermolecular FRET. The rise in FRET, therefore, can be used as an index for allele-specific ddNTP incorporation or probe ligation. Real time monitoring of FRET greatly increases the sensitivity and reliability of these assays. Change in FRET can also be measured by end-point reading when appropriate controls are included in the experiment. FRET detection proves to be a robust method in homogeneous DNA diagnostic assays.


http://www.sciencedirect.com/science/article/B6T72-3VS7DNK-K/2/35ed21b14304b5012636dac15ffa95dd

Important requirements for molecular genetic epidemiological studies are economy, sample parallelism, convenience of setup and accessibility, goals inadequately met by existent approaches. We invented microplate array diagonal gel electrophoresis (MADGE) to gain simultaneously the advantages of simple setup, 96-well microplate compatibility, horizontal electrophoresis, and the resolution of polyacrylamide. At essentially no equipment cost (one simple plastic gel former), 10-100-fold savings on time for sample coding, liquid transfers, and data documentation, in addition to volume reductions and gel re-use, can be achieved. MADGE is compatible with ARMS, restriction analysis and other pattern analyses. CpG-PCR is a general PCR approach to CpG sites (10-20% of all human single base variation): both primers have 3' T, and are abutted to the CpG, forcing a TaqI restriction site if the CpG is intact. Typically, a 52 bp PCR product is then cut in half. CpG-PCR also illustrates that PAGE-MADGE readily permits analysis of 'ultrashort' PCRs. Melt-MADGE employs real-time-variable-temperature electrophoresis to examine duplex mobility during melting, achieving DGGE-like de novo mutation scanning, but with the conveniences of arbitrary programmability, MADGE compatibility and short
run time. This suite of methods enhances our capability to type or scan thousands of samples simultaneously, by 10-100-fold.


http://www.sciencedirect.com/science/article/B6T72-3W2T5PB-4/2/562777239b086ae2333a7189a09b0eb301

The Escherichia coli DNA mismatch repair protein, MutS, binds single base pair mismatches and short deletions in vivo and in vitro. To adapt this protein for mutation detection, a fusion protein of E. coli MutS with a biotinylated peptide domain has been constructed (MutSb). The biotinylation tag facilitates MutS detection and binding by avidin without significantly altering the DNA mismatch binding properties of MutS in vitro. We describe a novel and rapid mutation detection method with MutSb using streptavidin-coated magnetic beads and demonstrate that MutSb can also be used to remove mismatch containing DNA fragments from a mixture of DNA fragments in solution.


http://www.sciencedirect.com/science/article/B6T72-4840KXF-Y/2/9c8972b5c782f1d500c5c5dd1bda9aba

We have developed a method for the whole sequence amplification of yeast artificial chromosome (YAC) DNA excised from preparative pulsed-field gel electrophoresis using single unique primer-polymerase chain reaction procedures. We used seven contiguous YAC clones, which span 2 Mbp of the Huntington disease gene region on 4p16.3, to amplify the YAC DNAs. The average size of the amplified DNA was ~300 bp long, and 12 DNA markers located on the YAC clones positively hybridized with these amplified products, implying that the sequences of the YAC clones were comprehensively amplified by our procedures. These amplified YAC DNAs greatly facilitate the characterization of YAC clones, leading to the detailed analysis of the defined chromosomal region.


http://www.sciencedirect.com/science/article/B6T72-497C71D-7/2/8ec5523c376be722f9a41b9041d361a

Degenerate oligonucleotide primers were made to peptide sequences from hydroxylamine oxidoreductase (HAO) from Nitrosomonas europaea. The primers were used singly in PCR reactions to amplify portions of the gene for HAO from genomic DNA. Southern hybridizations using fragments amplified with each primer showed that they labeled the same genomic DNA fragments. The PCR-amplified fragments were successfully used to screen a gene library for clones containing the HAO gene. The method of isolating genes by PCR with single primers has general utility.

http://www.sciencedirect.com/science/article/B6T72-3XX6S3X-M/2/03c6a4faa66efed7cc35d143787f1d97

The SuperTth DNA polymerase from Thermus thermophilus exhibits template-independent terminal transferase (extendase) activity. This enzyme is proposed as a cheap alternative for both high performance PCR as well as quick T-vector cloning of amplicons, including reverse transcription and cDNA cloning.


http://www.sciencedirect.com/science/article/B6T72-3VS7DNK-8/2/bcfa8c41e26053ff1f54927dc2961c18

Large-scale screening for known polymorphisms will require techniques with few steps and the ability to automate each of these steps. In this regard, the 5' nuclease, or TaqMan, PCR assay is especially attractive. A fluorogenic probe, consisting of an oligonucleotide labeled with both a fluorescent reporter dye and a quencher dye, is included in a typical PCR. Amplification of the probe-specific product causes cleavage of the probe, generating an increase in reporter fluorescence. By using different reporter dyes, cleavage of allele-specific probes can be detected in a single PCR. The 5' nuclease assay has been successfully used to discriminate alleles that differ by a single base substitution. Guidelines have been developed so that an assay for any single nucleotide polymorphism (SNP) can be quickly designed and implemented. All assays are performed using a single reaction buffer and single thermocycling protocol. Furthermore, a standard method of analysis has been developed that enables automated genotype determination. Applications of this assay have included typing a number of polymorphisms in human drug metabolism genes.


http://www.sciencedirect.com/science/article/B6T72-3VS7DNK-9/2/7726ec68e808881a0c465159bbd67a26

We demonstrate that single-nucleotide differences in a DNA sequence can be detected in homogeneous assays using molecular beacons. In this method, the region surrounding the site of a sequence variation is amplified in a polymerase chain reaction and the identity of the variant nucleotide is determined by observing which of four differently colored molecular beacons binds to the amplification product. Each of the molecular beacons is perfectly complementary to one variant of the target sequence and each is labeled with a different fluorophore. To demonstrate the specificity of these assays, we prepared four template DNAs that only differed from one another by the identity of the nucleotide at one position. Four amplification reactions were prepared, each containing all four molecular beacons, but each initiated with only one of the four template DNAs. The results show that in each reaction a fluorogenic response was elicited from the molecular beacon that was perfectly complementary to the amplified DNA, but not from the three molecular beacons whose probe sequence mismatched the target sequence. The color of
the fluorescence that appeared in each tube during the course of the amplification indicated which nucleotide was present at the site of variation. These results demonstrate the extraordinary specificity of molecular beacons. Furthermore, the results illustrate how the ability to label molecular beacons with differently colored fluorophores enables simple multiplex assays to be carried out for genetic analysis.


http://www.sciencedirect.com/science/article/B6T72-4840M2G-2P/2/e12ab1d091466044db5a8c03c0132195

We have used analysis of ethidium-bromide-stained reverse transcriptase-polymerase chain reaction (RT-PCR) products to assess the effects of X-chromosome inactivation during spermatogenesis in the mouse. RT-PCR was performed on total RNA from eight different spermatogenic cell types, including premeiotic spermatogonia, meiotic spermatocytes, and postmeiotic spermatids, to detect transcripts from five different X-linked structural genes (Pgk-1, Zfx, Pdha-1, Hprt, and Phka) and two autosomal genes (Pgk-2 and [beta]-actin). Relative intensities of ethidium-bromide-strained RT-PCR products representing transcripts from each gene in each cell type were analyzed by densitometry using the Image program (version 1.4, NIH), and normalized against [beta]-actin values. These results suggest a coordinate inactivation of the X-linked loci at the onset of meiosis, followed by variable rates of decline of corresponding transcript levels reflecting differential mRNA stabilities and/or leaky expression after inactivation. Technically, these results indicate that analysis of ethidium-bromide-stained RT-PCR products can be used to provide a "semiquantitative" indication of relative levels of specific transcripts in a developing cell lineage without using radioactive probes to quantitate these products.


http://www.sciencedirect.com/science/article/B6T72-476VPM2-52/2/bc8c94e918d0f51a73cddf812255d61


http://www.sciencedirect.com/science/article/B6T72-4840M30-2V/2/60157c05cb23a511a4026cd9faaf80f

A new and potentially reliable method for the isolation of yeast artificial chromosome (YAC)-insert termini, termed junction trapping, is described. This method is based on simple partial digestions of the YAC-containing yeast strain, ligation to a plasmid vector, and selection of the termini by two rounds of polymerase chain reaction (PCR). To date, the method has generated both terminal sequences from each of nine YACs (100%) that have been examined.


http://www.sciencedirect.com/science/article/B6T72-4840KVB/2/56bdcc373c80e8ce9217e5d0eb5a8f

The potential usefulness of chromosome microdissection, the polymerase chain reaction (PCR), and dot blot hybridization as a quick screening method for determining the genetic composition of double minute chromosomes (DMs) was evaluated. DMs or abnormally banding regions (ABRs) were microdissected from multidrug-resistant hamster cell lines and amplified with PCR using primers specific for the hamster multidrug-resistance (MDR) gene, pgp 1. The microdissected-PCR-amplified products were shown to (a) hybridize to a 32P-labeled pCHP1 probe for the hamster MDR gene by using dot blot or Southern blot analysis and also (b) hybridize back to the chromosome region from which they were originally dissected by using fluorescent in situ hybridization. Microdissected/PCR-amplified DMs were also shown to hybridize to ABRs. When microdissected DMs and ABRs were amplified using hamster specific Alu primers, the resulting material was shown to hybridize with probes for hamster MDR and Alu. These results suggest that the DMs contained in these MDR hamster cell lines contain Alu-like sequences and the chromosome microdissection-PCR-hybridization approach might be used as a quick screening method for identifying genes amplified in DMs and ABRs in cell lines and human tumor samples.


http://www.sciencedirect.com/science/article/B6T72-4840M2G-2M/2/ee21f9d29b702e9c96c572cb7e7e9815

This article describes a method for determining whether a particular nucleic acid sequence is present in a sample and for discriminating between any two nucleic acid sequences if such sequences differ only by a single nucleotide. The method entails extension of a novel two-component primer on templates that may or may not include a target nucleic acid sequence. The 3' portion of the primer is complementary to a portion of the template adjacent to the target sequence (for example, the polymorphic nucleotide). The 5' portion of the primer is complementary to a different preselected nucleic acid sequence. Extension of the 3' portion of the primer with a labeled deoxynucleoside triphosphate yields a labeled extension product, but only if the template includes the target sequence. The presence of such a labeled primer-extension product is detected by hybridization of the 5' portion to the preselected sequence. The preselected sequence is immobilized on a solid support. The method has been applied to genotyping individuals for the two-allele polymorphism of the human tyrosinase gene.


Direct label alkaline phosphatase (AP) conjugated oligonucleotide probes (AP-DNA) were prepared to assess their utility for allele-specific detection of single base substitutions.
Oligonucleotide conjugates were designed to detect point mutations in the genes for lipoprotein lipase (LPL) and coagulation factor-V (FV). Genomic DNA samples, including ones known to harbor point mutations in the genes for LPL and FV, were prepared from whole blood and subjected to polymerase chain reaction (PCR). PCR products were analyzed by Southern hybridization with the allele-specific AP-DNA probes and restriction endonuclease analysis. Thermal profiles for hybridization indicate optimal allele-specific selectivity was achieved with temperatures ranging from 45\(\text{deg}\)C to 55\(\text{deg}\)C at a total Na+ concentration of 150 mM. Under these conditions the base changes studied were easily discriminated with allele specific hybridization signals in excess of 200:1 as estimated by scanning densitometry. Complete concordance was observed between hybridization and restriction analyses for 175 LPL and 201 FV clinical and reference samples. The total time for analysis of the PCR products was less than 2 h with a dot blot hybridization protocol.

**Genetics** (30)


http://www.genetics.org/cgi/content/abstract/161/2/813

Determination of parentage is fundamental to the study of biology and to applications such as the identification of pedigrees. Limitations to studies of parentage have stemmed from the use of an insufficient number of hypervariable loci and mismatches of alleles that can be caused by mutation or by laboratory error and that can generate false exclusions. Furthermore, most studies of parentage have been limited to comparisons of small numbers of specific parent-progeny triplets thereby precluding large-scale surveys of candidates where there may be no prior knowledge of parentage. We present an algorithm that can determine probability of parentage in circumstances where there is no prior knowledge of pedigree and that is robust in the face of missing data or mistyped data. We present data from 54 maize hybrids and 586 maize inbreds that were profiled using 195 SSR loci including simulations of additional levels of missing and mistyped data to demonstrate the utility and flexibility of this algorithm.


http://www.genetics.org/cgi/content/abstract/169/2/1009

Plant endo-(beta)-1,3-glucanases (EGases) degrade the cell wall polysaccharides of attacking pathogens and release elicitors of additional plant defenses. Isozymes EGaseA and EGaseB of soybean differ in susceptibility to a glucanase inhibitor protein (GIP1) produced by Phytophthora sojae, a major soybean pathogen. EGaseA, the major elicitor-releasing isozyme, is a high-affinity ligand for GIP1, which completely inhibits it, whereas EGaseB is unaffected by GIP1. We tested for departures from neutral evolution on the basis of partial sequences of EGaseA and EGaseB from 20 widespread accessions of Glycine soja (the wild progenitor of soybean), from 4 other Glycine species, and across dicotyledonous plants. G. soja exhibited little intraspecific variation at either locus. Phylogeny-based codon evolution models detected strong evidence of positive
selection on Glycine EGaseA and weaker evidence for selection on dicot EGases and Glycine EGaseB. Positively selected peptide sites were identified and located on a structural model of EGase bound to GIP1. Positively selected sites and highly variable sites were found disproportionately within 4.5 Å of bound GIP1. Low variation within G. soja EGases, coupled with positive selection in both Glycine and dicot lineages and the proximity of rapidly evolving sites to GIP1, suggests an arms race involving repeated adaptation to pathogen attack and inhibition.


http://www.genetics.org/cgi/content/abstract/167/2/941

Sequencing was used to investigate the origin of the D genome of the allopolyploid species Triticum aestivum and Aegilops cylindrica. A 247-bp region of the wheat D-genome Xwye838 locus, encoding ADP-glucopyrophosphorylase, and a 326-bp region of the wheat D-genome Gss locus, encoding granule-bound starch synthase, were sequenced in a total 564 lines of hexaploid wheat (T. aestivum, genome AABBDD) involving all its subspecies and 203 lines of Aegilops tauschii, the diploid source of the wheat D genome. In Ae. tauschii, two SNP variants were detected at the Xwye838 locus and 11 haplotypes at the Gss locus. Two haplotypes with contrasting frequencies were found at each locus in wheat. Both wheat Xwye838 variants, but only one of the Gss haplotypes seen in wheat, were found among the Ae. tauschii lines. The other wheat Gss haplotype was not found in either Ae. tauschii or 70 lines of tetraploid Ae. cylindrica (genomes CCDD), which is known to hybridize with wheat. It is concluded that both T. aestivum and Ae. cylindrica originated recurrently, with at least two genetically distinct progenitors contributing to the formation of the D genome in both species.


http://www.genetics.org/cgi/content/abstract/162/1/307

ROSA22 male mice are sterile due to a recessive gene-trap mutation that affects development of the spermatid flagellum. The defect involves the flagellar axoneme, which becomes unstable around the time of its assembly. Despite a subsequent complete failure in flagellar assembly, development of the spermatid head appears normal and the spermatid head is released at the correct stage in spermatogenesis. The mutation is pleiotropic. Although ROSA22 homozygote males have normal levels of circulating testosterone and display normal mating behavior, they do not exhibit intermale aggressive behavior and have reduced body fat. The mutated gene (Gtrgeo22) maps to mouse chromosome 10 and is closely flanked by two known genes, Madcam1 and Cdc34. Ribonuclease protection analysis indicates that expression of the flanking genes is unaffected by the mutation. Gtrgeo22 is expressed at low levels in epithelial cells in several tissues, as well as in testis and brain. Analysis of the peptide coding sequence suggests that Gtrgeo22 encodes a novel transmembrane protein, which contains dileucine and tyrosine-based motifs involved in intracellular sorting of transmembrane proteins. Analysis of the Gtrgeo22 gene product should provide novel insight into the molecular basis for intermale aggression and sperm flagellar development.

In a screen for suppressors of the Drosophila winglessPE4 nonsense allele, we isolated mutations in the two components that form eukaryotic release factor. eRF1 and eRF3 comprise the translation termination complex that recognizes stop codons and catalyzes the release of nascent polypeptide chains from ribosomes. Mutations disrupting the Drosophila eRF1 and eRF3 show a strong maternal-effect nonsense suppression due to readthrough of stop codons and are zygotically lethal during larval stages. We tested nonsense mutations in wg and in other embryonically acting genes and found that different stop codons can be suppressed but only a subset of nonsense alleles are subject to suppression. We suspect that the context of the stop codon is significant: nonsense alleles sensitive to suppression by eRF1 and eRF3 encode stop codons that are immediately followed by a cytidine. Such suppressible alleles appear to be intrinsically weak, with a low level of readthrough that is enhanced when translation termination is disrupted. Thus the eRF1 and eRF3 mutations provide a tool for identifying nonsense alleles that are leaky. Our findings have important implications for assigning null mutant phenotypes and for selecting appropriate alleles to use in suppressor screens.


The impact of ploidy on adaptation is a central issue in evolutionary biology. While many eukaryotic organisms exist as diploids, with two sets of gametic genomes residing in the same nucleus, most basidiomycete fungi exist as dikaryons in which the two genomes exist in separate nuclei that are physically paired and that divide in a coordinated manner during hyphal extension. To determine if haploid monokaryotic and dikaryotic mycelia adapt to novel environments under natural selection, we serially transferred replicate populations of each ploidy state on minimal medium for 18 months (~13,000 generations). Dikaryotic mycelia responded to selection with increases in growth rate, while haploid monokaryotic mycelia did not. To determine if the haploid components of the dikaryon adapt reciprocally to one another's presence over time, we recovered the intact haploid components of dikaryotic mycelia at different time points (without meiosis) and mated them with nuclei of different evolutionary histories. We found evidence for coadaptation between nuclei in one dikaryotic line, in which a dominant deleterious mutation in one nucleus was followed by a compensatory mutation in the other nucleus; the mutant nuclei that evolved together had the best overall fitness. In other lines, nuclei had equal or higher fitness when paired with nuclei of other histories, indicating a heterozygote advantage. To determine if genetic exchange occurs between the two nuclei of a dikaryon, we developed a 24-locus genotyping system based on single nucleotide polymorphisms to monitor somatic exchange. We observed genetic exchange and recombination between the nuclei of several different dikaryons, resulting in genotypic variation in these mitotic cell lineages.


The involucrin gene encodes a protein of terminally differentiated keratinocytes. Its segment of repeats, which represents up to 80% of the coding region, is highly polymorphic in mouse strains.
derived from wild progenitors. Polymorphism includes nucleotide substitutions, but is most strikingly due to the recent addition of variable number of repeats at a precise location within the segment of repeats. Each mouse taxon examined showed consistent and distinctive patterns of evolution of its variable region: very rapid changes in most M. m. domesticus alleles, slow changes in M. m. musculus and complete arrest in M. spretus. We conclude that changes in the variable region are controlled by the genetic background. One of the M. m. domesticus alleles (DIK-L), which is of M. m. musculus origin, has undergone a recent repeat duplication typical of M. m. domesticus. This suggests that the genetic background controls repeat duplications through trans-acting factors. Because the repeat pattern differs in closely related murine taxa, involucrin reveals with greater sensitivity than random nucleotide substitutions the evolutionary relations of the mouse and probably of all murids.


http://www.genetics.org/cgi/content/abstract/161/4/1561

Of the seven recognized species of the Anopheles gambiae complex, A. gambiae s.s. is the most widespread and most important vector of malaria. It is becoming clear that, in parts of West Africa, this nominal species is not a single panmictic unit. We found that the internal transcribed spacer (ITS) of the X-linked rDNA has two distinct sequences with three fixed nucleotide differences; we detected no heterozygotes at these three sites, even in areas of sympathy of the two ITS types. The intergenic spacer (IGS) of this region also displays two distinct sequences that are in almost complete linkage disequilibrium with the distinct ITS alleles. We have designated these two types as S/type I and M/type II. These rDNA types correspond at least partly to the previously recognized chromosomal forms. Here we expand the geographic range of sampling to 251 individuals from 38 populations. Outside of West Africa, a single rDNA type, S/type I, corresponds to the Savanna chromosomal form. In West Africa, both types are often found in a single local sample. To understand if these findings might be due to unusual behavior of the rDNA region, we sequenced the same region for 46 A. arabiensis, a sympatric sibling species. No such distinct discontinuity was observed for this species. Autosomal inversions in one chromosome arm (2R), an insecticide resistance gene on 2L, and this single X-linked region indicate at least two genetically differentiated subpopulations of A. gambiae. Yet, rather extensive studies of other regions of the genome have failed to reveal genetic discontinuity. Evidently, incomplete genetic isolation exists within this single nominal species.


http://www.genetics.org/cgi/content/abstract/162/4/1979

The clustering of telomeres on the nuclear envelope (NE) during meiotic prophase to form the bouquet arrangement of chromosomes may facilitate homologous chromosome synopsis. The pam1 (plural abnormalities of meiosis 1) gene is the first maize gene that appears to be required for telomere clustering, and homologous synopsis is impaired in pam1. Telomere clustering on the NE is arrested or delayed at an intermediate stage in pam1. Telomeres associate with the NE during the leptotene-zygotene transition but cluster slowly if at all as meiosis proceeds. Intermediate stages in telomere clustering including miniclusters are observed in pam1 but not in wild-type meiocytes. The tight bouquet normally seen at zygotene is a rare event. In contrast, the polarization of centromeres vs. telomeres in the nucleus at the leptotene-zygotene transition is the same in mutant and wild-type cells. Defects in homologous chromosome synopsis include...
incomplete synapsis, nonhomologous synapsis, and unresolved interlocks. However, the number of RAD51 foci on chromosomes in pam1 is similar to that of wild type. We suggest that the defects in homologous synapsis and the retardation of prophase I arise from the irregularity of telomere clustering and propose that pam1 is involved in the control of bouquet formation and downstream meiotic prophase I events.


http://www.genetics.org/cgi/content/abstract/167/1/367

The human X chromosome exhibits four "evolutionary strata," interpreted to represent distinct steps in the process whereby recombination became arrested between the proto X and proto Y. To test if this is a general feature of sex chromosome evolution, we studied the Z-W sex chromosomes of birds, which have female rather than male heterogamety and evolved from a different autosome pair than the mammalian X and Y. Here we analyze all five known gametologous Z-W gene pairs to investigate the "strata" hypothesis in birds. Comparisons of the rates of synonymous substitution and intronic divergence between Z and W gametologs reveal the presence of at least two evolutionary strata spread over the p and q arms of the chicken Z chromosome. A phylogenetic analysis of intronic sequence data from different avian lineages indicates that Z-W recombination ceased in the oldest stratum (on Zq; CHD1Z, HINTZ, and SPINZ) 102-170 million years ago (MYA), before the split of the Neoeaves and Eoaves. However, recombination continued in the second stratum (on Zp; UBAP2Z and ATP5A1Z) until after the divergence of extant avian orders, with Z and W diverging 58-85 MYA. Our data suggest that progressive and stepwise cessation of recombination is a general feature behind sex chromosome evolution.


http://www.genetics.org/cgi/content/abstract/161/1/231

The neural selector gene cut, a homeobox transcription factor, is required for the specification of the correct identity of external (bristle-type) sensory organs in Drosophila. Targets of cut function, however, have not been described. Here, we study bereft (bft) mutants, which exhibit loss or malformation of a majority of the interommatidial bristles of the eye and cause defects in other external sensory organs. These mutants were generated by excising a P element located at chromosomal location 33AB, the enhancer trap line E8-2-46, indicating that a gene near the insertion site is responsible for this phenotype. Similar to the transcripts of the gene nearest to the insertion, reporter gene expression of E8-2-46 coincides with Cut in the support cells of external sensory organs, which secrete the bristle shaft and socket. Although bft transcripts do not obviously code for a protein product, its expression is abolished in bft deletion mutants, and the integrity of the bft locus is required for (interommatidial) bristle morphogenesis. This suggests that disruption of the bft gene is the cause of the observed bristle phenotype. We also sought to determine what factors regulate the expression of bft and the enhancer trap line. The correct specification of individual external sensory organ cells involves not only cut, but also the lineage genes numb and tramtrack. We demonstrate that mutations of these three genes affect the expression levels at the bft locus. Furthermore, cut overexpression is sufficient to induce ectopic bft expression in the PNS and in nonneuronal epidermis. On the basis of these results, we propose that bft acts downstream of cut and tramtrack to implement correct bristle morphogenesis.

http://www.genetics.org/cgi/content/abstract/genetics.104.039735v1

The Wingless(Wg)/Wnt signal transduction pathway directs a variety of cell fate decisions in developing animal embryos. Despite the identification of many Wg pathway components to date, it is still not clear how these elements work together to generate cellular identities. In the ventral epidermis of Drosophila embryos, Wg specifies cells to secrete a characteristic pattern of denticles and naked cuticle that decorate the larval cuticle at the end of embryonic development. We have used the Drosophila ventral epidermis as our assay system in a series of genetic screens to identify new components involved in Wg signaling. Two mutant lines that modify wg-mediated epidermal patterning represent the first loss of function mutations in the RacGap50C gene. These mutations on their own cause increased stabilization of Armadillo and cuticle pattern disruptions that include replacement of ventral denticles with naked cuticle, which suggest that the mutant embryos suffer from ectopic Wg pathway activation. In addition, RacGap50C mutations interact genetically with naked cuticle and Axin, known negative regulators of the Wg pathway. These phenotypes suggest that the RacGap50C gene product participates in the negative regulation of Wg pathway activity.


http://www.genetics.org/cgi/content/abstract/161/4/1497

An F1 mapping population of the septoria tritici blotch pathogen of wheat, Mycosphaerella graminicola, was generated by crossing the two Dutch field isolates IPO323 and IPO94269. AFLP and RAPD marker data sets were combined to produce a high-density genetic linkage map. The final map contained 223 AFLP and 57 RAPD markers, plus the biological traits mating type and avirulence, in 23 linkage groups spanning 1216 cM. Many AFLPs and some RAPD markers were clustered. When markers were reduced to 1 per cluster, 229 unique positions were mapped, with an average distance of 5.3 cM between markers. Because M. graminicola probably has 17 or 18 chromosomes, at least 5 of the 23 linkage groups probably will need to be combined with others once additional markers are added to the map. This was confirmed by pulsed-field gel analysis; probes derived from 2 of the smallest linkage groups hybridized to two of the largest chromosome-sized bands, revealing a discrepancy between physical and genetic distance. The utility of the map was demonstrated by identifying molecular markers tightly linked to two genes of biological interest, mating type and avirulence. Bulked segregant analysis was used to identify additional molecular markers closely linked to these traits. This is the first genetic linkage map for any species in the genus Mycosphaerella or the family Mycosphaerellaceae.


http://www.genetics.org/cgi/content/abstract/164/2/685

The Louisiana iris species Iris brevicaulis and I. fulva are morphologically and karyotypically
distinct yet frequently hybridize in nature. A group of high-copy-number TY3/gypsy-like retrotransposons was characterized from these species and used to develop molecular markers that take advantage of the abundance and distribution of these elements in the large iris genome. The copy number of these IRRE elements (for iris retroelement), is \( \sim 1 \times 10^5 \), accounting for \( \sim 6-10\% \) of the \( \sim 10,000\)-Mb haploid Louisiana iris genome. IRRE elements are transcriptionally active in I. brevicaulis and I. fulva and their F1 and backcross hybrids. The LTRs of the elements are more variable than the coding domains and can be used to define several distinct IRRE subfamilies. Transposon display or S-SAP markers specific to two of these subfamilies have been developed and are highly polymorphic among wild-collected individuals of each species. As IRRE elements are present in each of 11 iris species tested, the marker system has the potential to provide valuable comparative data on the dynamics of retrotransposition in large plant genomes.


http://www.genetics.org/cgi/content/abstract/165/2/721

A new mutation has arisen in a colony of mice transgenic for human \( \{ \alpha \}- \)galactosidase. The mutation is independent of the transgenic insertion, autosomal dominant, and morphologically very similar to the classical wavy coat mutation, caracul (Ca), on chromosome 15. Therefore, we designated this locus the caracul Rinshoken (CaRin). Applying a positional cloning approach, we identified the mK6irs1/Krt2-6g gene as a strong candidate for CaRin because among five Ca alleles examined mutations always occurred in the highly conserved positions of the \( \{ \alpha \}\)-helical rod domain (1A and 2B subdomain) of this putative gene product. The most striking finding is that four independently discovered alleles, the three preexistent alleles CaJ, Ca9J, Ca10J, and our allele CaRin, all share one identical amino acid deletion (N 140 del) and the fifth, CamedJ, has an amino acid substitution (A 431 D). These findings indicate that a mutation hotspot exists in the Ca locus. Additionally, we describe a Ca mutant allele induced by ENU mutagenesis, which also possesses an amino acid substitution (L 424 W) in the mK6irs1/Krt2-6g gene. The identification of the Ca candidate gene enables us to further define the nature of the genetic pathway required for hair formation and provides an important new candidate that may be implicated in human hair and skin diseases.


http://www.genetics.org/cgi/content/abstract/168/3/1655

Cf resistance genes in tomato confer resistance to the fungal leaf pathogen Cladosporium fulvum. Both the well-characterized resistance gene Cf-9 and the related 9DC gene confer resistance to strains of C. fulvum that secrete the Avr9 protein and originate from the wild tomato species Lycopersicon pimpinellifolium. We show that 9DC and Cf-9 are allelic, and we have isolated and sequenced the complete 9DC cluster of L. pimpinellifolium LA1301. This 9DC cluster harbors five full-length Cf homologs, including orthologs of the most distal homologs of the Cf-9 cluster and three central 9DC genes. Two 9DC genes (9DC1 and 9DC2) have an identical coding sequence, whereas 9DC3 differs at its 3’ terminus. From a detailed comparison of the 9DC and Cf-9 clusters, we conclude that the Cf-9 and Hcr9-9D genes from the Cf-9 cluster are ancestral to the first 9DC gene and that the three 9DC genes were generated by subsequent intra- and intergenic unequal recombination events. Thus, the 9DC cluster has undergone substantial rearrangements in the central region, but not at the ends. Using transient transformation assays, we show that all
three 9DC genes confer Avr9 responsiveness, but that 9DC2 is likely the main determinant of Avr9 recognition in LA1301.

http://www.genetics.org/cgi/content/abstract/167/4/1611

The influence of duplicated sequences on chromosomal stability is poorly understood. To characterize chromosomal rearrangements involving duplicated sequences, we compared the organization of tandem repeats of the DUP240 gene family in 15 Saccharomyces cerevisiae strains of various origins. The DUP240 gene family consists of 10 members of unknown function in the reference strain S288C. Five DUP240 paralogs on chromosome I and two on chromosome VII are arranged as tandem repeats that are highly polymorphic in copy number and sequence. We characterized DNA sequences that are likely involved in homologous or nonhomologous recombination events and are responsible for intra- and interchromosomal rearrangements that cause the creation and disappearance of DUP240 paralogs. The tandemly repeated DUP240 genes seem to be privileged sites of gene birth and death.

http://www.genetics.org/cgi/content/abstract/166/1/99

Race-cultivar specialization during the interaction of the basidiomycete smut pathogen Ustilago hordei with its barley host was described in the 1940s. Subsequent genetic analyses revealed the presence of dominant avirulence genes in the pathogen that conform to the gene-for-gene theory. This pathosystem therefore presents an opportunity for the molecular genetic characterization of fungal genes controlling avirulence. We performed a cross between U. hordei strains to obtain 54 progeny segregating for three dominant avirulence genes on three differential barley cultivars. Bulked segregant analysis was used to identify RAPD and AFLP markers tightly linked to the avirulence gene UhAvr1. The UhAvr1 gene is located in an area containing repetitive DNA and this region is undetectable in cosmid libraries prepared from the avirulent parental strain. PCR and hybridization probes developed from the linked markers were therefore used to identify cosmid clones from the virulent (Uhavr1) parent. By walking on Uhavr1-linked cosmid clones, a nonrepetitive, nearby probe was found that recognized five overlapping BAC clones spanning 170 kb from the UhAvr1 parent. A contig of the clones in the UhAvr1 region was constructed and selected probes were used for RFLP analysis of the segregating population. This approach genetically defined an [-]80-kb region that carries the UhAvr1 gene and provided cloned sequences for subsequent genetic analysis. UhAvr1 represents the first avirulence gene cloned from a basidiomycete plant pathogen.

http://www.genetics.org/cgi/content/abstract/165/1/47

The double-strand break repair (DSBR) model of recombination predicts that heteroduplexes will
be formed in regions that flank the double-strand break (DSB) site and that the resulting intermediate is resolved to generate either crossovers or noncrossovers for flanking markers. Previous studies in Saccharomyces cerevisiae, however, failed to detect heteroduplexes on both sides of the DSB site. Recent physical studies suggest that some recombination events involve heteroduplex formation by a mechanism, synthesis-dependent strand annealing (SDSA), that is inherently asymmetric with respect to the DSB site and that leads exclusively to noncrossovers of flanking markers. Below, we demonstrate that many of the recombination events initiated at the HIS4 recombination hotspot are consistent with a variant of the DSBR model in which the extent of heteroduplex on one side of the DSB site is much greater than that on the other. Events that include only one flanking marker in the heteroduplex (unidirectional events) are usually resolved as noncrossovers, whereas events that include both flanking markers (bidirectional events) are usually resolved as crossovers. The unidirectional events may represent SDSA, consistent with the conclusions of others, although other possibilities are not excluded. We also show that the level of recombination reflects the integration of events initiated at several different DSB sites, and we identify a subset of gene conversion events that may involve break-induced replication (BIR) or repair of a double-stranded DNA gap.

http://www.genetics.org/cgi/content/abstract/161/1/143

Immature spermatids from Caenorhabditis elegans are stimulated by an external activation signal to reorganize their membranes and cytoskeleton to form crawling spermatozoa. This rapid maturation, termed spermiogenesis, occurs without any new gene expression. To better understand this signal transduction pathway, we isolated suppressors of a mutation in the spe-27 gene, which is part of the pathway. The suppressors bypass the requirement for spe-27, as well as three other genes that act in this pathway, spe-8, spe-12, and spe-29. Eighteen of the suppressor mutations are new alleles of spe-6, a previously identified gene required for an early stage of spermatogenesis. The original spe-6 mutations are loss-of-function alleles that prevent major sperm protein (MSP) assembly in the fibrous bodies of spermatocytes and arrest development in meiosis. We have isolated the spe-6 gene and find that it encodes a predicted protein-serine/threonine kinase in the casein kinase 1 family. The suppressor mutations appear to be reduction-of-function alleles. We propose a model whereby SPE-6, in addition to its early role in spermatocyte development, inhibits spermiogenesis until the activation signal is received. The activation signal is transduced through SPE-8, SPE-12, SPE-27, and SPE-29 to relieve SPE-6 repression, thus triggering the formation of crawling spermatozoa.

http://www.genetics.org/cgi/content/abstract/166/2/807

Genomic sequences provide powerful new tools in genetic analysis, making it possible to combine classical genetics with genomics to characterize the genes in a particular chromosome region. These approaches have been applied successfully to the euchromatin, but analysis of the heterochromatin has lagged somewhat behind. We describe a combined genetic and bioinformatic approach to the base of the right arm of the Drosophila melanogaster second chromosome, at the boundary between pericentric heterochromatin and euchromatin. We used resources provided by the genome project to derive a physical map of the region, examine gene density, and estimate the number of potential genes. We also carried out a large-scale genetic
screen for lethal mutations in the region. We identified new alleles of the known essential genes and also identified mutations in 21 novel loci. Fourteen complementation groups map proximal to the assembled sequence. We used PCR to map the endpoints of several deficiencies and used the same set of deficiencies to order the essential genes, correlating the genetic and physical map. This allowed us to assign two of the complementation groups to particular "computed/curated genes" (CGs), one of which is Nipped-A, which our evidence suggests encodes Drosophila Tra1/TRRAP.


http://www.genetics.org/cgi/content/abstract/169/1/197

We studied microsatellite frequency and distribution in 21.76-Mb random genomic sequences, 0.67-Mb BAC sequences from the Z chromosome, and 6.3-Mb EST sequences of Bombyx mori. We mined microsatellites of $\geq 15$ bases of mononucleotide repeats and $\geq 5$ repeat units of other classes of repeats. We estimated that microsatellites account for 0.31% of the genome of B. mori. Microsatellite tracts of A, AT, and ATT were the most abundant whereas their number drastically decreased as the length of the repeat motif increased. In general, tri- and hexanucleotide repeats were overrepresented in the transcribed sequences except TAA, GTA, and TGA, which were in excess in genomic sequences. The Z chromosome sequences contained shorter repeat types than the rest of the chromosomes in addition to a higher abundance of AT-rich repeats. Our results showed that base composition of the flanking sequence has an influence on the origin and evolution of microsatellites. Transitions/transversions were high in microsatellites of ESTs, whereas the genomic sequence had an equal number of substitutions and indels. The average heterozygositis value for 23 polymorphic microsatellite loci surveyed in 13 diverse silkworm strains having 2-14 alleles was 0.54. Only 36 (18.2%) of 198 microsatellite loci were polymorphic between the two divergent silkworm populations and 10 (5%) loci revealed null alleles. The microsatellite map generated using these polymorphic markers resulted in 8 linkage groups. B. mori microsatellite loci were the most conserved in its immediate ancestor, B. mandarina, followed by the wild saturniid silkworm, Antheraea assama.


http://www.genetics.org/cgi/content/abstract/165/2/799

An RFLP genomic subtraction was used to isolate male-specific sequences in the species Silene latifolia. One isolated fragment, SLP2, shares similarity to a portion of the Activator (Ac) transposase from Zea mays and to related proteins from other plant species. Southern blot analysis of male and female S. latifolia genomic DNA shows that SLP2 belongs to a low-copy-number repeat family with two Y-linked copies. Screening of a S. latifolia male genomic library using SLP2 as a probe led to the isolation of five clones, which were partially sequenced. One clone contains two large open reading frames that can be joined into a sequence encoding a putative protein of 682 amino acids by removing a short intron. Database searches and phylogenetic analysis show that this protein belongs to the hAT superfamily of transposases, closest to Tag2 (Arabidopsis thaliana), and contains all of the defined domains critical for the activity of these transposases. PCR with genomic and cDNA templates from S. latifolia male, female, and hermaphrodite individuals revealed that one of the Y-linked copies is transcriptionally active and alternatively spliced. This is the first report of a transcriptionally active transposable element (TE) family in S. latifolia and the first DNA transposon residing on a plant Y chromosome.
The potential activity and regulation of this TE family and its use for Y chromosome gene discovery is discussed.


http://www.genetics.org/cgi/content/abstract/166/1/307

The gray, short-tailed opossum, Monodelphis domestica, is the most extensively used, laboratory-bred marsupial resource for basic biologic and biomedical research worldwide. To enhance the research utility of this species, we are building a linkage map, using both anonymous markers and functional gene loci, that will enable the localization of quantitative trait loci (QTL) and provide comparative information regarding the evolution of mammalian and other vertebrate genomes. The current map is composed of 83 loci distributed among eight autosomal linkage groups and the X chromosome. The autosomal linkage groups appear to encompass a very large portion of the genome, yet span a sex-average distance of only 633.0 cM, making this the most compact linkage map known among vertebrates. Most surprising, the male map is much larger than the female map (884.6 cM vs. 443.1 cM), a pattern contrary to that in eutherian mammals and other vertebrates. The finding of genome-wide reduction in female recombination in M. domestica, coupled with recombination data from two other, distantly related marsupial species, suggests that reduced female recombination might be a widespread metatherian attribute. We discuss possible explanations for reduced female recombination in marsupials as a consequence of the metatherian characteristic of determinate paternal X chromosome inactivation.


http://www.genetics.org/cgi/content/abstract/161/2/773

High levels of inheritable resistance to phosphate in Rhyzopertha dominica have recently been detected in Australia and in an effort to isolate the genes responsible for resistance we have used random amplified DNA fingerprinting (RAF) to produce a genetic linkage map of R. dominica. The map consists of 94 dominant DNA markers with an average distance between markers of 4.6 cM and defines nine linkage groups with a total recombination distance of 390.1 cM. We have identified two loci that are responsible for high-level resistance. One provides \(~50x\) resistance to phosphine while the other provides 12.5x resistance and in combination, the two genes act synergistically to provide a resistance level 250x greater than that of fully susceptible beetles. The haploid genome size has been determined to be 4.76 x 108 bp, resulting in an average physical distance of 1.2 Mbp per map unit. No recombination has been observed between either of the two resistance loci and their adjacent DNA markers in a population of 44 fully resistant F5 individuals, which indicates that the genes are likely to reside within 0.91 cM (1.1 Mbp) of the DNA markers.


http://www.genetics.org/cgi/content/abstract/169/3/1403
Loline alkaloids are produced by mutualistic fungi symbiotic with grasses, and they protect the host plants from insects. Here we identify in the fungal symbiont, Neotyphodium uncinatum, two homologous gene clusters (LOL-1 and LOL-2) associated with loline-alkaloid production. Nine genes were identified in a 25-kb region of LOL-1 and designated (in order) lolF-1, lolC-1, lolD-1, lolO-1, lolA-1, lolU-1, lolP-1, lolT-1, and lolE-1. LOL-2 contained the homologs lolC-2 through lolE-2 in the same order and orientation. Also identified was lolF-2, but its possible linkage with either cluster was undetermined. Most lol genes were regulated in N. uncinatum and N. coenophilium, and all were expressed concomitantly with loline-alkaloid biosynthesis. A lolC-2 RNA-interference (RNAi) construct was introduced into N. uncinatum, and in two independent transformants, RNAi significantly decreased lolC expression (P < 0.01) and loline-alkaloid accumulation in culture (P < 0.001) compared to vector-only controls, indicating involvement of lolC in biosynthesis of lolines. The predicted LolU protein has a DNA-binding site signature, and the relationships of other lol-gene products indicate that the pathway has evolved from various different primary and secondary biosynthesis pathways.


http://www.genetics.org/cgi/content/abstract/164/1/259

The rate of mutation for nucleotide substitution is generally higher among males than among females, likely owing to the larger number of DNA replications in spermatogenesis than in oogenesis. For insertion and deletion (indel) mutations, data from a few human genetic disease loci indicate that the two sexes may mutate at similar rates, possibly because such mutations arise in connection with meiotic crossing over. To address origin- and sex-specific rates of indel mutation we have conducted the first large-scale molecular evolutionary analysis of indels in noncoding DNA sequences from sex chromosomes. The rates are similar on the X and Y chromosomes of primates but about twice as high on the avian Z chromosome as on the W chromosome. The fact that indels are not uncommon on the nonrecombining Y and W chromosomes excludes meiotic crossing over as the main cause of indel mutation. On the other hand, the similar rates on X and Y indicate that the number of DNA replications (higher for Y than for X) is also not the main factor. Our observations are therefore consistent with a role of both DNA replication and recombination in the generation of short insertion and deletion mutations. A significant excess of deletion compared to insertion events is observed on the avian W chromosome, consistent with gradual DNA loss on a nonrecombining chromosome.


http://www.genetics.org/cgi/content/abstract/167/1/377

Understanding the population genetic factors that shape genome variability is pivotal to the design and interpretation of studies using large-scale polymorphism data. We analyzed patterns of polymorphism and divergence at Z-linked and autosomal loci in the domestic chicken (Gallus gallus) to study the influence of mutation, effective population size, selection, and demography on levels of genetic diversity. A total of 14 autosomal introns (8316 bp) and 13 Z-linked introns (6856 bp) were sequenced in 50 chicken chromosomes from 10 highly divergent breeds. Genetic variation was significantly lower at Z-linked than at autosomal loci, with one segregating site every 39 bp at autosomal loci ($\theta_W = 5.8 \pm 2.0$) and one every 156 bp on the Z chromosome ($\theta_W = 1.4 \pm 0.4$). This difference may in part be due to a low male effective population size arising from skewed reproductive success among males, evident both in
the wild ancestor—the red jungle fowl—and in poultry breeding. However, this effect cannot
entirely explain the observed three- to fourfold reduction in Z chromosome diversity. Selection, in
particular selective sweeps, may therefore have had an impact on reducing variation on the Z
chromosome, a hypothesis supported by the observation of heterogeneity in diversity levels
among loci on the Z chromosome and the lower recombination rate on Z than on autosomes.
Selection on sex-linked genes may be particularly important in organisms with female
heterogamety since the heritability of sex-linked sexually antagonistic alleles advantageous to
males is improved when fathers pass a Z chromosome to their sons.


http://www.genetics.org/cgi/content/abstract/168/1/215

Colias eurytheme and C. philodice are sister species with broad sympatry in North America. They
hybridize frequently and likely share a significant portion of their genomes through introgression.
Both taxa have been ecologically well characterized and exploited to address a broad spectrum
of evolutionary issues. Using AFLP markers, we constructed the first linkage map of Colias
butterflies. The map is composed of 452 markers spanning 2541.7 cM distributed over 51 linkage
groups (40 major groups and 11 small groups with 2-4 markers). Statistical tests indicate that
these AFLP markers tend to cluster over the map, with the coefficient of variation of interval sizes
being 1.236 (95% C.I. is 1.234-1.240). This nonrandom marker distribution can account for the
nonequivalence between the number of linkage groups and the actual haploid chromosome
number (N = 31). This study presents the initial step for further marker-assisted research on
Colias butterflies, including QTL and introgression analyses. Further investigation of the genomes
will help us understand better the roles of introgression and natural selection in the evolution of
hybridizing species and devise more appropriate strategies to control these pests.

Wieczorek, D. J., L. Didion, et al. (2002). "Alterations of the Portal Protein, gpB, of Bacteriophage
{lambda} Suppress Mutations in cosQ, the Site Required for Termination of DNA Packaging." Genetics 161(1): 21-31.

http://www.genetics.org/cgi/content/abstract/161/1/21

The cosQ site of bacteriophage {lambda} is required for DNA packaging termination. Previous
studies have shown that cosQ mutations can be suppressed in three ways: by a local suppressor
within cosQ, an increase in the length of the {lambda} chromosome, and missense mutations
affecting the prohead's portal protein, gpB. In the present work, revertants of a set of lethal cosQ
mutants were screened for suppressors. Seven new cosQ suppressors affected gene B, which
encodes the portal protein of the prohead. All seven were allele-nonspecific suppressors of cosQ
mutations. Experiments with several phages having two cosQ suppressors showed that the
suppression effects were additive. Furthermore, these double suppressors had minimal effects on
the growth of cosQ+ phages. These trans-acting suppressors affecting the portal protein are
proposed to allow the mutant cosQ site to be more efficiently recognized, due to the slowing of
the rate of translocation.

Genome Res. (26)

http://www.genome.org/cgi/content/abstract/14/7/1232

LTR-containing retrotransposons reverse transcribe their RNA genomes, and the resulting cDNAs are integrated into the genome by the element-encoded integrase protein. The yeast LTR retrotransposon Ty1 preferentially integrates into a target window upstream of tDNAs (tRNA genes) in the yeast genome. We investigated the nature of these insertions and the target window on a genomic scale by analyzing several hundred de novo insertions upstream of tDNAs in two different multicopy gene families. The pattern of insertion upstream of tDNAs was nonrandom and periodic, with peaks separated by ~80 bp. Insertions were not distributed equally throughout the genome, as certain tDNAs within a given family received higher frequencies of upstream Ty1 insertions than others. We showed that the presence and relative position of additional tDNAs and LTRs surrounding the target tDNA dramatically influenced the frequency of insertion events upstream of that target.


http://www.genome.org/cgi/content/abstract/15/2/214

Inversions breaking the 1041 bp int1h-1 or the 9.5-kb int22h-1 sequence of the F8 gene cause hemophilia A in 1/30,000 males. These inversions are due to homologous recombination between the above sequences and their inverted copies on the same DNA molecule, respectively, int1h-2 and int22h-2 or int22h-3. We find that (1) int1h and int22h duplicated more than 25 million years ago; (2) the identity of the copies (>99%) of these sequences in humans and other primates is due to gene conversion; (3) gene conversion is most frequent in the internal regions of int22h; (4) breakpoints of int22h-related inversions also tend to involve the internal regions of int22h; (5) sequence variations in a sample of human X chromosomes defined eight haplotypes of int22h-1 and 27 of int22h-2 plus int22h-3; (6) the latter two sequences, which lie, respectively, 500 and 600 kb telomeric to int1h-1 are five-fold more identical when in cis than when in trans, thus suggesting that gene conversion may be predominantly intrachromosomal; (7) int1h, int22h, and flanking sequences evolved at a rate of about 0.1% substitutions per million years during the divergence between humans and other primates, except for int1h during the human-chimpanzee divergence, when its rate of evolution was significantly lower. This is reminiscent of the slower evolution of palindrome arms in the male specific regions of the Y chromosome and we propose, as an explanation, that intrachromosomal gene conversion and cosegregation of the duplicated regions favors retention of the ancestral sequence and thus reduces the evolution rate.


http://www.genome.org/cgi/content/abstract/14/2/287

Genomic copy number alterations are a feature of many human diseases including cancer. We have evaluated the effectiveness of an oligonucleotide array, originally designed to detect single-nucleotide polymorphisms, to assess DNA copy number. We first showed that fluorescent signal from the oligonucleotide array varies in proportion to both decreases and increases in copy
number. Subsequently we applied the system to a series of 20 cancer cell lines. All of the putative homozygous deletions (10) and high-level amplifications (12; putative copy number >4) tested were confirmed by PCR (either qPCR or normal PCR) analysis. Low-level copy number changes for two of the lines under analysis were compared with BAC array CGH; 77% (n = 44) of the autosomal chromosomes used in the comparison showed consistent patterns of LOH (loss of heterozygosity) and low-level amplification. Of the remaining 10 comparisons that were discordant, eight were caused by low SNP densities and failed in both lines. The studies demonstrate that combining the genotype and copy number analyses gives greater insight into the underlying genetic alterations in cancer cells with identification of complex events including loss and reduplication of loci.


http://www.genome.org/cgi/content/abstract/13/5/925

We report the validation of a new assay for typing single nucleotide polymorphisms (SNPs) that takes advantage of the 3’-to-5’ exonuclease proofreading activity of many DNA polymerases. The assay uses one or more primers labeled on the 3’ nucleotide base, and can be implemented in a variety of formats including a one-step PCR reaction that allows SNP typing directly from genomic DNA samples. The detection of genotypes can be accomplished by means of fluorescence detection on assays that have been purified to remove excess primer, or by means of fluorescence polarization without any additional cleanup. We also demonstrate that the Exo-Proofreading SNP assay can be used on pooled samples to obtain allele frequency data.


http://www.genome.org/cgi/content/abstract/12/3/414

Nuclear microsatellite loci (2- to 5-bp tandem repeats) would seem to be ideal markers for population genetic monitoring because of their abundant polymorphism, wide dispersal in vertebrate genomes, near selective neutrality, and ease of assessment; however, questions about their mode of generation, mutation rates and ascertainment bias have limited interpretation considerably. We have assessed the patterns of genomic diversity for ninety feline microsatellite loci among previously characterized populations of cheetahs, lions and pumas in recapitulating demographic history. The results imply that the microsatellite diversity measures (heterozygosity, allele reconstitution and microsatellite allele variance) offer proportionate indicators, albeit with large variance, of historic population bottlenecks and founder effects. The observed rate of reconstruction of new alleles plus the growth in the breadth of microsatellite allele size (variance) was used here to develop genomic estimates of time intervals following historic founder events in cheetahs (12,000 yr ago), in North American pumas (10,000-17,000 yr ago), and in Asiatic lions of the Gir Forest (1000-4000 yr ago).[Supplemental material available online at http://rex.nci.nih.gov/lgd/front_page.htm and at http://www.genome.org.]


http://www.genome.org/cgi/content/abstract/12/9/1428
A DNA mutation detection protocol able to identify and characterize a previously unknown change in a given sequence in a rapid, efficient, sensitive, and inexpensive manner is required to take advantage of the resources now available to researchers through the genome sequencing projects. We have developed a method based on base-specific cleavage of polymerase chain reaction (PCR) products and then separation of the fragments by matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), which can meet these criteria. Differences are seen as the presence, absence, or mass change of peaks corresponding to fragments affected by the base difference. This technique is shown through the detection of a polymorphism in the 3’ untranslated region of IL12p40 from a double-stranded PCR product, and the detection of a single nucleotide polymorphism between two mouse strains. The sensitivity of the technique can be increased with the use of postsource decay, which enables differentiation of two fragments of identical mass but different sequence. The level of specificity and the rapid sample analysis time lend this technique to the mass screening of individuals for sequence changes and, in combination with MS sequencing methods, could be used to facilitate rapid resequencing of DNA.


http://www.genome.org/cgi/content/abstract/12/11/1651

Human chromosome 2 was formed by the head-to-head fusion of two ancestral chromosomes that remained separate in other primates. Sequences that once resided near the ends of the ancestral chromosomes are now interstitially located in 2q13-2q14.1. Portions of these sequences had duplicated to other locations prior to the fusion. Here we present analyses of the genomic structure and evolutionary history of >600 kb surrounding the fusion site and closely related sequences on other human chromosomes. Sequence blocks that closely flank the inverted arrays of degenerate telomere repeats marking the fusion site are duplicated at many, primarily subtelomeric, locations. In addition, large portions of a 168-kb centromere-proximal block are duplicated at 9pter, 9p11.2, and 9q13, with 98%-99% average sequence identity. A 67-kb block on the distal side of the fusion site is highly homologous to sequences at 22qter. A third ~100-kb segment is 96% identical to a region in 2q11.2. By integrating data on the extent and similarity of these paralogous blocks, including the presence of phylogenetically informative repetitive elements, with observations of their chromosomal distribution in nonhuman primates, we infer the order of the duplications that led to their current arrangement. Several of these duplicated blocks may be associated with breakpoints of inversions that occurred during primate evolution and of recurrent chromosome rearrangements in humans.[Supplemental material is available online at http://www.genome.org. The following individuals kindly provided reagents, samples, or unpublished information as indicated in the paper: T. Newman, C. Harris, and J. Young.]


http://www.genome.org/cgi/content/abstract/12/11/1663

Various portions of the region surrounding the site where two ancestral chromosomes fused to form human chromosome 2 are duplicated elsewhere in the human genome, primarily in subtelomeric and pericentromeric locations. At least 24 potentially functional genes and 16 pseudogenes reside in the 614-kb of sequence surrounding the fusion site and paralogous segments on other chromosomes. By comparing the sequences of genomic copies and
transcripts, we show that at least 18 of the genes in these paralogous regions are transcriptionally
active. Among these genes are new members of the cobalamin synthetase W domain (CBWD)
and forkhead domain FOXD4 gene families. Copies of RPL23A and SNRPA1 on chromosome 2
are retrotransposed-processed pseudogenes that were included in segmental duplications; we
find 53 RPL23A pseudogenes in the human genome and map the functional copy of SNRPA1 to
15qter. The draft sequence of the human genome also provides new information on the location
and intron-exon structure of functional copies of other 2q-fusion genes (PGM5, retina-specific
F379, helicase CHLR1, and acrosin). This study illustrates that the duplication and rearrangement
of subtelomeric and pericentromeric regions have functional relevance to human biology; these
processes can change gene dosage and/or generate genes with new functions.[Supplemental
material is available online at http://www.genome.org. Sequence data reported in this paper have
been deposited in GenBank and assigned the following accession nos.: AF452722, AF452723,
and AF452724.]


http://www.genome.org/cgi/content/abstract/12/9/1357

Olfaction is of considerable importance to many insects in behaviors critical for survival and
reproduction, including location of food sources, selection of mates, recognition of colony con-
specifics, and determination of oviposition sites. An ubiquitous, but poorly understood, component
of the insect's olfactory system is a group of odorant-binding proteins (OBPs) that are present at
high concentrations in the aqueous lymph surrounding the dendrites of olfactory receptor
neurons. OBPs are believed to shuttle odorants from the environment to the underlying odorant
receptors, for which they could potentially serve as odorant presenters. Here we show that the
Drosophila genome carries 51 potential OBP genes, a number comparable to that of its odorant-
receptor genes. We find that the majority (73%) of these OBP-like genes occur in clusters of as
many as nine genes, in contrast to what has been observed for the Drosophila odorant-receptor
genes. Two of the presumptive OBP gene clusters each carries an odorant-receptor gene. We
also report an intriguing subfamily of 12 putative OBPs that share a unique C-terminal structure
with three conserved cysteines and a conserved proline. Members of this subfamily have not
previously been described for any insect. We have performed phylogenetic analyses of the OBP-
related proteins in Drosophila as well as other insects, and we discuss the duplication and
divergence of the genes for this large family.[The sequence data from this study have been
submitted to FlyBase. Annotations for these sequences are available as supplementary material
at http://www.genome.org.]

Analysis." Genome Res. 12(9): 1401-1407.

http://www.genome.org/cgi/content/abstract/12/9/1401

Fluorescence resonance energy transfer (FRET) is a powerful tool for detecting spatial
relationships between macromolecules, one use of which is the tracking of DNA hybridization
status. The process involves measuring changes in fluorescence as FRET donor and acceptor
moieties are brought closer together or moved farther apart as a result of DNA
hybridization/denaturation. In the present study, we introduce a new version of FRET, which we
term induced FRET (iFRET), that is ideally suited for melting curve analysis. The innovation
entails using a double-strand, DNA-specific intercalating dye (e.g., SYBR Green I) as the FRET
donor, with a conventional FRET acceptor affixed to one of the DNA molecules. The SNP
genotyping technique dynamic allele specific hybridization (DASH) was used as a platform to
compare iFRET to two alternative fluorescence strategies, namely, the use of the intercalating dye alone and the use of a standard FRET pair (fluorescein as donor, 6-rhodamine as acceptor). The iFRET configuration combines the advantages of intercalating dyes, such as high signal strengths and low cost, with maintaining the specificity and multiplex potential afforded by traditional FRET detection systems. Consequently, iFRET represents a fresh and attractive schema for monitoring interactions between DNA molecules.


http://www.genome.org/cgi/content/abstract/13/5/916

Genotyping technologies need to be continually improved in terms of their flexibility, cost-efficiency, and throughput, to push forward genome variation analysis. To this end, we have leveraged the inherent simplicity of dynamic allele-specific hybridization (DASH) and coupled it to recent innovations of centrifugal arrays and iFRET. We have thereby created a new genotyping platform we term DASH-2, which we demonstrate and evaluate in this report. The system is highly flexible in many ways (any plate format, PCR multiplexing, serial and parallel array processing, spectral-multiplexing of hybridization probes), thus supporting a wide range of application scales and objectives. Precision is demonstrated to be in the range 99.8-100%, and assay costs are 0.05 USD or less per genotype assignment. DASH-2 thus provides a powerful new alternative for genotyping practice, which can be used without the need for expensive robotics support.


http://www.genome.org/cgi/content/abstract/12/6/985

Messenger RNAs that have the stability determinants, adenylate uridylate-rich elements (AREs), in their 3' untranslated region (UTR) code for key products that regulate early and transient biological responses. We used a computational laboratory approach for amplification of large, including full-length, protein-coding regions for ARE genes. Statistical analysis of the initiation regions in the 5' UTR of ARE-mRNAs was performed. Accordingly, several 5' primers and a single universal 3' primer that targeted the initiation consensuses and ARE regions, respectively, were designed. Using optimized conditions, the primers were able to enrich and amplify large protein-coding regions for the ARE gene family. The selective amplification of ARE cDNAs was verified using specific polymerase chain reactions (PCRs) to known ARE mRNA molecules and monitoring the abundance of the non-ARE [beta]-actin signal. A mini-library from the amplified ARE products was constructed for further confirmation of ARE selection. Distinct ARE amplified cDNA pools were selectively generated by distinct 5' primers. The biological utility of the method was shown with differential display. The up-regulation of several ARE-mRNAs, including the full-length coding region of the small inducible cytokine A4 (SCYA4) gene, was shown in endotoxin-stimulated monocytic cells. The integrated computational and laboratory approach should lead to enhanced capability for discovery and expression analysis of early and transient response genes.


http://www.genome.org/cgi/content/abstract/13/8/1966

As a step toward the goal of adding the cattle genome to those available for multispecies comparative genome analysis, 40,224 cattle BAC clones were end-sequenced, yielding 60,547 sequences (BAC end sequences, BESs) after trimming with an average read length of 515 bp. Cattle BACs were anchored to the human and mouse genome sequences by BLASTN search, revealing 29.4% and 10.1% significant hits (E < e-5), respectively. More than 60% of all cattle BES hits in both the human and mouse genomes are located within known genes. In order to confirm in silico predictions of orthology and their relative position on cattle chromosomes, 84 cattle BESs with similarity to sequences on HSA11 were mapped using a cattle-hamster radiation hybrid (RH) panel. Resulting RH maps of BTA15 and BTA29 cover ~85% of HSA11 sequence, revealing a complex patchwork shuffling of segments not explained by a simple translocation followed by internal rearrangements. Overlay of the mouse conserved syntenies onto HSA11 revealed that segmental boundaries appear to be conserved in all three species. The BAC clone-based comparative map provides a foundation for the evolutionary analysis of mammalian karyotypes and for sequencing of the cattle genome.


http://www.genome.org/cgi/content/abstract/12/12/1885

Fish-odor syndrome or Trimethylaminuria (OMIM #602079) in humans is an inborn error of metabolism associated with a characteristic fishy body odor due to elevated levels of trimethylamine (TMA) in body fluids. It is caused by loss-of-function mutations in FMO3 encoding flavin-containing mono-oxygenase 3. A fishy off-flavor is occasionally observed in cow's milk and it has been established recently that this phenotype is due to elevated TMA levels. Here, we report that fishy off-flavor in cow's milk is caused by a nonsense mutation (R238X) in the bovine FMO3 ortholog. RT-PCR analysis indicated that the mutant transcript is present in a very low amount. The mutation was found to be surprisingly common (q = 0.155) in one breed of cattle. [The sequence data described in this paper have been submitted to GenBank with accession nos. AF488417-AF488422. The following individuals kindly provided reagents, samples, or unpublished information as indicated in the paper: K. Sandberg and I. Hansson.]


http://www.genome.org/cgi/content/abstract/14/3/414

The analysis of single nucleotide polymorphisms (SNPs) is increasingly utilized to investigate the genetic causes of complex human diseases. Here we present a high-throughput genotyping platform that uses a one-primer assay to genotype over 10,000 SNPs per individual on a single oligonucleotide array. This approach uses restriction digestion to fractionate the genome, followed by amplification of a specific fractionated subset of the genome. The resulting reduction in genome complexity enables allele-specific hybridization to the array. The selection of SNPs was primarily determined by computer-predicted lengths of restriction fragments containing the SNPs, and was further driven by strict empirical measurements of accuracy, reproducibility, and average call rate, which we estimate to be >9.5%, >99.9%, and >95%, respectively. With average heterozygosity of 0.38 and genome scan resolution of 0.31 cM, the SNP array is a viable
alternative to panels of microsatellites (STRs). As a demonstration of the utility of the genotyping platform in whole-genome scans, we have replicated and refined a linkage region on chromosome 2p for chronic mucocutaneous candidiasis and thyroid disease, previously identified using a panel of microsatellite (STR) markers.


http://www.genome.org/cgi/content/abstract/14/3/478

We report 80,388 ESTs from 23 Atlantic salmon (Salmo salar) cDNA libraries (61,819 ESTs), 6 rainbow trout (Oncorhynchus mykiss) cDNA libraries (14,544 ESTs), 2 chinook salmon (Oncorhynchus tshawytscha) cDNA libraries (1317 ESTs), 2 sockeye salmon (Oncorhynchus nerka) cDNA libraries (1243 ESTs), and 2 lake whitefish (Coregonus clupeaformis) cDNA libraries (1465 ESTs). The majority of these are 3' sequences, allowing discrimination between paralogs arising from a recent genome duplication in the salmonid lineage. Sequence assembly reveals 28,710 different S. salar, 8981 O. mykiss, 1085 O. tshawytscha, 520 O. nerka, and 1176 C. clupeaformis putative transcripts. We annotate the submitted portion of our EST database by molecular function. Higher- and lower-molecular-weight fractions of libraries are shown to contain distinct gene sets, and higher rates of gene discovery are associated with higher-molecular weight libraries. Pyloric caecum library group annotations indicate this organ may function in redox control and as a barrier against systemic uptake of xenobiotics. A microarray is described, containing 7356 salmonid elements representing 3557 different cDNAs. Analyses of cross-species hybridizations to this cDNA microarray indicate that this resource may be used for studies involving all salmonids.


http://www.genome.org/cgi/content/abstract/13/1/122

We have developed a unique comprehensive mouse radiation hybrid (RH) map of nearly 23,000 markers integrating data from three international genome centers and over 400 independent laboratories. We have cross-referenced this map to the 0.5-cM resolution recombination-based Jackson Laboratory (TJL) backcross panel map, building a complete set of RH framework chromosome maps based on a high density of known-ordered anchor markers. We have systematically typed markers to improve coverage and resolve discrepancies, and have reanalyzed data sets as needed. The cross-linking of the RH and recombination maps has resulted in a highly accurate genome-wide map with consistent marker order. We have compared these linked framework maps to the Ensembl mouse genome sequence assembly, and show that they are a useful medium resolution tool for both validating sequence assembly and elucidating chromosome biology. [Supplemental material is available online at www.genome.org.]


http://www.genome.org/cgi/content/abstract/14/2/267

http://www.genome.org/cgi/content/abstract/13/8/1944

Peptide mass-signature genotyping (PMSG) is a scanning genotyping method that identifies mutations and polymorphisms by translating the sequence of interest in more than one reading frame and measuring the masses of the resulting peptides by mass spectrometry. PMSG was applied to the RDS/peripherin gene of 16 individuals from a family exhibiting autosomal dominant macular degeneration. The method revealed an A[-&gt;T] transversion in the 5' splice site of intron 2 that is the likely cause of the disease. It also revealed four different minihaplotypes in exon 3 that represent particular combinations of SNPs at four different locations. This study demonstrates the utility of PMSG for identifying and characterizing point mutations and local minihaplotypes that are not readily analyzed by other approaches.


http://www.genome.org/cgi/content/abstract/14/11/2357

Despite recent advances in linear whole genome amplification of intact DNA/RNA, amplification of degraded nucleic acids in an unbiased fashion remains a serious challenge for genetic diagnosis. We describe a new whole genome amplification procedure, RCA-RCA (Restriction and Circularization-Aided Rolling Circle Amplification), which retains the allelic differences among degraded amplified genomes while achieving almost complete genome coverage. RCA-RCA utilizes restriction digestion and whole genome circularization to generate genomic sequences amenable to rolling circle amplification. When intact genomic DNA is used, RCA-RCA retains gene-amplification differences (twofold or higher) between complex genomes on a genome-wide scale providing highly improved concordance with unamplified material as compared with other amplification methodologies including multiple displacement amplification. Using RCA-RCA, formalin-fixed samples of modest or substantial DNA degradation were successfully amplified and screened via array-CGH or Taqman PCR that displayed retention of the principal gene amplification features of the original material. Microsatellite analysis revealed that RCA-RCA amplified genomic DNA is representative of the original material at the nucleotide level. Amplification of cDNA is successfully performed via RCA-RCA and results to unbiased gene expression analysis (R2 = 0.99). The simplicity and universal applicability of RCA-RCA make it a
powerful new tool for genome analysis with unique advantages over previous amplification technologies.


http://www.genome.org/cgi/content/abstract/12/4/555

To accelerate the molecular analysis of behavior in the honey bee (Apis mellifera), we created expressed sequence tag (EST) and cDNA microarray resources for the bee brain. Over 20,000 cDNA clones were partially sequenced from a normalized (and subsequently subtracted) library generated from adult A. mellifera brains. These sequences were processed to identify 15,311 high-quality ESTs representing 8912 putative transcripts. Putative transcripts were functionally annotated (using the Gene Ontology classification system) based on matching gene sequences in Drosophila melanogaster. The brain ESTs represent a broad range of molecular functions and biological processes, with neurobiological classifications particularly well represented. Roughly half of Drosophila genes currently implicated in synaptic transmission and/or behavior are represented in the Apis EST set. Of Apis sequences with open reading frames of at least 450 bp, 24% are highly diverged with no matches to known protein sequences. Additionally, over 100 Apis transcript sequences conserved with other organisms appear to have been lost from the Drosophila genome. DNA microarrays were fabricated with over 7000 EST cDNA clones putatively representing different transcripts. Using probe derived from single bee brain mRNA, microarrays detected gene expression for 90% of Apis cDNAs two standard deviations greater than exogenous control cDNAs.[The sequence data described in this paper have been submitted to Genbank data library under accession nos. BI502708-BI517278. The sequences are also available at http://titan.biotec.uiuc.edu/bee/honeybee_project.htm.]


http://www.genome.org/cgi/content/abstract/13/5/932

The analysis of human genetic variations such as single nucleotide polymorphisms (SNPs) has great applications in genome-wide association studies of complex genetic traits. We have developed an SNP genotyping method based on the primer extension assay with fluorescence quenching as the detection. The template-directed dye-terminator incorporation with fluorescence quenching detection (FQ-TDI) assay is based on the observation that the intensity of fluorescent dye R110- and R6G-labeled acycloterminators is universally quenched once they are incorporated onto a DNA oligonucleotide primer. By comparing the rate of fluorescence quenching of the two allelic dyes in real time, we have extended this method for allele frequency estimation of SNPs in pooled DNA samples. The kinetic FQ-TDI assay is highly accurate and reproducible both in genotyping and in allele frequency estimation. Allele frequencies estimated by the kinetic FQ-TDI assay correlated well with known allele frequencies, with an r2 value of 0.993. Applying this strategy to large-scale studies will greatly reduce the time and cost for genotyping hundreds and thousands of SNP markers between affected and control populations.

We report here a new mechanism for allelic discrimination--allele-specific Holliday Junction formation. The Holliday Junction (HJ) is a unique DNA structure that can be formed in a sequence-nonspecific manner by routine PCR. To cause the PCR-based HJ formation to occur in an allele-specific manner, the PCR primers are manipulated such that an extra mismatch next to a SNP of interest is introduced between a target and a reference amplicon and a GC-clamp is added. Based on this new mechanism, novel SNP genotyping methods were developed, including a homogeneous fluorescence polarization (FP) competition assay that requires neither labeled primers/probes nor expensive enzymes/substrates. Using this novel genotyping technology, we were able to convert >95% of SNP sequences into genotyping assays that work well under a universal set of assay conditions and achieved 100% accuracy in clinical samples.


Comparative genome analysis is a powerful tool that can facilitate the reconstruction of the evolutionary history of the genomes of modern-day species. The model plant Arabidopsis thaliana with its n = 5 genome is thought to be derived from an ancestral n = 8 genome. Pairwise comparative genome analyses of A. thaliana with polyploid and diploid Brassicaceae species have suggested that rapid genome evolution, manifested by chromosomal rearrangements and duplications, characterizes the polyploid, but not the diploid, lineages of this family. In this study, we constructed a low-density genetic linkage map of Arabidopsis lyrata ssp. lyrata (A. l. lyrata; n = 8, diploid), the closest known relative of A. thaliana (MRCA [~5 Mya], using A. thaliana-specific markers that resolve into the expected eight linkage groups. We then performed comparative Bayesian analyses using raw mapping data from this study and from a Capsella study to infer the number and nature of rearrangements that distinguish the n = 8 genomes of A. l. lyrata and Capsella from the n = 5 genome of A. thaliana. We conclude that there is strong statistical support in favor of the parsimony scenarios of 10 major chromosomal rearrangements separating these n = 8 genomes from A. thaliana. These chromosomal rearrangement events contribute to a rate of chromosomal evolution higher than previously reported in this lineage. We infer that at least seven of these events, common to both sets of data, are responsible for the change in karyotype and underlie genome reduction in A. thaliana.


The forced swim test (FST) and tail suspension test (TST) are widely used and well established screening paradigms for antidepressants. A variety of antidepressive agents are known to reduce immobility time in both FST and TST. To identify genetic determinants of immobility duration in both tests, we analyzed 560 F2 mice from an intercross between C57BL/6 (B6) and C3H/He (C3) strains. Composite interval mapping revealed five major loci (suggestive and significant linkage) affecting immobility in the FST, and four loci for the TST. The quantitative trait loci (QTL) on chromosomes 8 and 11 overlap between the two behavioral measures. Genome-wide interaction analysis, which was developed to identify locus pairs that may contribute epistatically to a phenotype, detected two pairs of chromosomal loci for the TST. The QTL on chromosome 11 and
its associated epistatic TST-QTL on chromosome X encode [gamma]-aminobutyric acid type A (GABAA) receptor subunits as candidates. Sequence and expression analyses of these genes from the two parental strains revealed a significantly lower expression of the [alpha]1 subunit gene in the frontal cortex of B6 mice compared to C3 mice. The present quantitative trait study should open up avenues for identifying novel molecular targets for antidepressants and unraveling the complex genetic mechanisms of depressive and anxiety disorders.


http://www.genome.org/cgi/content/abstract/13/2/173

Association studies of candidate genes with complex traits have generally used one or a few single nucleotide polymorphisms (SNPs), although variation in the extent of linkage disequilibrium (LD) within genes markedly influences the sensitivity and precision of association studies. The extent of LD and the underlying haplotype structure for most candidate genes are still unavailable. We sampled 193 blacks (African-Americans) and 160 whites (European-Americans) and estimated the intragenic LD and the haplotype structure in four genes of the renin-angiotensin system. We genotyped 25 SNPs, with all but one of the pairs spaced between 1 and 20 kb, thus providing resolution at small scale. The pattern of LD within a gene was very heterogeneous. Using a robust method to define haplotype blocks, blocks of limited haplotype diversity were identified at each locus; between these blocks, LD was lost owing to the history of recombination events. As anticipated, there was less LD among blacks, the number of haplotypes was substantially larger, and shorter haplotype segments were found, compared with whites. These findings have implications for candidate-gene association studies and indicate that variation between populations of European and African origin in haplotype diversity is characteristic of most genes. [The sequence data described in this paper are available in GenBank under the following accession nos: AGT, MIM 106150; Renin, MIM 179820; ACE, MIM 106180; Angiotensin receptor I, MIM 106165. Supplementary material is available online at http://www.genome.org.]

Genomics (105)


http://www.sciencedirect.com/science/article/B6WG1-47TF6BT-3/2/037449c7379a083e7c7a5dc5f7670ae

We isolated a candidate choriocarcinoma suppressor gene from a PCR-based subtracted fragmentary cDNA library between normal placental villi and the choriocarcinoma cell line CC1. This gene comprises an open reading frame of 219 nt encoding 73 amino acids and contains a homeodomain as a consensus motif. This gene, designated NECC1 (not expressed in choriocarcinoma clone 1), is located on human chromosome 4q11-q12. NECC1 expression is ubiquitous in the brain, placenta, lung, smooth muscle, uterus, bladder, kidney, and spleen. Normal placental villi expressed NECC1, but all choriocarcinoma cell lines examined and most of
the surgically removed choriocarcinoma tissue samples failed to express it. We transfected this gene into choriocarcinoma cell lines and observed remarkable alterations in cell morphology and suppression of in vivo tumorigenesis. Induction of CSH1 (chorionic somatomammotropin hormone 1) by NECC1 expression suggested differentiation of choriocarcinoma cells to syncytiotrophoblasts. Our results suggest that loss of NECC1 expression is involved in malignant conversion of placental trophoblasts.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4N/2/f7891522e50770bac39f3b422467aaad


http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-40/2/35d2728a62bf6b4c6d88648b104a314a0

A characteristic translocation t(2;13)(q35;q14) has been previously identified in the pediatric soft tissue tumor alveolar rhabdomyosarcoma. We have assembled a panel of lymphoblast, fibroblast, and somatic cell hybrid cell lines with deletions and unbalanced translocations involving chromosome 2 to develop a physical map of the distal 2q region. Twenty-two probes were localized on this physical map by Southern blot analysis of the mapping panel. The position of these probes with respect to the t(2;13) rhabdomyosarcoma breakpoint was then determined by quantitative Southern blot analysis of an alveolar rhabdomyosarcoma cell line with two copies of the derivative chromosome 13 and one copy of the derivative chromosome 2 and by analysis of somatic cell hybrid clones derived from an alveolar rhabdomyosarcoma cell line. We demonstrate that the t(2;13) breakpoint is situated within a map interval delimited by the distal deletion breakpoint in fibroblast line GM09892 and the t(X;2) breakpoint in somatic cell hybrid GM11022. Furthermore, from a comparison of our data with the linkage map of the syntenic region on mouse chromosome 1, we conclude that the t(2;13) breakpoint is most closely flanked by loci INHA and ALPI within this map interval.


http://www.sciencedirect.com/science/article/B6WG1-471W7HX-7S/2/1d767bf2581fa2b9a62502be6d8200bb

Proteins with seven transmembrane segments (7TM) define a superfamily of receptors (7TM receptors) sharing the same topology: an extracellular N-terminus, three extramembranous loops on either side of the plasma membrane, and a cytoplasmic C-terminal tail. Upon ligand binding, cytoplasmic portions of the activated receptor interact with heterotrimeric G-coupled proteins to induce various second messengers. A small group, recently recognized on the basis of homologous primary amino acid sequences, comprises receptors to hormones of the secretin/vasoactive intestinal peptide/glucagon family, parathyroid hormone and parathyroid hormone-related peptides, growth hormone-releasing factor, corticotropin-releasing factor, and calcitonin. A cDNA, extracted from a neuroectodermal cDNA library, was predicted to encode a
new 886-amino-acid protein with three distinct domains. The C-terminal third contains the seven hydrophobic segments and characteristic residues that allow the protein to be readily aligned with the various hormone receptors in the family. Six egf-like modules, at the N-terminus of the predicted mature protein, are separated from the transmembrane segments by a serine/threonine-rich domain, a feature reminiscent of mucin-like, single-span, integral membrane glycoproteins with adhesive properties. Because of its unique characteristics, this putative egf module-containing, mucin-like hormone receptor has been named EMR1. Southern analysis of a panel of somatic cell hybrids and fluorescence in situ hybridization have assigned the EMR1 gene to human chromosome 19p13.3.


An automated gridding procedure for the inoculation of yeast and bacterial clones in high-density arrays has been developed. A 96-pin inoculating tool compatible with the standard microtiter plate format and an eight-position tablet have been designed to fit the Biomek 1000 programmable robotic workstation (Beckman Instruments). The system is used to inoculate six copies of 80 x 120-mm filters representing a total of ~20,000 individual clones in approximately 3 h. High-density arrays of yeast artificial chromosome (YAC) and cosmid clones have been used for rapid large-scale hybridization screens of ordered libraries. In addition, an improved PCR library screening strategy has been developed using strips cut from the high-density arrays to prepare row and column DNA pools for PCR analysis. This strategy eliminates the final hybridization step and allows identification of a single clone by PCR in 2 days. The development of automated gridding technology will have a significant impact on the establishment of fully versatile screening of ordered library resources for genomic studies.


The ubiquitous nature of the Alu sequence throughout the human genome forms the basis of an assay we present here for analyzing the human chromosome content of human x rodent somatic cell hybrids. A human-specific Alu primer was used both to amplify sequences and to 32P label the products in a polymerase chain reaction (PCR) technique. Unlabeled inter-Alu PCR products from two series of human x rodent hybrids were used to prepare dot blots which were probed with labeled inter-Alu products prepared from between 103 and 104 hybrid cells. In the first series we demonstrate that a labeled inter-Alu probe from the hybrid DL18ts, containing a single chromosome 18, on a dot blot hybridized only with those inter-Alu products containing chromosome 18. Similar specificity for human chromosome 5 was shown when a Southern blot of the PCR products was hybridized with a probe made from the hybrid HHW 213, which contains only chromosome 5p. Using a dot blot from a second series of control hybrids, 15 of which contained single human chromosomes, hybridization of a labeled probe from the hybrid 18X4-1 was shown to react specifically with the controls that expressed chromosome 18. Application of the technique reported here allows simple and rapid characterization of the human chromosome content in human x rodent hybrids.

http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-3S/2/0b0c579a867314913cc65a76a712385f

The human PDGFA gene, encoding the A chain of platelet-derived growth factor, has been previously cloned and characterized, but two conflicting chromosomal localizations have been presented. To resolve this controversy, we have now performed nonisotopic in situ hybridization using new genomic PDGFA subclones and analyzed somatic cell hybrid DNAs for the presence of human PDGFA by polymerase chain reaction. The results confirm our previous assignment of PDGFA to chromosome 7p22. New sequence data from the PDGFA locus have been obtained and analyzed. An unusual minisatellite, which includes an evolutionarily conserved protein-coding region of exon 4, was found within IVS4. The minisatellite includes an embedded polymorphic pentanucleotide microsatellite repeat. Analysis of this polymorphism and in situ hybridization both locate PDGFA outside the monosomic region in a patient with a de novo deletion of the short arm of chromosome 7 [del (7)(p22.1-pter)].


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-50/2/58fc929e81918e36fa81d959b883fdec

Four cosmid clones containing putative pseudogenes for human aldehyde dehydrogenase (Aldose reductase) were isolated from libraries made to two individuals. These clones show different patterns on digestion with restriction endonucleases and probably represent distinct and separate loci. The DNA sequence of one of the putative pseudogenes (cosmid AR.F) was determined, and comparisons demonstrate 89.7% homology with the cDNA sequence of the functional aldose reductase gene. This pseudogene sequence contains no intronic sequences, whereas the functional aldose reductase has nine introns. In addition, the homology disappears in region 5' to the transcription start site for the cDNA, implying that regulatory elements such as the promoter are missing from this pseudogene. The pseudogene defined by cosmid AR.F has been mapped to chromosome 3 by polymerase chain reaction using amplimers specific for this pseudogene to amplify DNA from somatic cell hybrids.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4K/2/68b26433171dae6f8ca011c3934766e1

We constructed a yeast artificial chromosome (YAC) contig spanning the genes encoding Kit (Kit), the platelet-derived growth factor [alpha] receptor (Pdgfra), and fetal liver kinase 1 (Flk1), three members of a receptor tyrosine kinase gene family located in the central portion of mouse chromosome 5. The orientation of YAC clones and the extent of their overlap was determined by “probe content mapping,” that is, hybridization analysis of YAC clones using the available gene probes and YAC end sequences. For four YAC clones, which constitute a minimal set spanning 1.8 Mb, a detailed restriction map was constructed. This map, in conjunction with the previously published long-range restriction map, indicates the order, the physical distances, and the relative transcriptional orientations of the Pdgfra, Kit, and Flk1 genes. The YAC clones and corresponding YAC end probes presented here provide an important resource for the molecular analysis of a cluster of developmental mutations, namely dominant white spotting (W), patch (Ph), recessive spotting (rs), and rump-white (Rw), associated with this chromosomal region.


We have cloned human genes that are encoded in the region 17q12-q23 and expressed in breast tissue using interspecific somatic cell hybrids and subtractive hybridization. Two mouse microcell hybrids containing fragments of human chromosome 17 with a nonoverlap region at 17q12-q23 were generated by microcell transfer. Radiolabeled cDNA was synthesized from the hybrid cell containing the 17q12-q23 interval and was subtracted with an excess of RNA from the hybrid cell lacking the interval. Resulting cDNA probes enriched for sequences from 17q12-q23 were used to screen a human premenopausal breast cDNA library, and 60 cDNAs were identified. Three of these cDNAs mapped to the hybrid cell nonoverlap region. These cDNAs were expressed in mammary epithelial cell hybrids, although none appeared to be breast-specific. Sequence analysis of the cDNAs revealed that clone 93A represents a previously unidentified gene, clone 98C has homology to an expressed sequence tag from goat mammary tissue, and clone 200A is identical to the human homologue of the Drosophila melanogaster flightless-I gene. These genes map outside a 1-cM region linked to early onset familial breast cancer but may be useful genetic markers in the 17q12-q23 region.


Human SSX was first identified as the gene involved in the t(X;18) translocation in synovial sarcoma. SSX is a multigene family, with 9 complete genes on chromosome Xp11. Normally expressed almost exclusively in testis, SSX mRNA is expressed in various human tumors, defining SSX as a cancer/testis antigen. We have now cloned the mouse ortholog of SSX. Mouse SSX genes can be divided into Ssxα and Ssxβ subfamilies based on sequence homology. Ssxα has only one member, whereas 12 Ssxβ genes, Ssxβ1 to Ssxβ12, were identified by cDNA cloning from mouse testis and mouse tumors. Both Ssxα and Ssxβ are located on chromosome X and show tissue-restricted mRNA expression to testis among normal tissues. All putative human
and mouse SSX proteins share conserved KRAB and SSX-RD domains. Mouse tumors were found to express some, but not all, Ssxb genes, similar to the SSX activation in human tumors.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-9J/2/1762076d2a2eef2ed35f8f8cc4c5161

A sequence tagged site (STS)-based approach has been used to construct a 2.6-Mb contig in yeast artificial chromosomes (YACs) spanning the human dystrophin gene. Twenty-seven STSs were used to identify and overlap 34 YAC clones. A DNA fingerprint of each clone produced by direct Alu-PCR amplification of YAC colonies and the isolation of YAC insert ends by vectorette PCR were used to detect overlaps in intron 1 (280 kb) where no DNA sequence data were available, thereby achieving closure of the map. This study has evaluated methods for mapping large regions of the X chromosome and provides a valuable resource of the dystrophin gene in cloned form for detailed analysis of gene structure and function in the future.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-6R/2/bba82d0d391c0c161076a04628b37f88

Human DNA segments from discrete chromosomal regions were generated by utilizing Alu-element-based polymerase chain reaction (Alu-PCR) of an irradiation-fusion hybrid containing approximately 10 to 15 Mb of human DNA. Following cloning into a plasmid vector, a subset of the clones was used to generate sequence tagged sites (STSs) de novo. By means of a panel of hybrids containing portions of the human X chromosome, the STSs were shown to localize to two chromosomal regions, Xq24-Xq26 and Xcen-Xq13, reflecting the presence in the irradiation-fusion hybrid of two human chromosome fragments. These results demonstrate that high densities of STSs can be rapidly and efficiently generated from defined regions of the human genome using Alu-PCR.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8F5-20/2/31524960512c989a971cbde2d2a74983

A CA repeat has been found on the human X chromosome within 16 kb of the gene encoding properdin P factor (PFC) and has been shown to be a highly informative marker. Two more polymorphic CA repeats were found in a cosmid containing DXS228. The CA repeats, and other markers from proximal Xp, were mapped genetically in CEPH families and the likely order of markers was established as Xpter-(DXS7, MAO-A, DXS228)-(PFC, DXS426)-(TIMP, OATL1)-DXS255-Xcen. This places PFC in the region Xp11.3-Xp11.23, thus refining previous in situ hybridization data. Two yeast artificial chromosomes (YACs) (440 and 390 kb) contain both PFC and DXS426, and one of them (440 kb) also contains TIMP. This confirms the genetic order TIMP-(PFC, DXS426). PFC and TIMP are located on the same 100-kb Sall/Pvul fragment of the...
440-kb YAC. Given the genetic orientation of TIMP and (PFC, DXS426), this YAC can now serve as a starting point for directional walking toward disease genes located in Xp11.3-Xp11.2 such as retinitis pigmentosa (RP2) and Wiskott-Aldrich syndrome.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-37/2/3a0ce6a563f3be3029dca14780f8251

Forty-three sequences containing simple sequence repeats or microsatellites were generated from an M13 library of total genomic mouse DNA. These sequences were analyzed for size variation using the polymerase chain reaction and gel electrophoresis without the need for radiolabeling. Seventy-two percent of the sequences showed allelic size variations between different inbred strains of mouse and the wild mouse, Mus spretus; and 53% showed variation between inbred strains. Thirty-seven percent were variant between B6/J and DBA/2J, and 81% of these were resolved using minigel agarose electrophoresis alone. This approach is a useful way of generating the large number of variants that are needed to create high resolution maps of the mouse genome.


http://www.sciencedirect.com/science/article/B6WG1-4DNHS2F-R6/2/f22bf99cda9fe1ba28974f53342cede8

The creation of a comprehensive genetic map in human has been limited by the lack of highly polymorphic markers spaced evenly throughout the human genome. We have utilized yeast artificial chromosomes (YAC) containing large human DNA inserts to help identify highly polymorphic (CA)n repeats at a chosen locus. The DNA of a YAC containing the locus was subcloned in M13 vectors, and the recombinants were screened at high stringency to detect preferentially long (CA)n repeats (n > 20). These repeats, which are the most likely to be highly polymorphic, were then studied to confirm both the level of polymorphism and their precise genetic location. This strategy has permitted the identification of a new, highly polymorphic CA repeat (77% heterozygosity) at the T cell receptor [alpha] chain (TCRA) locus on chromosome 14q. It provides a powerful marker for assessing the role of this locus in the susceptibility to autoimmune and infectious diseases. This approach should permit the development of highly polymorphic markers at any targeted locus and rapidly improve the current human genetic map.


http://www.sciencedirect.com/science/article/B6WG1-4DXB903-4W2/f66a4ae413cea1efe9220786867e6993

A strategy for the isolation of DNA probes from small numbers of flow-sorted human chromosomes has been developed. A lymphoblastoid cell line carrying the 22q- derivative chromosome product of the constitutional t(11;22) translocation was used as the source of
chromosomes. Synthetic oligonucleotide primers, based on the consensus Alu sequence, were used to amplify inter-Alu sequence from 500 flow-sorted 22q- derivative chromosomes. The amplified sequences were cloned into a plasmid vector by blunt-end ligation, yielding clones with inserts in the range of 400 to 1000 bp. Approximately 70% of these clones hybridized to human DNA as single-copy probes. To identify clones derived from chromosome 11, the library was screened with a heterogeneous probe prepared by Alu-PCR amplification from the DNA of a somatic cell hybrid containing one homolog of chromosome 11. All the positive clones found were mapped to within the q23-q25 region of chromosome 11 known to be translocated onto the 22q- derivative chromosome. Further mapping studies showed that most of these probes (7/8) lay between the breakpoints for the t(4;11) translocation of acute lymphocytic leukemia and the t(11;22) of Ewing sarcoma. Thus, the use of Alu-PCR on the small derivative chromosome 22q- has provided a greatly enriched source of probes to region 11q23, a part of the genome that is currently of great interest. This approach will be particularly appropriate to small numbers of chromosomes when high specificity rather than total representation is required.


http://www.sciencedirect.com/science/article/B6WG1-4DNHPYR-C9/2/9a8e597b22efb03a5a3c638348055759

A strategy for the rapid isolation from rodent hybrids of human chromosome-specific probes by enzymatic amplification is described. Synthetic oligonucleotide primers based on the consensus Alu sequence were used to amplify inter-Alu sequence from total human genomic DNA and from a somatic cell hybrid, PNTS-1, containing one homolog of chromosome 5 as its only human complement. Direct sequence analysis of the products from human genomic DNA confirmed their inter-Alu structure and provided a novel means for the examination of the 5' end of the Alu consensus. The amplified sequences from the somatic cell hybrid DNA were cloned into a plasmid vector by blunt-end ligation, yielding clones with inserts in the range 300 to 1000 bp. More than 80% of these clones carried inserts that behaved essentially as single-copy human sequences. Hybridization of a selection of these clones to human DNA, hamster DNA, and the original hybrid DNA confirmed that they were derived from chromosome 5. Direct sequence analysis of the vector/insert boundaries in two clones confirmed that inter-Alu sequences had been cloned. This approach has significant advantages over other methods of isolating chromosome-specific probes from hybrid cells, enabling direct separation and cloning of human DNA probes that can be readily used for mapping studies.


http://www.sciencedirect.com/science/article/B6WG1-4DXK9Y3-3B/2/76b0ef9d0d47461ba755b63946fdaf8d

We have developed a rapid method of generating and simultaneously mapping interrepeat polymerase chain reaction products using DNA from interspecific backcross animals derived from mating C57BL/6J and Mus spretus mice. This method is based on the high degree of B1, B2, and L1 dispersed repeat position polymorphism found between these two species of mouse. We have mapped 13 new loci to 9 different chromosomes and have found no evidence of clustering among these loci. The advantages of this approach are that no prior knowledge of sequence is required, a single PCR reaction generates many markers which can be mapped simultaneously, and only 50 ng of each backcross DNA (a finite resource) is required. We anticipate that many more markers remain to be characterized in this valuable new source of polymorphism.

http://www.sciencedirect.com/science/article/B6WG1-48NX5NW-1/2/56e2ebf047d70f30a46bfc324cb561d4

The linkage disequilibrium (LD) pattern within the adenosine deaminase (ADA) gene was analyzed by studying 13 polymorphic loci in 137 families from two European and three African populations. Evidence for the presence of a 12-kb meiotic crossover hot spot, spanning part of the first and the second intron and flanked by regions of reduced recombination activity, was obtained. Moreover, segregation analysis of 113 informative meioses revealed two recombination events that are internal or overlap the 12-kb region, thus suggesting a recombination rate for the hot-spot region about 50-fold higher than the mean rate across the human genome. Within the hot spot, a 144-bp palindromic sequence was also identified and its possible involvement in the recombination process is discussed. The 12-kb region characterized by the low degree of LD does not include the 3.2-kb region that is deleted, as a result of recurrent unequal homologous recombination between two Alu elements, in patients affected by autosomal severe combined immunodeficiency. This observation provides the first evidence for an absence of correlation between hot spots of equal and unequal homologous recombination.


Monosomy 7 and deletions of 7q are recurring leukemia-associated cytogenetic abnormalities that correlate with adverse outcomes in children and adults. We describe a 2.52-Mb genomic DNA contig that spans a commonly deleted segment of chromosome band 7q22 identified in myeloid malignancies. This interval currently includes 14 genes, 19 predicted genes, and 5 predicted pseudogenes. We have extensively characterized the FBXL13, NAPE-PLD, and SVH genes as candidate myeloid tumor suppressors. FBXL13 encodes a novel F-box protein, SVH is a member of a gene family that contains Armadillo-like repeats, and NAPE-PLD encodes a phospholipase D-type phosphodiesterase. Analysis of a panel of leukemia specimens with monosomy 7 did not reveal mutations in these or in the candidate genes LRRC17, PRO1598, and SRPK2. This fully sequenced and annotated contig provides a resource for candidate myeloid tumor suppressor gene discovery.


http://www.sciencedirect.com/science/article/B6WG1-4DP5JHW-2D/2/3894e23d3b60dc4ed647c6f09a9b4d55

The human amylase gene cluster includes a (CA)n repeat sequence immediately upstream of the [gamma]-actin pseudogene associated with the AMY2B gene. Analysis of this (CA)n repeat by PCR amplification of genomic DNA from the 40 families of the Centre d'Etude du Polymorphisme Humain (CEPH) reference panel revealed extensive polymorphism. A total of six alleles with (CA)n lengths of 16-18 repeats were found. The average heterozygosity for this polymorphism
was 0.70. Multipoint linkage analysis showed that the amylase gene cluster is located distal to the nerve growth factor [beta]-subunit gene (NGFB) and is within 1 cM of the anonymous locus D1S10. The amylase (CA)n repeat provides a convenient marker for both the physical and the genetic maps of human chromosome 1p.


http://www.sciencedirect.com/science/article/B6WG1-471W704-2N/2/06b2df9fa3995acf1e549ae295425a

The enzymes of the 17[beta]-hydroxysteroid dehydrogenase (17[beta]-HSD) gene family are responsible for a key step in the formation and degradation of androgens and estrogens: catalyzing the interconversion of 17-ketosteroids and their active 17[beta]-hydroxysteroid counterparts. The structure of human type II 17[beta]-HSD cDNA was recently reported. This enzyme catalyzes the interconversion of [Delta]4-androstenedione and testosterone, androstanedione and dihydrotestosterone, and estrone and 17[beta]-estradiol, whereas type I 17[beta]-HSD catalyzes exclusively the interconversion of estrogens. To locate the HSD17B2 gene, the novel dinucleotide CA repeat sequence found 571 bp downstream from the end of exon 1 was genotyped into eight CEPH reference families by PCR. Two-point linkage analysis was performed between the latter polymorphism and the 2066 microsatellite markers of Genethon. The maximal pairwise lod score (Zmax = 33.3) with a maximal recombination fraction ([theta]max) of 0.008 was obtained with the marker D16S422 located on 16q24.1-q24.2. To define further the localization of the HSD17B2 gene, we constructed a high-resolution genetic map of the region flanking the polymorphic HSD17B2 gene including eight Genethon markers. The order of the HSD17B2 gene and markers is qter-D16S516 -- D16S504 -- D16S507 -- D16S505 -- D16S511 -- [HSD17B2--D16S422]--D16S520--D16S413--tel.


http://www.sciencedirect.com/science/article/B6WG1-4DNHRHP-HX/2/377e1df023d83639664c5a229467e231

Trimeric and tetrameric short tandem repeats (STRs) represent a rich source of highly polymorphic markers in the human genome that may be studied with the polymerase chain reaction (PCR). We report the analysis of a multilocus genotype survey of 97-380 chromosomes in U.S. Black, White, Mexican-American, and Asian populations at five STR loci located on chromosomes 1, 4, 11, and X. The heterozygote frequencies of the loci ranged from 0.36 to 0.91 and the number of alleles from 6 to 20 for the 20 population and locus combinations. Relative allele frequencies exhibited differences between populations and unimodal, bimodal, and complex distributions. Although deviations were noted at some locus-population test combinations, genotype data from the loci were consistent overall with Hardy-Weinberg equilibrium by three tests. Population subheterogeneity within each ethnic group was not detected by two additional tests. No mutations were detected in a total of 860 meioses for two loci studied in the CEPH kindreds and five loci studied in other families. An indirect estimate of the mutation rates gave values from 2.3 x 10-5 to 15.9 x 10-5 for the five loci. Higher mutation rates appear to be associated with greater numbers of tandem repeats in the core motif. The most frequent genotype for all five loci combined appears to have a frequency of 7.59 x 10-4. Together, these results suggest that trimeric and tetrameric STR loci are useful markers for the study of new mutations and genetic linkage analysis and for application to personal identification in the
medical and forensic sciences.


http://www.sciencedirect.com/science/article/B6WG1-4DNHS2F-NX/2/c128dcfe37105557e88b5718092f4255

We have used screening with the polymerase chain reaction and chemical mismatch detection of amplified cDNA to detect and characterize deletions and point mutations in six Hunter Syndrome patients. A high degree of mutational heterogeneity was observed. The first patient is completely deleted for the gene coding for [alpha]-iduronate sulfate sulfatase, while the second has a point mutation that creates a stop codon. The third patient shows a point mutation that creates a novel splice site that is preferentially utilized and results in partial loss of one exon in the RNA. Patients 4, 5, and 6 have point mutations resulting in single amino acid substitutions. Four of the six single-base changes observed in this study were examples of transitions of the highly mutable dinucleotide CpG to TpG. This study has demonstrated a procedure capable of detecting all types of mutation that affect the function of the IDS protein and should enable direct carrier and prenatal diagnosis for Hunter syndrome families.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-3J/2/59e6e560df74c722c4407579e7d08564

A porcine repetitive DNA sequence has been isolated from an intron of the glucose phosphate isomerase gene. The copy number of this and related sequences was estimated to be approximately 105 copies per genome. The sequence possesses all the characteristics of short interspersed elements (SINEs) described in other mammals: The repeat is 300 bp in length, has an poly(A) stretch, and contains insertion duplication sites. Homology to seven other porcine sequences, which also have the characteristics of SINEs, has been demonstrated. Primer oligonucleotides, based on conserved regions in the SINE sequences, have been synthesized. Using these primers, PCR-mediated specific amplification of porcine sequences was demonstrated from pig x mouse and pig x hamster hybrid cell lines. Cloning and sequencing of some amplified porcine sequences verify that the sites of priming are SINE sequences.


http://www.sciencedirect.com/science/article/B6WG1-471W6W8-14/2/bbf59b9c51822d6aa89b42b9c009c3f1

In our effort to identify BRCA1, 22 genes were cloned from a 1-Mb region of chromosome 17q21 defined by meiotic recombinants in families with inherited breast and/or ovarian cancer. Subsequent discovery of another meiotic recombinant narrowed the region to ~650 kb. Genes were cloned from fibroblast and ovarian cDNA libraries by direct screening with YACs and cosmids. The more than 400 cDNA clones so identified were mapped to cosmids, YACs, and P1
clones and to a chromosome 17 somatic panel informative for the BRCA1 region. Clones that mapped back to the region were hybridized to each other and consolidated into clusters reflecting 22 genes. Ten genes were known human genes, 5 were human homologs of known genes, and 7 were novel. Each gene was sequenced, compared to genes in the databases to find homologies, and analyzed for mutations in BRCA1-linked families and tumors. Eight mutations were found in tumors or families and not in controls. In the gene encoding [alpha]-N-acetylglucosaminidase, ~100 kb proximal to the 650-kb linked region, somatic nonsense, missense, and splice junction mutations occurred in 3 breast tumors, but not in these patients’ germline DNA nor in controls. In an ets-related oncogene in the linked region, a missense mutation cosegregated with breast cancer in one family and was not observed in controls. In a human homolog of a yeast pre-mRNA splicing factor, 3 different mutations cosegregated with breast cancer in 3 families and were not observed in controls. In these and the other genes in the region, 36 polymorphic variants were observed in both cases and controls.


http://www.sciencedirect.com/science/article/B6WG1-4DXB997-7X/2/0b8f0869b103b1c2cfc8f8360d1d0ad9

Dinucleotide repeats constitute so-called microsatellites of the human and other eukaryotic genomes. Microsatellite polymorphisms can be identified through the amplification of the microsatellite DNA using the polymerase chain reaction (PCR), followed by resolution of the amplified DNA fragments on a polyacrylamide sequencing gel. We performed a preliminary sequence database search to identify bovine sequences containing (CA)n, (AC)n, (GT)n, or (TG)n blocks, with n >= 6. This search yielded 10 sequences containing one or two of the specified repeat blocks and often additional dinucleotide repeat blocks. One of the microsatellite-containing regions has been sequenced twice from independent clones and the reported sequences showed variation in the number of repeats. PCR-amplified fragments of another sequence, the gene for steroid 21-hydroxylase, ranged from 186 to 216 nucleotides in 43 unrelated animals. The database search, as well as the hypervariable microsatellite in the bovine steroid 21-hydroxylase gene, indicates that dinucleotide blocks may be an abundant source of DNA polymorphism in cattle.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-44/2/8951b4e39c1f855e6b99399ab150cbb8

Several mutations have been identified in the first nucleotide binding fold (NBF) of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene. We have analyzed the DNA sequences of exons 10 and 11 in five different mammalian species, marmoset, mouse, cow, pig, and sheep; the amino acid conservation studied for nine disease mutations; and two "benign" mutations. For exon 10, 87% homology at the DNA level and 93.5% at the amino acid level were found for these species. For exon 11, the lowest homology (70%), as found in mouse and the highest in marmoset (93%), whereas the amino acid sequence conservation ranged from 82.5 to 100%. All codons involved in CF mutations are highly conserved throughout evolution.
The major mutation in the cystic fibrosis (CF) gene is a 3-bp deletion ([Delta]F508 in exon 10. About 50% of the CF chromosomes in Southern Europe carry this mutation, while other previously described mutations account for less than 4%. To identify other common mutations in CF patients from the Mediterranean area, we have sequenced, exon by exon, 16 chromosomes that did not show the [Delta]F508 deletion from a selected panel of eight unrelated CF patients. We describe here one missense and one nonsense mutation, and four sequence polymorphisms. We have also found two previously reported mutations in three chromosomes. Overall, these mutations may account for about 20% of CF alleles in the Italian and Spanish populations. No other mutations were detected in 10 out of 16 CF chromosomes after analyzing about 90% of the coding region of the CF gene, and 39 out of 54 intron/exon boundaries. Therefore, about 26% of CF mutations remain to be identified. In addition we provide the intron/exon boundary sequences for exons 4 to 9. These results together with previously reported linkage data suggest that in the Mediterranean populations further mutations may lie in the promoter region, or in intron sequences not yet analyzed.

The Lesch-Nyhan (LN) syndrome is a genetically lethal human neurological disease that results from mutations that inactivate the hypoxanthine phosphoribosyltransferase (HPRT) gene. The elucidation of the complete DNA sequence of the human HPRT gene locus has enabled the construction of multiple oligonucleotide primer sets for the simultaneous in vitro amplification of all nine HPRT exons. The multiplex polymerase chain reaction provides a facile assay for the detection of HPRT exon deletions and the reaction products can be analyzed by direct automated fluorescent DNA sequencing to identify subtle alterations in the gene. Alterations have been identified in the HPRT genes from 15 independent LN cases, and 10 LN family studies were performed. The sequencing method uses solid supports and is sufficiently simple and sensitive to be a favored approach for LN diagnosis. LN heterozygotes can be diagnosed without reference to the affected male. In addition, these procedures will be useful for somatic mutagenesis studies.

Expressed sequence tags (ESTs) provide useful molecular landmarks for physical mapping and identify the position of an expressed region in the genome. The use of subtracted cDNA libraries enriched for tissue-specific genes as a source of ESTs should reduce the repetitive isolation of constitutively expressed sequences. We report here the sequence tags from the 3'-end region of 58 new directionally cloned cDNAs from a subtracted human retinal pigment epithelium (RPE) cell line library. Eight of the cDNAs have been assigned to human chromosomes using PCR-
based EST assays. Chromosomal mapping of subtracted RPE cDNA clones may also help in identifying candidate genes for inherited eye diseases.


http://www.sciencedirect.com/science/article/B6WG1-471W6W8-S/2/81aaa4e11b383875ab00866281a2b506

The paradigm of sequence-tagged site (STS)-content mapping involves the systematic assignment of STSs to individual cloned DNA segments. To date, yeast artificial chromosomes (YACs) represent the most commonly employed cloning system for constructing STS maps of large genomic intervals, such as whole human chromosomes. For developing a complete YAC-based STS-content map of human chromosome 7, we wished to utilize a limited set of YAC clones that were highly enriched for chromosome 7 DNA. Toward that end, we have assembled a human chromosome 7 YAC resource that consists of three major components: (1) a newly constructed library derived from a human–hamster hybrid cell line containing chromosome 7 as its only human DNA; (2) a chromosome 7-enriched sublibrary derived from the CEPH mega-YAC collection by Alu-polymerase chain reaction (Alu-PCR)-based hybridization; and (3) a set of YACs isolated from several total genomic libraries by screening for >125 chromosome 7 STSs. In particular, the hybrid cell line-derived YACs, which comprise the majority of the clones in the resource, have a relatively low chimera frequency (10-20%) based on mapping isolated insert ends to panels of human–hamster hybrid cell lines and analyzing individual clones by fluorescence in situ hybridization. An efficient strategy for polymerase chain reaction (PCR)-based screening of this YAC resource, which totals 4190 clones, has been developed and utilized to identify corresponding YACs for >600 STSs. The results of this initial screening effort indicate that the human chromosome 7 YAC resource provides an average of 6.9 positive clones per STS, a level of redundancy that should support the assembly of large YAC contigs and the construction of a high-resolution STS-content map of the chromosome.


http://www.sciencedirect.com/science/article/B6WG1-4DXB5C5-19/2/06a683aae993e4d7d418281a497233f8

Basic to the development of long-range physical maps of DNA are the detection and localization of landmarks within recombinant clones. Sequence-tagged sites (STSs), which are short stretches of DNA that can be specifically detected by the polymerase chain reaction (PCR), can be used as such landmarks. Our interest is to construct physical maps of whole human chromosomes by localizing STSs within yeast artificial chromosome (YAC) clones. Here we describe a generalized strategy for the systematic generation of large numbers of STSs specific for human chromosome 7. These STSs can be detected by PCR assays developed following the sequencing of anonymous pieces of chromosome 7 DNA, which was derived from flow-sorted chromosomes or from lambda clones made from DNA of a human-hamster hybrid cell line. Our approach for STS generation is tailored for the development of PCR assays capable of screening a large YAC library. In this study, we report the generation of 100 new STSs specific to human chromosome 7.

http://www.sciencedirect.com/science/article/B6WG1-4DNHS2F-NY/2/a6e90c169ced7dc943d2ae31ba890506

Somatic cell hybrids retaining the deleted chromosome 17 from 15 unrelated Smith-Magenis syndrome (SMS) del(17)(p11.2p11.2) patients were obtained by fusion of patient lymphoblasts with thymidine kinase-deficient rodent cell lines. Seventeen sequence-tagged sites (STSs) were developed from anonymous markers and cloned genes mapping to the short arm of chromosome 17. The STSs were used to determine the deletion status of these loci in these and four previously described human chromosome 17-retaining hybrids. Ten STSs were used to identify 28 yeast artificial chromosomes (YACs) from the St. Louis human genomic YAC library. Four of the 17 STSs identified simple repeat polymorphisms. The order and location of deletion breakpoints were confirmed and refined, and the regional assignment of several probes and cloned genes were determined. The cytogenetic band locations and relative order of six markers on 17p were established by fluorescence in situ hybridization mapping to metaphase chromosomes. The latter data confirmed and supplemented the somatic cell hybrid results. Most of the hybrids derived from [del(17)(p11.2p11.2)] patients demonstrated a similar pattern of deletion for the marker loci and were deleted for D17S446, D17S258, D17S29, D17S71, and D17S445. However, one of them demonstrated a unique pattern of deletion. This patient is deleted for several markers known to recognize a large DNA duplication associated with Charcot-Marie-Tooth (CMT) disease type 1A. These data suggest that the proximal junction of the CMT1A duplication is close to the distal breakpoint in [del(17)(p11.2p11.2)] patients.


http://www.sciencedirect.com/science/article/B6WG1-471W79Y-69/2/e6b3013b2a3a3715b1269b6fdd99ca82

We have determined the structure of the human CBFB gene, which encodes the [beta] subunit of the heterodimeric transcription factor core binding factor (CBF). This gene becomes fused to the MYH11 gene encoding smooth muscle myosin heavy chain by an inversion of chromosome 16 that occurs in the M4Eo subtype of acute myeloid leukemia. The CBFB gene contains 6 exons and spans 50 kb. The gene is highly conserved in animal species as distant as Drosophila, and the exon boundaries are in locations identical to those of the murine Cbfb homologue. The CBFB promoter region has typical features of a housekeeping gene, including high G+C content, high frequency of CpG dinucleotides, and lack of canonical TATA and CCAAT boxes. This gene has a single transcriptional start site, 345 nucleotides upstream of the beginning of the coding region. The human and mouse CBFB promoters show conservation of several transcriptional regulatory sequence motifs, including binding sites for Sp1, Ets family members, and Myc, but do not contain any CBF binding sites. The 5' end of the human CBFB gene also contains a highly polymorphic, transcribed CGG repeat that is not present in the murine homologue.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-
Interspersed repetitive sequence polymerase chain reaction (IRS-PCR) has become a powerful tool for the rapid generation of DNA probes from human chromosomes present in rodent somatic cell hybrids. We have constructed a somatic cell hybrid containing a major portion of the mouse X chromosome in a human background (clone 8.0). IRS-PCR was developed for the specific amplification of mouse DNA using either of two primers from the rodent-specific portion of the murine B1 repeat. Amplification was subsequently performed with clone 8.0 and a subclone, 8.1/1, which retains a small murine X-chromosomal fragment including Hprrt and the Gdx locus. A total of 15-20 discrete PCR products ranging in size from 3000 bp were obtained from clone 8.0 with each primer. In clone 8.1/1, a subset of these bands plus some additional bands were observed. Nine bands amplified from clone 8.1/1 have been excised from gels and used as probes on Southern blots. All of the fragments behaved as single-copy probes and detected domesticus/spretus variation. They have been regionally mapped using an interspecific backcross. The probe locations are compatible with those of markers known to be present in clone 8.1/1. These results demonstrate the feasibility of this method as applied to the mouse genome and the high likelihood of generating useful DNA probes from a targeted region.


We report a comparative map of canine chromosome 1 (CFA1) incorporating single nucleotide polymorphisms (SNPs) and insertion/deletion (indel) polymorphisms, developed by using cross-species primers, radiation hybrid analysis, and pool-and-sequence identification of genetic variations. Fifty-five genes were chosen with relatively even spacing (approximately 3 Mb between the human homologues) and were mapped to CFA1, with 49 of these being new assignments. Evolutionary chromosomal breakpoints between CFA1 and the corresponding human chromosomes (HSA6, HSA9, HSA18, and HSA19) were located within 1 to 5 Mb based upon the human genome sequence. The process of identifying the evolutionary chromosomal breakpoints between CFA1 and the relevant human chromosomes led to an improvement in the comparative maps of CFA7, CFA12, and CFA29 through the mapping of 21 additional genes. A manual pool-and-sequence method was used to identify 79 SNPs, 9 small indels, 7 simple tandem repeats, and 2 polymorphic SINE insertions within the genes mapped. The cross-species primers can also be used in the manner described here to improve the comparative maps for other mammalian species.


Sterol regulatory element binding protein-1 (SREBP1) and SREBP2 are structurally related proteins that control cholesterol homeostasis by stimulating transcription of sterol-regulated genes, including those encoding the low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl CoA synthase. SREBP1 and SREBP2 are 47% identical, and they share a novel structure comprising a transcriptionally active NH2-terminal basic helix--loop--helix--leucine zipper
(bHLH-Zip) domain followed by a membrane attachment domain. Cleavage by a sterol-regulated protease frees the bHLH-Zip domain from the membrane and allows it to enter the nucleus. SREBP1 exists in several forms, possibly as a result of alternative splicing at both the 5’ and the 3’ ends of the mRNA. The genes for SREBP1 (SREBF1) and SREBP2 (SREBF2) have not been studied. In this paper we describe the cloning and characterization of the human SREBF1 gene. The gene is 26 kb in length and has 22 exons and 20 introns. The 5’ and 3’ sequences that differ between the two SREBP1 cDNAs are encoded by discrete exons, confirming the hypothesis that they result from alternative splicing. The chromosomal locations of human SREBF1 and SREBF2 were determined by analysis of human-rodent somatic cell hybrids and fluorescence in situ hybridization. The SREBF1 gene mapped to the proximal short arm of chromosome 17 (17p11.2), and the SREBF2 gene was localized to the long arm of chromosome 22 (22q13).


http://www.sciencedirect.com/science/article/B6WG1-4B667HD-1/2/6b31393608f34cc119d495ccf9a23394

Xcat mice display X-linked congenital cataracts and are a mouse model for the human X-linked cataract disease Nance Horan syndrome (NHS). The genetic defect in Xcat mice and NHS patients is not known. We isolated and sequenced a BAC contig representing a portion of the Xcat critical region. We combined our sequencing data with the most recent mouse sequence assemblies from both Celera and public databases. The sequence of the 2.2-Mb Xcat critical region was then analyzed for potential Xcat candidate genes. The coding regions of the seven known genes within this area (Rai2, Rbbp7, Ctps2, Calb3, Grpr, Reps2, and Syap1) were sequenced in Xcat mice and no mutations were detected. The expression of Rai2 was quantitatively identical in wild-type and Xcat mutant eyes. These results indicate that the Xcat mutation is within a novel, undiscovered gene.


http://www.sciencedirect.com/science/article/B6WG1-4DXK9Y3-2H/2/7757def909f0cd302ec5c965ea83a8e89

A 680-kb yeast artificial chromosome (YAC) that contains a functional copy of the human hypoxanthine phosphoribosyltransferase (HPRT) gene has been isolated. This YAC, yHPRT, and another YAC, yXY837, which contains the 3’ end of the HPRT gene, have been mapped with restriction enzymes that cleave human DNA infrequently. The HPRT gene lies near the center of yHPRT. Fusion of yHPRT-containing yeast spheroplasts with mouse L A-9 cells, which are HPRT-negative, gives rise to HPRT-positive colonies. These colonies contain the human HPRT gene and express human HPRT mRNA. Fusion of yeast with mammalian cells is an efficient way of testing the integrity and functionality of human DNA contained in YACs.


http://www.sciencedirect.com/science/article/B6WG1-4CVV6RK-
Haplotype-based human genome research is important in identifying disease susceptibility genes efficiently. Although haplotype reconstruction by statistical methods is widely used, direct haplotype determination by molecular techniques has also been developed as a complementary method for statistical estimation. In this study, we demonstrate a molecular haplotyping method making use of single-strand conformation polymorphism (SSCP) gels. We identified 10 common SNPs and a dinucleotide insertion/deletion polymorphism within 2-kb region upstream of the transcription initiation site of MUC5B and determined haplotype structure, dividing the region into two DNA fragments. Real haplotypes were determined unambiguously by our SSCP-based analysis with fragments longer than 1 kb. Haplotypes reconstructed from diploid genotypes in the same region by the statistical methods including EM algorithm were also evaluated. Direct comparison between statistical estimation and direct determination of haplotypes revealed that major haplotypes containing multiple marker sites showing strong LD are estimated in great accuracy but that a variety of haplotypes reflecting weak LD are not reconstructed precisely enough. Our data can be helpful in implementing molecular haplotyping or statistical estimation, since usage of these methods may be determined depending on the haplotype structures.

http://www.sciencedirect.com/science/article/B6WG1-4FP1J0W-1/2/01996f6e737288ff0180ffcf8b9e47f

Holoprosencephaly (HPE) is the most common developmental field defect in patterning of the human prosencephalon and associated craniofacial structures. The genetics is complex, with 12 loci defined on 11 chromosomes. We defined a locus for HPE (HPE8) on human chromosome 14q13 between markers D14S49 and AFM205XG5, by mapping deletion intervals of affected subjects with proximal chromosome 14q interstitial cytogenetic deletions. A 35-BAC contig was built by chromosome walking. By annotation of the 2.82-Mb minimal critical region, we identified 28 possible genes. Seven genes were expressed in human fetal brain: NPAS3, SNX6, C14ORF11, C14ORF10, PAX9, NKX2.1, and C14ORF19, the last an apparent gene fragment. Molecular embryology, animal modeling, and human mutation studies were reported elsewhere for PAX9 and NKX2.1. We focused on three genes, SNX6, NPAS3, and C14ORF11, as potential candidates for HPE. Genomic structure, human expression patterns, protein cellular localization, and embryonic expression patterns of orthologous murine genes were determined, showing that the three genes have properties similar to those of known HPE genes.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-2N/2/f89e7e389e9de7b06263388dd5e9c986

We have developed the "shotgun polymerase chain reaction," a method for obtaining a large number of DNA markers specific to a giant DNA fragment, which facilitates analysis of a particular chromosomal region. We applied this method to a giant NotI fragment which carries the immunoglobulin lambda constant region on chromosome 22. NotI digests of chromosome 22 flow-sorted from human B-lymphoblastoid cell line GM130B were size fractionated by pulsed-field gel electrophoresis. Preliminary Southern hybridization analysis revealed that the immunoglobulin lambda constant region was conveyed on 1.4- and 1.3-Mb NotI fragments in this cell line. The agarose gel corresponding to 1.2 to 1.5 Mb in size was excised into slices and subjected to polymerase chain reaction to identify gel slices containing NotI fragments carrying Ke-Oz+, a
subtype of the immunoglobulin lambda constant region. From the NotI fragment thus identified, a large number of small DNA segments were amplified through the ligation-mediated random polymerase chain reaction method. The amplified products were cloned and analyzed for chromosomal origin and localization to particular NotI fragments. Seven of eighteen clones originated from the 1.4-Mb NotI fragment of chromosome 22 in GM130B cells, which appears to be exactly the same as detected by a probe for the immunoglobulin lambda constant region.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4J/2/4ef10ff56ac6e73756529c4f554e3935  


http://www.sciencedirect.com/science/article/B6WG1-471W7HX-89/2/741f76a522ca806d5ab629f20af0dd1e  

The phenol-preferring sulfotransferases aryl sulfotransferase IV and N-hydroxyarylamine sulfotransferase catalyze sulfate conjugation of N-hydroxy-2-acetyl-aminofluorene, a metabolite capable of causing hepatocarcinogenesis in rats. We utilized published cDNA sequences of these sulfotransferases to type the progeny of two multilocus crosses and determined that the genes, aryl sulfotransferase (Stp) and N-hydroxyaryl-amine sulfotransferase (Stp2), map to positions on mouse chromosomes 7 and 17.


http://www.sciencedirect.com/science/article/B6WG1-4DNHRPW-KR/2/b0283427ca96e31d88dcd5b1b52031e5  

The members of the carcinoembryonic antigen (CEA)/pregnancy-specific glycoprotein (PSG) gene family have a characteristic N-terminal domain that is homologous to the immunoglobulin variable region. We have estimated the size of the PSG subfamily by identification of N-domain exons from isolated genomic clones and from total genomic DNA through PCR amplification and DNA sequence determination. The PSG subfamily contains at least 11 different genes. For 7 of these, two DNA sequences differing from each other in 1 to 4 nucleotides were detected. Most likely, they represent different alleles. They are PSG1, PSG2, PSG3, PSG4, PSG5, PSG6, PSG7, PSG8, PSG11, PSG12, and PSG13. Six of the N-domain sequences described here are new. All of the PSGs except PSG1, PSG4, and PSG8 contained the arginine-glycine-aspartic acid sequence at position 93-95 corresponding to the complementarity determining region 3 of immunoglobulin. Parsimony analysis of 24 CEA and PSG sequences using 12 members of the immunoglobulin gene superfamily as out-groups to root the family free shows that the N-domain of the CEA group genes evolved in one major branch and the PSG group genes in the other.

The creation of a physical map of chromosome 18 will be useful for the eventual identification of specific chromosomal regions that are critical in the occurrence of Edwards syndrome, the 18q-syndrome, and the 18p-syndrome. To begin the investigation of these syndromes, a physical map has been constructed to order random DNA fragments to specific portions of chromosome 18. A set of somatic cell hybrids that retain deletions or translocations involving chromosome 18 has been isolated and characterized. Over 200 lambda phage from a chromosome 18-specific library have been localized to 11 distinct regions of chromosome 18 using the chromosomal breakpoints present in the somatic cell hybrids.


Nonsense-mediated mRNA decay (NMD) is a eukaryotic quality-control mechanism that detects and degrades aberrant transcripts prematurely terminating translation. NMD may be elicited by
intergenic transcripts that contain premature termination codons (PTCs), but chimeric mRNAs of genes that have introns of identical phase would be predicted to lack PTCs and escape NMD. We examined intron phase I-containing HLA class II genes for the presence of intergenic mRNAs and found an extraordinary diversity of correctly spliced and polyadenylated intergenic transcripts. They lacked a significant homology at the chimeric joins and had no PTCs. Their expression levels were very low and positively correlated with the expression of natural transcripts. In contrast, pair-wise mixtures of separately transcribed plasmids carrying full-length HLA-DQB1, -DQA1, -DRB1, and -DRA cDNAs produced only hybrid molecules that lacked canonical exon boundaries, had homologous chimeric joins, and occasionally contained PTCs, implicating in vitro artifacts generated by template switching of Taq polymerase and reverse transcriptase. A differential exon structure of hybrid molecules observed in vitro and in cellular RNA preparations suggests that intergenic mRNAs with canonical exon boundaries arise in vivo during exon joining and/or transcription. Since the observed intergenic mRNAs may encode mixed class II heterodimers that were previously shown to present antigens it will be interesting to determine functional properties of such molecules in future studies.

http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-3D/2/72e29a21091f1da3423af6b1b7b884c56

Highly informative dinucleotide repeat polymorphisms were identified at the T-complex-associated-testes-expressed-1 (TCTE1) locus on human chromosome 6p. Electrophoresis of single-stranded DNA on native gels facilitated the analysis of the dinucleotide polymorphisms. Linkage mapping positions this marker midway between the centromere and HLA with recombination fractions as follows: D6Z1-0.21-TCTE1-0.24-HLA. Two-color fluorescence in situ hybridization places TCTE1 proximal to CRIL171 (D6S19). Together, linkage and in situ hybridization indicate that the order of the loci is D6Z1-D6S4-D6S90-TCTE1-D6S19-D6S29-HLA-telomere. A sequence tagged site (STS) was established, and three yeast artificial chromosome (YAC) clones were identified for the TCTE1 locus.

http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-32/2/0ebab682eaa6af6d2c42b6108e0f430ba

The systematic screening of yeast artificial-chromosome (YAC) libraries is the limiting step in many physical mapping projects. To improve the screening throughout for a human YAC library, we designed an automatable strategy to identify YAC clones containing a specific segment of DNA. Our approach combines amplification of the target sequence from pooled YAC DNA by the polymerase chain reaction (PCR) with detection of the sequence by an ELISA-based oligonucleotide-ligation assay (OLA). The PCR-OLA approach eliminates the use of radioactive isotopes and gel electrophoresis, two of the major obstacles to automated YAC screening. Furthermore, the use of the OLA to test for the presence of sequences internal to PCR primers provides an additional level of sensitivity and specificity in comparison to methods that rely solely on the PCR.
Formation of tooth enamel is a poorly understood biological process. In this study we describe a 9-bp deletion in exon 2 of the amelogenin gene (AMGX) causing X-linked hypoplastic amelogenesis imperfecta, a disease characterized by defective enamel. The mutation results in the loss of 3 amino acids and exchange of 1 in the signal peptide of the amelogenin protein. This deletion in the signal peptide probably interferes with translocation of the amelogenin protein during synthesis, resulting in the thin enamel observed in affected members of the family. We compare this mutation to a previously reported mutation in the amelogenin gene that causes a different disease phenotype. The study illustrates that molecular analysis can help explain the various manifestations of a tooth disorder and thereby provide insights into the mechanisms of tooth enamel formation.

Amelogenesis imperfecta is characterized by the defective formation of tooth enamel. Here we present evidence that the X-linked form of this disorder (AIH1) is caused by a structural alteration in one of the predominant proteins in enamel, amelogenin. Southern blot analysis revealed a deletion extending over 5 kb of the amelogenin gene in males with the hypomineralization form of the AIH1. Carrier females were heterozygous for the molecular defect. The deletion appears to include at least two exons of the amelogenin gene and the extent of the deletion was verified by PCR analysis. The mutation was shown to segregate with the disease among 15 analyzed individuals belonging to the same kindred. Our results link a defect in the amelogenin gene to the abnormal formation of enamel. We thus conclude that the amelogenin protein has a role in biomineralization of tooth enamel.

Sonic Hedgehog (SHH) plays a fundamental role in numerous developmental processes including morphogenesis of limbs, nervous system, and teeth. Using a Bayesian alignment algorithm for phylogenetic footprinting we analyzed [not, vert, similar]28 kb of noncoding DNA in the SHH locus of human and mouse. This showed that the length of conserved noncoding sequences (4196 nt) shared by these species was approximately 3 times larger than the SHH coding sequence (1386 nt). Most segments were located in introns (53%) or within 2-kb regions upstream (16%) or downstream (20%) of the first and last SHH codon. Even though regions more than 2 kb upstream or downstream of the first and last SHH codon represented 57% (16 kb) of the sequence compared, they accounted for only 11% (494 nt) of the total length of conserved noncoding segments. One region of 650 nt downstream of SHH was identified as a putative
scaffold/matrix attachment region (SMAR). Human-mouse analysis was complemented by sequencing in apes, monkeys, rodents, and bats, thus further confirming the evolutionary conservation of some segments. Gel-shift assays indicated that conserved segments are targeted by nuclear proteins and showed differences between two cell types that expressed different levels of SHH, namely human endothelial cells and breast cancer cells. The relevance of these findings with respect to regulation of SHH expression during normal and pathologic development is discussed.


http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-3C/2/178c28d1400e0f91aa5ac33c957e5374

Ninety-three phage clones identified by hybridization with a C2---H2 zinc finger sequence probe have been grouped into 23 genetic loci. Partial sequencing verified that each locus belonged to the zinc finger family. Oligonucleotide primer pairs were developed from these sequences to serve as STS markers for these loci. One or more clones from each locus was mapped onto human metaphase chromosomes by fluorescence in situ hybridization. Several loci map to identical chromosomal regions, indicating the possible presence of multigene clusters. Zinc finger loci were found to reside predominantly either in telomeric regions or in chromosomal bands known to exhibit chromosome fragility. Chromosome 19 carries a disproportionate fraction (10 of 23) of the mapped zinc finger loci.


http://www.sciencedirect.com/science/article/B6WG1-471W704-2D/2/fea25740948a480efc5c38c390aca4a47

Isolation of DNA segments adjacent to known sequences is a tedious task in genome-related research. We have developed an efficient PCR strategy that overcomes the shortcomings of existing methods and can be automated. This strategy, thermal asymmetric interlaced (TAIL)-PCR, utilizes nested sequence-specific primers together with a shorter arbitrary degenerate primer so that the relative amplification efficiencies of specific and nonspecific products can be thermally controlled. One low-stringency PCR cycle is carried out to create annealing site(s) adapted for the arbitrary primer within the unknown target sequence bordering the known segment. This sequence is then preferentially and geometrically amplified over nontarget ones by interspersion of high-stringency PCR cycles with reduced-stringency PCR cycles. We have exploited the efficiency of this method to expedite amplification and sequencing of insert end segments from P1 and YAC clones for chromosome walking. In this study we present protocols that are amenable to automation of amplification and sequencing of insert end sequences directly from cells of P1 and YAC clones.


http://www.sciencedirect.com/science/article/B6WG1-4DP5JB1-
The Duchenne muscular dystrophy locus is remarkable in that it shows a high mutation rate and the majority of mutations found are deletions. These deletions are generated as meiotic as well as mitotic events and occur preferentially in the central region of the gene. Nothing is known so far about the mechanisms involved. This paper reports the first sequencing of deletion junctions in the dystrophin gene. The data from a study of two patients with deletions in the central region of dystrophin show the breakpoints to lie in regions of introns in which stretches of dA-dT are seen. The relationship between these observations and possible mechanisms for the mutations is discussed.


http://www.sciencedirect.com/science/article/B6WG1-4D3B142-2/2/a045171993669e66ec46d2d2a8bb2af1

Human carboxylesterases 1 and 2 (CES1 and CES2) catalyze the hydrolysis of many exogenous compounds. Alterations in carboxylesterase sequences could lead to variability in both the inactivation of drugs and the activation of prodrugs. We resequenced CES1 and CES2 in multiple populations (n = 120) to identify single-nucleotide polymorphisms and confirmed the novel SNPs in healthy European and African individuals (n = 190). Sixteen SNPs were found in CES1 (1 per 300 bp) and 11 in CES2 (1 per 630 bp) in at least one population. Allele frequencies and estimated haplotype frequencies varied significantly between African and European populations. No association between SNPs in CES1 or CES2 was found with respect to RNA expression in normal colonic mucosa; however, an intronic SNP (IVS10-88) in CES2 was associated with reduced CES2 mRNA expression in colorectal tumors. Functional analysis of the novel polymorphisms described in this study is now warranted to identify putative roles in drug metabolism.


http://www.sciencedirect.com/science/article/B6WG1-4DNRHHP-J7/2/7889511d55e6d49c278b8de28949a0d0

A compound imperfect dinucleotide repeat element, [CA]4TTTGT[CT]7[CA]9AA[CA]4CCACATA[CA]3, was found approximately 10 kb 3' to the human glucokinase gene (GCK) from analysis of contiguous genomic DNA obtained from a bacteriophage [lambda] chromosome walk. Direct human genomic sequencing revealed the source of polymorphism to be variable numbers of CT and CA repeats. Altogether six alleles that range in length from +10 to -15 nucleotides compared to the most common (Z) allele have been identified. Alleles Z, Z + 2, and Z + 4 were present in American Blacks, Pima Indians, and Caucasians, with somewhat varied frequencies among the group. Two alleles, Z + 10 and Z - 15, appear to be unique to American Blacks, while a Z + 6 allele was observed only in the Caucasian population studied. Observed heterozygosity of the polymorphism in the CEPH reference pedigree collection is 44% and the PIC 0.44. The polymorphism is assayed by PCR amplification and resolution of 32P-end-labeled products (ranging in length from 180 to 205 bp) on denaturing polyacrylamide sequencing gels. Using the PCR assay, the human glucokinase gene was physically localized to chromosome 7 in a panel of rodent/human somatic cell lines. Genetic analysis in CEPH pedigrees placed the dinucleotide repeat element, and thereby the human
The glucokinase gene, on chromosome 7p between TCRG and a RFLP locus D7S57. The glucokinase dinucleotide repeat genetic marker can now be used to assess the role of the glucokinase gene in diabetes by population association studies. In addition, this repeat marker and others flanking it on chromosome 7 can be used in linkage studies with families segregating the disorder.


http://www.sciencedirect.com/science/article/B6WG1-4DNHRHP-J8/2/79fa73bc1fd6456acac29a59086a234e

A new high-resolution genetic linkage map for human chromosome 7p has been constructed. The map is composed of 47 loci (54 polymorphic systems), 19 of which are uniquely placed with odds of at least 1000:1. Four genes are represented, including glucokinase (GCK, ATP- hexose-6-phosphatotransferase, EC 2.7.1.2) which was mapped via a (CA)n dinucleotide repeat polymorphism. The sex-average map measures 94.4 cM and the male and female maps measure 73.2 and 116.1 cM, respectively. We believe that the genetic map extends nearly the full length of the short arm of chromosome 7 since a centromere marker has been incorporated, and the most distal marker, D7S21, has been cytogenetically localized by in situ hybridization to 7p22-pter. The average marker spacing is 2 cM, and the largest interval between uniquely placed markers is 13 cM (sex-average map). Overall, female recombination was observed to be about 1.5 times that of males, and a statistically significant sex-specific recombination frequency was found for a single interval. The map is based on genotypic data gathered from 40 CEPH reference pedigrees and was constructed using the CRI-MAP program package. The map presented here represents a combined and substantially expanded dataset compared to previously published chromosome 7 maps, and it will serve as a "baseline" genetic map that should prove useful for future efforts to develop a 1-cM map and for construction of a contiguous clone-based physical map for this chromosome.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-9H/2/b33da104ee4e53584e6e79586c3208c5

A contig of 36 overlapping yeast artificial chromosome (YAC) clones has been constructed for the complete Duchenne muscular dystrophy (DMD) gene in Xp21. The YACs were isolated from a human 48,XXXX YAC library using the DMD cDNA and brain promoter fragments as hybridization probes. The YAC clones were characterized for exon content using HindIII or EcoRI digests, hybridization of individual DMD cDNA probes, and polymerase chain reaction (PCR) amplification of specific exons near the 5’ end of the gene. For comparison to the known long-range restriction map of the DMD gene, YAC clones were digested with SfiI and hybridized with DMD cDNA probes. The combined analysis of the exon content and the SfiI map allowed an approximately 3.2-Mb YAC contig to be constructed. The complete 2.4-Mb DMD gene could be represented in a minimum set of 7 overlapping YAC clones.

We have determined the genetic stability of three independent intragenic human HPRT gene duplications and the structure of each duplication at the nucleotide sequence level. Two of the duplications were isolated as spontaneous mutations from the HL60 human myeloid leukemia cell line, while the third was originally identified in a Lesch-Nyhan patient. All three duplications are genetically unstable and have a reversion rate approximately 100-fold higher than the rate of duplication formation. The molecular structures of these duplications are similar, with direct duplication of HPRT exons 2 and 3 and of 6.8 kb (HL 60 duplications) or 13.7 kb (Lesch-Nyhan duplication) of surrounding HPRT sequence. Nucleotide sequence analyses of duplication junctions revealed that the HL60-derived duplications were generated by unequal homologous recombination between clusters of Alu repeats contained in HPRT introns 1 and 3, while the Lesch-Nyhan duplication was generated by the nonhomologous insertion of duplicated HPRT DNA into HPRT intron 1. These results suggest that duplication substrates of different lengths can be generated from the human HPRT exon 2-3 region and can undergo either homologous or nonhomologous recombination with the HPRT locus to form gene duplications.


We have determined the nucleotide sequences of 10 intragenic human HPRT gene deletion junctions isolated from thioguanine-resistant PSV811 Werner syndrome fibroblasts or from HL60 myeloid leukemia cells. Deletion junctions were located by fine structure blot hybridization mapping and then amplified with flanking oligonucleotide primer pairs for DNA sequence analysis. The junction region sequences from these 10 HPRT mutants contained 13 deletions ranging in size from 57 bp to 19.3 kb. Three DNA inversions of 711, 368, and 20 bp were associated with tandem deletions in two mutants. Each mutant contained the deletion of one or more HPRT exon, thus explaining the thioguanine-resistant cellular phenotype. Deletion junction and donor nucleotide sequence alignments suggest that all of these HPRT gene rearrangements were generated by the nonhomologous recombination of donor DNA duplexes that share little nucleotide sequence identity. This result is surprising, given the potential for homologous recombination between copies of repeated DNA sequences that constitute approximately a third of the human HPRT locus. No difference in deletion structure or complexity was observed between deletions isolated from Werner syndrome or from HL60 mutants. This suggests that the Werner syndrome deletion mutator uses deletion mutagenesis pathway(s) that are similar or identical to those used in other human somatic cells.


Several mammalian sialidases have been cloned so far and here we describe the identification and expression of a new member of the human sialidase gene family. The NEU4 gene, identified by searching sequence databases for entries showing homologies to the human cytosolic sialidase NEU2, maps in 2q37 and encodes a 484-residue protein. The polypeptide contains all
the typical sialidase amino acid motifs and, apart from an amino acid stretch that appears unique among mammalian sialidases, shows a high degree of homology for NEU2 and the plasma membrane-associated (NEU3) sialidases. RNA dot-blot analysis showed a low but wide expression pattern, with the highest level in liver. Transient transfection in COS7 cells allowed the detection of a sialidase activity toward the artificial substrate 4MU-NeuAc in the acidic range of pH. Immunofluorescence staining and Western blot analysis demonstrated the association of NEU4 with the inner cell membranes.


http://www.sciencedirect.com/science/article/B6WG1-4FDJ6VR-1/2/aaa2d8c75c81d642052dcbdb84c92877

Four different transcripts of the Mecp2 gene can be distinguished by the length of the 3' untranslated region generated by usage of alternative polyadenylation sites. In situ hybridization analyses encompassing embryonic to 20-week postnatal age showed that transcripts are expressed in the central nervous system, with a progressive restriction during development culminating in localized strong expression in the cerebral cortex, olfactory bulb, hippocampal formation, and internal granule and Purkinje layer of the cerebellum. Real-time RT-PCR measurements of Mecp2 transcript levels showed variations with mouse age in two distinctive patterns that are unique to the central nervous system and the visceral organs, respectively. The 10-kb mRNA is the predominant form expressed in the brain in contrast to the shorter species expressed in the lung and liver. The developmental profile of Mecp2 mRNA highlights a potential tissue-specific function of the 3'UTR in the regulation of MeCP2 protein synthesis in response to the age-specific requirement of MeCP2 function during the life of the mouse.


http://www.sciencedirect.com/science/article/B6WG1-471W6W8-V/2/32e2c15b0a2486998edf44d9768fb724

Laminin 5 consists of three polypeptides, [alpha]3, [beta]3, and [gamma]2, encoded by the genes LAMA3, LAMB3, and LAMC2, respectively. In this study, we have elucidated the exon–intron organization of the human LAMB3 gene. Characterization of five overlapping [lambda] phage DNA clones revealed that the gene was approximately 29 kb in size. Subsequent sequence data revealed that the gene consisted of 23 exons that varied from 64 to 379 bp in size, accounting for the full-length cDNA with an open reading frame of 3516 bp encoding 1172 amino acids. Comparison of the LAMB3 gene structure with the previously characterized LAMB1 gene revealed that LAMB3 was considerably more compact. Knowledge of the exon–intron organization of the LAMB3 gene will facilitate elucidation of mutations in patients with the junctional forms of epidermolysis bullosa, some of which have been associated with mutations in the laminin 5 genes.

We have previously reported the isolation of a genomic clone encoding human liver-specific peroxisomal alanine:glyoxylate aminotransferase (AGT, EC 2.6.1.44), the deficient enzyme in primary hyperoxaluria type 1 (PH1) (P. E. Purdue, Y. Takada, and C. J. Danpure, J. Cell Biol. 111: 2341-2351, 1990). This clone has now been characterized, revealing that the coding sequence is distributed among 11 exons covering 10 kb. The nucleotide sequences of each exon have been determined, confirming that this clone corresponds to previously characterized AGT cDNA (Y. Takada, N. Kaneko, H. Esumi, P. E. Purdue, and C. J. Danpure, Biochem. J. 268: 517-520, 1990). In addition, to provide sequence data for the design of exon-specific PCR primers, the intron sequences immediately flanking each exon have been determined. Furthermore, in an attempt to identify putative transcriptional control sequences we have determined the sequence of 1.25 kb directly upstream of the cDNA 5' end. The results of genomic Southern blotting indicate that human AGT is probably encoded by a single copy gene, and a combination of in situ hybridization and PCR analysis of rodent/human somatic cell hybrids suggests that this gene is located on chromosome 2q36-q37. The gene symbol AGXT has been assigned for this locus.


We report the molecular characterization of two novel galactosemia mutations that exhibit different molecular phenotypes. Both are of the missense type with low or no residual enzyme activity. The R148W mutation results in an unstable protein, although messenger RNA is still produced. In contrast, the L195P mutation produces stable but inactive immunoreactive protein. The R148W mutation alters an amino acid that is not evolutionarily conserved, while the L195P mutation affects a well-conserved residue nine amino acids downstream from the putative active site nucleophile. These mutations provide evidence that different mechanisms can result in galactosemia: destabilizing mutations in any given area of the protein and missense mutations in conserved domains of the enzyme resulting in low or no activity. These two mutant alleles represent the fifth and sixth galactosemia mutations and confirm the hypothesis that galactosemia results from a multiplicity of mutations at the molecular level.


cDNA-AFLP is a genome-wide expression analysis technology that does not require any prior knowledge of gene sequences. This PCR-based technique combines a high sensitivity with a high specificity, allowing detection of rarely expressed genes and distinguishing between homologous genes. In this report, we validated quantitative expression data of 110 cDNA-AFLP fragments in yeast with DNA microarrays and GeneChip data. The best correlation was found between cDNA-AFLP and GeneChip data. The cDNA-AFLP data revealed a low number of inconsistent profiles that could be explained by gel artifact, overexposure, or mismatch amplification. In addition, 18 cDNA-AFLP fragments displayed homology to genomic yeast DNA,
but could not be linked unambiguously to any known ORF. These fragments were most probably derived from 5' or 3' noncoding sequences or might represent previously unidentified ORFs. Genes liable to cross hybridization showed identical results in cDNA-AFLP and GeneChip analysis. Three genes, which were readily detected with cDNA-AFLP, showed no significant expression in GeneChip experiments. We show that cDNA-AFLP is a very good alternative to microarrays and since no preexisting biological or sequence information is required, it is applicable to any species.


http://www.sciencedirect.com/science/article/B6WG1-4DXB88G-3/2/44e01632b9ec0f453c1d8393105de481

The highly polymorphic VNTR locus pYNZ32 has been more extensively characterized, and its analysis converted to a rapid PCR-based format. DNA sequencing in the areas within and flanking the repeated segment allowed the design of specific amplification primers. The repeated region of pYNZ32 consists of an imperfectly duplicated 27-bp motif, 16 bases of which are more highly conserved. Allelic products from PCR amplification were resolved into nine different size classes ranging from approximately 1400 to 2200 bp. Additional polymorphism was revealed when the amplified products were analyzed by restriction enzyme digestion. Both the overall size variation and the internal sequence polymorphism were used to determine a heterozygosity value of 86% for YNZ32 in 50 unrelated individuals. The rapid analysis and improved resolution of amplified alleles on agarose gels, and the internal variability within YNZ32, increase its diagnostic utility as a VNTR and as a linkage marker for the nearby Huntington disease gene.


http://www.sciencedirect.com/science/article/B6WG1-4DXB8RP-41/2/a04b9dbc9ae4e819ae503b114ec59def

Physical mapping of human chromosome 16 has been undertaken using somatic cell hybrid DNAs as templates for polymerase chain reaction (PCR) deletion analysis of sequence tagged sites (STSS). A panel of 29 somatic cell hybrids was analyzed, confirming and refining previous chromosome 16 breakpoint orders and distinguishing between the locations of breakpoints in new hybrids. Ten STS markers were coamplified in three multiplex reactions allowing the rapid, simultaneous deletion analysis of nine different loci. The locations of the protamine (PRM1), sialophorin (SPN), complement component receptor 3A (CR3A), NAD(P)H menadione oxidoreductase 1 (NMOR1), and calbindin (CALB2) genes were refined.


http://www.sciencedirect.com/science/article/B6WG1-45GMFRM-6J/2/b7ba7f182024dbd2145eb9d5018a907e
Focal nonepidermolytic palmoplantar keratoderma (NEPPK), or tylosis, is an autosomal, dominantly inherited disorder of the skin that manifests as focal thickening of the palmar and plantar surfaces. In three families studied, the skin disorder cosegregates with esophageal cancer and oral lesions. New haplotype analysis, presented here, places the tylosis esophageal cancer (TOC) locus between D17S1839 and D17S785. Envoplakin (EVPL) is a protein component of desmosomes and the cornified envelope that is expressed in epidermal and esophageal keratinocytes and has been localized to the TOC region. Mutation analysis of EVPL in the three affected families failed to show tylosis-specific mutations, and haplotype analysis of three intragenic sequence polymorphisms of the EVPL gene placed it proximal to D17S1839. Confirmation of the exclusion of EVPL as the TOC gene by location was obtained by integration of the genetic and physical mapping data using radiation hybrid, YAC, BAC, and PAC clones. This new physical map will allow further identification of candidate genes underlying NEPPK associated with esophageal cancer, which may also be implicated in the development of sporadic squamous cell esophageal carcinoma and Barrett's adenocarcinoma.


http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-33/2/3f906168eb4ca6a2871077063d94d771

The structure of the 3' one-third of the dystrophin gene has not previously been established. We have used vectorette PCR on a yeast artificial chromosome containing part of the human dystrophin gene to determine that there are 20 exons in this region and to characterize adjacent intron sequences of each one. Combined with previous information on the remainder of the gene, this study shows that the coding sequence is distributed between 79 exons. We have used PCR between exons to measure the distances that separate the more closely clustered exons. Vectorette PCR products were used as probes on Southern blots to assign all the 3' exons to genomic HindIII fragments that are commonly detected in the analysis of dystrophin gene deletions. The results will be useful for determining the effect of genomic deletions on the translational reading frame, for setting up genomic PCR assays to confirm point mutations, for analyzing splice site mutations, and for investigating potential cis-acting elements involved in tissue-specific alternative splicing. Vectorette PCR using primers derived from cDNA sequence represents an efficient and widely applicable method for establishing gene structure and obtaining intron sequence flanking exons, starting from a genomic clone and a cDNA sequence.


http://www.sciencedirect.com/science/article/B6WG1-4938JNP-1/2/75e2966a49ef4c264e7ff8742a9bbe3

We developed a simple method, based on the TaqMan technology, for fast genotyping of insertion/deletion polymorphisms of known location. The genotypes of 22 CEPH individuals, previously ascertained by conventional methods, were confirmed in the new assay without manual, time-consuming, post-PCR analysis. We propose to expand the application of TaqMan probes for population screening of insertion/deletion polymorphisms in which the exact endpoints of the insertion/deletion are known. The method can be applied to polymorphisms of any size and can be used for different applications such as diagnostics, genome variation, and species identification.

The fine structure of the Chinese hamster hypoxanthine guanine phosphoribosyltransferase (HPRT) gene has been determined; the gene has nine exons and is dispersed over 36 kb DNA. Exons 2-9 are contained within overlapping [lambda] bacteriophage clones and exon 1 was obtained by an inverse polymerase chain reaction (PCR). All the exons have been sequenced, together with their immediate flanking regions, and these sequences compared to those of the mouse and human HPRT genes. Sequences immediately flanking all exons but the first show considerable homology between the different species but the region around exon 1 is less conserved, apart from the preserved location of putative functional elements. Oligonucleotide primers derived from sequences flanking the HPRT gene exons were used to amplify simultaneously seven exon-containing fragments in a multiplex PCR. This simple procedure was used to identify total and partial gene deletions among Chinese hamster HPRT-deficient mutants. The multiplex PCR is quicker to perform than Southern analysis, traditionally used to study such mutants, and also provides specific exon-containing fragments for further analysis. The Chinese hamster HPRT gene is often used as a target for mutation studies in vitro because of the ease of selection of forward and reverse mutants; the information presented here will enhance the means of investigating molecular defects within this gene.


The dopamine D2 receptor gene (gene symbol DRD2) is a candidate gene for schizophrenia because the potency of certain neuroleptics correlates with their affinity for this receptor. Seven regions of likely functional significance including the coding sequences and the splice junctions were fully sequenced in the dopamine D2 receptor of 14 schizophrenics (and partially in several others) meeting DSM-III-R diagnostic criteria and in four unaffected non-Caucasians (97 kb of total sequence). No structural changes were found, suggesting that alteration in the structure of the dopamine D2 receptor is not commonly involved in the etiology of schizophrenia. However, two common and one uncommon intragenic polymorphisms were found. At least one of the polymorphisms was informative for linkage in 70% of Caucasians and 78% of Koreans.


Zebrafish is one of the best model organisms for investigating gene functions in vertebrates. By 4,5',8-trimethylpsoralen mutagenesis, we isolated a zebrafish mutant, vibrato, with defects in the
spontaneous contraction and touch response. Whole genome subtraction between the wild-type and the mutant genomes by representational difference analysis yielded polymorphic markers tightly linked to the vibrato locus. Using these markers, we constructed a high-resolution physical map and localized the vibrato locus within a genomic region of 720 kb. Direct cDNA selection with the contig led to the identification of a novel gene, solo, encoding a protein with SEC14 and spectrin repeat domains. These domains of Solo shared significant amino acid sequence identities with those of mammalian Trio and Karilin. In addition, we found the zebrafish orthologs for mammalian TTN, COL5A2, and CED-6 in the vibrato region. Mapping of these genes localized human chromosomal regions possibly involved in motor disorders. Our results suggest that representational difference analysis provides an efficient way to isolate mutated genomic regions in zebrafish.


http://www.sciencedirect.com/science/article/B6WG1-48TKF2F-7/2/a2a26d0d030d87c02893c5afa2015358

Here we report the identification of a novel transcript containing SNF2, PHD-finger, RING-finger, helicase, and linker histone domains mapping to the q24 band region of human chromosome 6. These domains are characteristic of several DNA repair proteins, transcription factors, and helicases. We have cloned both human and mouse homologs of this novel gene using interexon PCR and RACE technologies. The human cDNA, termed SHPRH, is 6018 bp and codes for a putative protein of 1683 amino acids. The mouse cDNA, termed Shprh, is 7225 bp and codes for a putative protein of 1616 amino acids. The deduced amino acid sequences of the two proteins share 86% identity. Both genes are expressed ubiquitously, with a transcript size of ~7.5 kb. Mapping of this gene to 6q24, a region reported to contain a tumor suppressor locus, prompted us to evaluate SHPRH by mutation analysis in tumor cell lines. We have identified one truncating and three missense mutations, thus suggesting SHPRH as a possible candidate for the tumor suppressor gene.


http://www.sciencedirect.com/science/article/B6WG1-4DNHRXG-NJ/2/67be5165b93fc36e75c1bf14c5c3b480


http://www.sciencedirect.com/science/article/B6WG1-4DNHP53-4R/2/5690eedef6767d6c1fb06c879d5d6546

A strategy for the analysis of yeast artificial chromosome (YAC) clones that relies on polymerase chain reaction (PCR) amplification of small restriction fragments from isolated YACs following adapter ligation was developed. Using this method, termed YACadapt, we have amplified several YACs from a human Xq24-qter library and have used the PCR products for physical mapping by somatic cell hybrid deletion analysis and fluorescent in situ hybridization. One YAC, RS46, was
mapped to band Xq27.3, near the fragile X mutation. The PCR product is an excellent renewable source of YAC DNA for analyses involving hybridization of YAC inserts to a variety of DNA/RNA sources.


Aspartylglucosaminuria (AGU) is a recessively inherited lysosomal disease caused by inadequate aspartylglucosaminidase (AGA) activity. The disease is prevalent in the genetically isolated Finnish population. We have used a new method, solid-phase minisequencing, to determine the frequency of two missense mutations in the AGA gene in this population. In samples from 70% of the Finnish AGU families, we found that the two nucleotide changes were always associated, and they were identified in 98% of the AGU alleles analyzed. Thus, the high prevalence of AGU in the Finnish population is the consequence of a founder effect of one ancient mutation. The identification of asymptomatic carriers by the minisequencing test proved to be unequivocal. The method also allowed quantification of a mutated nucleotide sequence present in less than 1% of a sample. The frequency of AGU carriers in this population was 1/36 when estimated by quantifying the mutated AGU allele in a pooled leukocyte sample from 1350 normal Finnish individuals.


Annexin IV (placental anticoagulant protein II) is a member of the annexin or lipocortin family of calcium-dependent phospholipid-binding proteins. A cDNA for human annexin IV was isolated from a placental library that is 675 bases longer in the 3' untranslated region than previously reported, indicating the existence of alternative mRNA processing for this gene. Genomic Southern blotting with a cDNA probe indicated a gene size of 18-56 kb. Primers developed for polymerase chain reaction (PCR) allowed amplification of a 1.6-kb portion of the ANX4 gene. DNA sequence analysis showed that this PCR product contained a single intron with exon-intron boundaries in exactly the same position as in the mouse annexin I and annexin II genes. PCR analysis of a somatic cell hybrid panel mapped the ANX4 gene to chromosome 2, and in situ hybridization with a cDNA probe showed a unique locus for ANX4 at 2p13. This study provides further evidence that genes for the annexins are dispersed throughout the genome but are similar in size and exon-intron organization.


Schizophrenia is a common neuropsychiatric disorder of uncertain etiology that is believed to
result from the interaction of environmental factors and multiple genes. To identify new genes predisposing to schizophrenia, numerous groups have focused on CAG-repeat-containing genes. We previously reported a CAG repeat polymorphism that was shown to be associated with both the severity of the phenotype and the response to medication in schizophrenic patients. In this article, we now report the genomic structure of this gene, the retinoic acid inducible-1 gene (RAI1), and present its characterization. This gene, located on chromosome 17p11.2, comprises six exons coding for a 7.6-kb mRNA. The RAI1 gene is highly homologous to its mouse counterpart and it is expressed at high levels mainly in neuronal tissues.


http://www.sciencedirect.com/science/article/B6WG1-471W72M-4P/2/b6aa0e0b90f1717b060d76036dac5b58


http://www.sciencedirect.com/science/article/B6WG1-47TF6BT-4/2/19bbe69bbf0ed3aa0f61269644f1460

By microarray assay we identified ESTs (expressed sequence tags) whose expression was predominantly increased in the affected skin of patients with psoriasis vulgaris. Among them, a full-length cDNA sequence corresponding to one of those ESTs (AI829641) was isolated by screening of cultured human keratinocyte cDNA libraries. This cDNA encodes a novel member of the Ly-6/uPAR superfamily, designated SLURP-2 (secreted Ly-6/uPAR related protein 2). SLURP-2 has an open reading frame of 97 amino acids containing 10 conserved cysteine residues. SLURP-2 has a single functional copy within the LY6 superfamily gene cluster at chromosome 8q24.3. RT-PCR (reverse transcriptase-polymerase chain reaction) expression analysis revealed that SLURP-2 was expressed in multiple tissues, mainly in the epithelial cells including the skin and keratinocytes, but not in spleen or bone marrow. Comparison of the expression of this gene among the psoriatic lesional and nonlesional skin of patients and the normal skin of healthy individuals detected by quantitative real-time RT-PCR analysis disclosed that SLURP-2 was up-regulated threefold in psoriatic lesional skin. These findings suggest that SLURP-2 may be involved in the pathophysiology of psoriasis through its role in keratinocyte hyperproliferation and/or T cell differentiation/activation.


http://www.sciencedirect.com/science/article/B6WG1-4C0V55V-1/2/2398a7daa70c3b8446aadbc8756d87e9

The Intersectin 1 (ITSN1) protein functions in clathrin-mediated endocytosis and in MAP kinase signaling. The complex domain structure comprises two EH and five SH3 domains in the short isoform, plus RhoGEF, pleckstrin, and putative calcium-interaction domains in the long isoform. Alternative splicing of exon 20, affecting the SH3A domain, has been shown in rat and that of exons 25 + 26, affecting the SH3C domain, has been shown in human and rat. Here we report 7
novel splice variants of the human and mouse ITSN1 genes and demonstrate conservation of alternative splicing affecting SH3A and SH3C in mouse. The novel variants encode transcripts with altered EH domain spacing and RhoGEF domain structure and possible targets of nonsense-mediated decay. Eight and 16 protein variants of the short and long ITSN1 isoforms, respectively, are predicted. These isoforms likely serve to modulate the many complex protein interactions and functions of ITSN1.


http://www.sciencedirect.com/science/article/B6WG1-4DNHRHP-JB/2/041950847b6868a39d1abe13da86344b

The role of genetic variation in isoenzyme gene families is often poorly appreciated. We report here on the determination of DNA sequences and typing of genetic variability in four creatine kinase B (CKB) gene loci in different inbred strains of mice. The unique functional murine CKB gene was found to be nearly identical to the previously characterised rat and human sequences in both size and exon-intron structure. In this gene, approximately 0.5% allelic nucleotide positions as well as the lengths of simple A-rich and [TG]n repetitive elements located at the 5' and 3' sides of the transcribed segment, differed between inbred strains of mice. Preliminary experiments suggest that this sequence divergence is of importance for design of gene targeting strategies involving homologous DNA recombination. The three additional CKB-like gene loci in mice all had the characteristics of processed pseudogenes. By Southern blot analysis we could demonstrate that both the type and number of pseudogenes differed between inbred strains. Analysis of the CKB gene sequences enabled us to speculate about the evolutionary history of this highly polymorphic subfamily of genes.

http://www.sciencedirect.com/science/article/B6WG1-4FP1J0W-2/2/bab0fc462e19805a602890aa971a1d9c

Human [beta] defensins contribute to the first line of defense against infection of the lung. Polymorphisms in these genes are therefore potential modifiers of the severity of lung disease in cystic fibrosis. Polymorphisms were sought in the human [beta]-defensin genes DEFB1, DEFB4, DEFB103A, and DEFB104 in healthy individuals and cystic fibrosis (CF) patients living in various European countries. DEFB1, DEFB4, and DEFB104 were very polymorphic, but DEFB103A was not. Within Europe, differences between control populations were found for some of the frequent polymorphisms in DEFB1, with significant differences between South-Italian and Czech populations. Moreover, frequent polymorphisms located in DEFB4 and DEFB104 were not in Hardy Weinberg equilibrium in all populations studied, while those in DEFB1 were in Hardy Weinberg equilibrium. Sequencing of a monochromosomal chromosome 8 mouse-human hybrid cell line revealed signals for multiple alleles for some loci in DEFB4 and DEFB104, but not for DEFB1. This indicated that more than one DEFB4 and DEFB104 gene was present on this chromosome 8, in agreement with recent findings that DEFB4 and DEFB104 are part of a repeat region. Individual DEFB4 and DEFB104 PCR amplification products of various samples were cloned and sequenced. The results showed that one DNA sample could contain more than two haplotypes, indicating that the various repeats on one chromosome were not identical. Given the higher complexity found in the genomic organization of the DEFB4 and DEFB104 genes, association studies with CF lung disease severity were performed only for frequent polymorphisms located in DEFB1. No association with the age of first infection by Pseudomonas aeruginosa or with the FEV1 percentage at the age of 11-13 years could be found.
The gene mutated in chorea-acanthocytosis (CHAC; approved gene symbol VPS13A) encodes chorein, a protein similar to yeast Vps13p. We detected several similar putative human proteins by BLAST analysis of chorein. We characterized the structure of three new genes encoding these CHAC-similar proteins, located on chromosomes 1p36, 8q22, and 15q21. The most similar gene in yeast to all four human genes is Vps13, and therefore the human genes were named VPS13A (CHAC, 9q21), VPS13B (8q22), VPS13C (15q21), and VPS13D (1p36). VPS13B has recently been reported as COH1, altered in Cohen syndrome. For each gene, we describe several alternative splicing variants; at least two transcripts per gene are major forms. The expression pattern of these genes is ubiquitous, with some tissue-specific differences between several transcript variants. Protein sequence comparisons suggest that intramolecular duplications have played an important role in the evolution of this gene family.

We have designed and evaluated a series of class-specific (Aves), order-specific (Rodentia), and species-specific (equine, canine, feline, rat, hamster, guinea pig, and rabbit) polymerase chain reaction (PCR)-based assays for the identification and quantitation of DNA using amplification of genome-specific short and long interspersed elements. Using SYBR Green-based detection, the minimum effective quantitation levels of the assays ranged from 0.1 ng to 0.1 pg of starting DNA template. Background cross-amplification with DNA templates derived from sixteen other species was negligible prior to 30 cycles of PCR. The species-specificity of the PCR amplicons was further demonstrated by the ability of the assays to accurately detect known quantities of species-specific DNA from mixed (complex) sources. The 10 assays reported here will help facilitate the sensitive detection and quantitation of common domestic animal and bird species DNA from complex biomaterials.

Treacher Collins syndrome is an autosomal dominant disorder of abnormal craniofacial development. Linkage analysis was performed in Treacher Collins families with restriction fragment length or microsatellite polymorphisms associated with eight loci previously mapped to 5q31->qter. Positive lod scores were obtained for four loci, D5S119, D5S207, D5S209, and D5S210, which map to 5q31.3->q33.3. The Treacher Collins syndrome locus was linked closest to locus D5S210, which is associated with microsatellite polymorphisms, with a maximum lod score of 8.65 at [theta] = 0.02. The Treacher Collins syndrome locus was excluded from locus
ADRB2R, which maps to 5q31->q32, and loci D5S22, D5S61, and D5S43, which map to 5q34->qter. There was no evidence for genetic heterogeneity among eight families with variable expression of the condition.


http://www.sciencedirect.com/science/article/B6WG1-4DBCBNW-1/2/c95fb4280115112e7565739e611b3520

Infantile nephronophthisis is associated with cystic kidneys, situs inversus, and INVS mutations. The function of the INVS product, inversin, is unknown but evidence suggests there are multiple inversin isoforms with differing molecular weights, cellular localization patterns, and binding partners. We used Northern blots, RT-PCR, and sequence analysis to identify alternative INVS transcripts. Northern blots probed with Invs cDNA detected four bands in normal mouse kidney. RT-PCR of mouse kidney RNA revealed Invs transcripts with skipping of exon 5, 11, or 13. We sequenced canine (MDCK-II cells) INVS and determined that the corresponding full-length protein shares identity with mouse (74%) and human (84%) inversin. Canine INVS produces a transcript that skips exon 12. Exon skips cause loss of inversin protein motifs, including ankyrin repeats, IQ domains, destruction boxes, and nuclear localization signals. Identification of INVS splice variants will help us determine which inversin protein motifs contribute to left-right asymmetry and kidney development.


http://www.sciencedirect.com/science/article/B6WG1-4D5XCJP-3/2/0b130def73c3c13f91f6631057e6e4f6

Amplicons are frequently found in human tumor genomes, but the mechanism of their generation is still poorly understood. We previously measured the replication timing of the genes along the entire length of human chromosomes 11q and 21q and found that many "disease-related" genes are located in timing-transition regions. In this study, further scrutiny of the updated replication-timing map of human chromosome 11q revealed that both amplicons on human chromosomal bands 11q13 and 11q22 are located in the early/late-switch regions of replication timing in two human cell lines (THP-1 and Jurkat). Moreover, examination of synteny in the human and mouse genomes revealed that synteny breakage in both genomes occurred primarily at the early/late-switch regions of replication timing that we had identified. In conclusion, we found that the early/late-switch regions of replication timing coincided with "unstable" regions of the genome.


http://www.sciencedirect.com/science/article/B6WG1-4DXB57P-8/2/85db262021c41c5b6ff7a1f8861325c1

Abundant human interspersed repetitive DNA sequences of the form (dC-dA)n. (dG-dT)n have been shown to exhibit length polymorphisms. Examination of over 100 human (dC-dA)n. (dG-dT)n sequences revealed that the sequences differed from each other both in numbers of repeats
and in repeat sequence type. Using a set of precise classification rules, the sequences were divided into three categories: perfect repeat sequences without interruptions in the runs of CA or GT dinucleotides (64% of total), imperfect repeat sequences with one or more interruptions in the run of repeats (25%), and compound repeat sequences with adjacent tandem simple repeats of a different sequence (11%). Informativeness of (dC-dA)n. (dG-dT)n markers in the perfect sequence category was found to increase with increasing average numbers of repeats. PIC values ranged from 0 at about 10 or fewer repeats to above 0.8 for sequences with about 24 or more repeats. (dC-dA)n. (dG-dT)n polymorphisms in the imperfect sequence category showed lower informativeness than expected on the basis of the total numbers of repeats. The longest run of uninterrupted CA or GT repeats was found to be the best predictor of informativeness of (dC-dA)n. (dG-dT)n polymorphisms regardless of the repeat sequence category.


http://www.sciencedirect.com/science/article/B6WG1-4DXB5C5-1V/2/a7117b21bd7f5912b120741622b24f38

Thirteen moderately to highly informative microsatellite DNA polymorphisms based on (dC-dA)n. (dG-dT)n repeats were mapped to segments of human chromosome 5 using both linkage analysis and a panel of somatic cell hybrids which contained rearranged chromosomes. The markers were distributed throughout most of the length of the chromosome from the regions p15.3-p15.1 to q33.3-qter. Maps of the sites of meiotic recombination within the reference families proved particularly useful for the purpose of integrating new polymorphisms into the existing linkage map.


http://www.sciencedirect.com/science/article/B6WG1-471W7HX-82/2/8727cc75d255e548a733d2e1777ddc16

Physical mapping of small genomic DNA fragments or expressed sequences by in situ hybridization is typically limited by the size of the target DNA sequence. Isolation of large insert DNA clones from libraries containing the target DNA sequence facilitates physical mapping by fluorescence in situ hybridization and allows rapid assignment of genes to cytogenetic bands. Here, we demonstrate the scheme by mapping the human protooncogene trk (NTRK1), a tyrosine kinase receptor type I gene that has earlier been assigned to two different cytogenetic loci. Large DNA insert library screening was carried out by in vitro DNA amplification using oligonucleotide primers flanking exon 4 of trk. The scheme presented here can easily be generalized to map physically very small nonrepetitive genomic DNA fragments or incomplete cDNAs.


http://www.sciencedirect.com/science/article/B6WG1-4D10KHN-1/2/198682806ad797a174fb122f01bb8adf
Members of the human UDP-glucuronosyltransferase 2B family are located in a cluster on chromosome 4q13 and code for enzymes whose gene products are responsible for the normal catabolism of steroid hormones. Two members of this family, UGT2B15 and UGT2B17, share over 95% sequence identity. However, UGT2B17 exhibits broader substrate specificity due to a single amino acid difference. Using gene-specific primers to explore the genomic organization of these two genes, it was determined that UGT2B17 is absent in some human DNA samples. The gene-specific primers demonstrated the presence or absence of a 150 kb genomic interval spanning the entire UGT2B17 gene, revealing that UGT2B17 is present in the human genome as a deletion polymorphism linked to UGT2B15. Furthermore, it is shown that the UGT2B17 deletion polymorphism shows Mendelian segregation and allele frequencies that differ between African Americans and Caucasians.


As a first step towards verifying the candidate status of DGAT1 as the causal gene for milk fat percentage in cattle, we constructed a bovine BAC contig spanning 576 kb of the chromosomal region containing DGAT1. High content of NotI sites (21 within the contig) indicated that the region is gene-rich. Twenty-three genes neighboring DGAT1 were mapped, including two bovine cDNA sequences that have no orthologous sequences within the NCBI sequence databases. On average, 2015 bp for each of the 23 neighboring genes were sequenced and entered into EMBL. Likewise, 10 new STS markers were established by BAC-end sequencing. Within the genes and STS markers, 55 polymorphisms were discovered. These will form the basis of future linkage disequilibrium studies to test whether any genes neighboring DGAT1 are associated with variation in milk fat percentage, thereby testing the candidate status of DGAT1.


The telomeric region of chromosome 9p is paralogous to the pericentromeric regions of chromosome 9 as well as to 2q13, the site of an ancestral telomere-telomere fusion. These paralogous regions span approximately 200 kb and contain seven transcriptional units, including the previously identified CBWD, FOXD4, PGM5, F379, CXYorf1, and two human Unigene clusters, Hs.115173 and Hs.189170. Within these gene duplicates, the number of expressed paralogous loci varies, from one in PGM5 to all three in CBWD and Hs.115173. FOXD4 shows the most dramatic changes among its paralogs. Two independent insertion/deletion changes created four different carboxy ends of these intronless genes, two of which are within the 2q13 locus. A comparison of KA/KS values among functional paralogs shows these genes evolved rapidly in primates. This study shows the importance of paralogous regions in the generation of transcriptional diversity and highlights the significance that large-scale telomeric duplication may play in this process.


http://www.sciencedirect.com/science/article/B6WG1-482YXT2-1/2/673df987acffa3e46e7b807fd83f9da1

We have recently reported a new pathogen discovery approach, "computational subtraction". With this approach, non-human transcripts are detected by sequencing cDNA libraries from infected tissue and eliminating those transcripts that match the human genome. We show now that this method is experimentally feasible. We generated a cDNA library from a tissue sample of post-transplant lymphoproliferative disorder (PTLD). 27,840 independent cDNA sequences were filtered by computational subtraction against the known human sequence to identify 32 nonmatching transcripts. Of these, 22 (0.1%) were found to be amplifiable from both infected and noninfected samples and were inferred to be human DNA not yet contained in the available human genome sequence. The remaining 10 sequences could be amplified only from Epstein-Barr virus (EBV)-infected tissues. All 10 corresponded to the known EBV sequence. This proof-of-principle experiment demonstrates that computational subtraction can detect pathogenic microbes in primary human-diseased tissue.


http://www.sciencedirect.com/science/article/B6WG1-4B3NM01-2/2/055c7d3de394cebbdfe6f30e7e1defa2

MEST is one of the imprinted genes in human. With the assistance of our integration map and the complete sequence in the registry, we mapped a total of 16 genes/transcripts at the 1.5-Mb MEST-flanking region at 7q32. This region has been suggested to form an imprinted gene cluster, because MEST and its three flanking genes/transcripts (MESTIT1, CPA4, and COPG2IT1) were reported to be imprinted, although two (TSGA14 and COPG2) were shown to escape imprinting. In this study, 10 other genes/transcripts were examined for their imprinting status in human fetal tissues. The results indicated that 8 genes/transcripts (NRF1, UBE2H, HSPC216, KIAA0265, FLJ14803, CPA2, CPA1, and DKFZp667F0312) were expressed biallelically. The imprinting status of two (TSGA13 and CPA5) was not conclusive, because of their weak and/or tissue-specific expression and inconstant results. These findings provided evidence that only 4 of the 16 genes/transcripts located to the region show monoallelic expression, while others are not involved in imprinting. Therefore, it is less likely that the MEST-flanking 7q32 region forms a large imprinted domain.


http://www.sciencedirect.com/science/article/B6WG1-4DYM8X6-4F/2/f4c423de52dc4c49235fe2c92f0b5054

A method based on the differential screening of a chromosome-specific cosmids library with amplified inter-Alu sequences obtained from a set of somatic cell hybrids has been developed to target the isolation of probes from predefined subchromosomal regions. As a model system, we have used a chromosome 22-specific cosmids library and four cell hybrids containing different parts of this chromosome. The procedure has identified cosmids that demonstrate differential hybridization signals with Alu-PCR products from these cell hybrids. We show, by in situ hybridization or individual mapping, that their hybridization pattern is indicative of their
sublocalization on chromosome 22, thus resulting in a large enrichment factor for the isolation of probes from specific small chromosome subregions. Depending on the local Alu-sequence density, from 3 to 10 independent loci per megabase of genome can thus be identified.

**Glycobiology** (2)


http://glycob.oupjournals.org/cgi/content/abstract/14/3/219

Galectins are a family of (beta)-galactoside-binding lectins that on synthesis are either translocated into the nucleus or released to the extracellular space. Their developmentally regulated expression, extracellular location, and affinity for extracellular components (such as laminin and fibronectin) suggest a role in embryonic development, but so far this has not been unequivocally established. Zebrafish constitute an ideal model for developmental studies because of their external fertilization, transparent embryos, rapid growth, and availability of a large collection of mutants. As a first step in addressing the biological roles in zebrafish embryogenesis, we identified and characterized members of the three galectin types: three protogalectins (Drgal1-L1, Drgal1-L2, Drgal1-L3), one chimera galectin (Drgal3), and one tandem-repeat galectin (Drgal9-L1). Like mammalian prototype galectin-1, Drgal1-L2 preferentially binds to N-acetyllactosamine. Genomic structure of Drgal1-L2 revealed four exons, with the exon-intron boundaries conserved with the mammalian galectin-1. Interestingly, this gene also encodes an alternatively spliced form of Drgal1-L2 that lacks eight amino acids near the carbohydrate-binding domain. Zebrafish galectins exhibited distinct patterns of temporal expression during embryo development. Drgal1-L2 is expressed postbud stage, and its expression is strikingly specific to the notochord. In contrast, Drgal1-L1 is expressed maternally in the oocytes. Drgal1-L3, Drgal3, and Drgal9-L1 are expressed both maternally and zygotically, ubiquitously in the adult tissues. The distinct temporal and spatial patterns of expression of members of the zebrafish galectin repertoire suggest that each may play distinct biological roles during early embryogenesis.


http://glycob.oupjournals.org/cgi/content/abstract/12/2/85

We report here the purification, characterization, and cDNA cloning of a novel N-acetylgalactosamine-specific lectin from starfish, Asterina pectinifera. The purified lectin showed 19-kDa, 41-kDa, and 60-kDa protein bands on SDS-PAGE, possibly corresponding to a monomer, homodimer, and homotrimer. Interestingly, on 4-20% native PAGE the lectin showed at least nine protein bands, among which oligomers containing six to nine subunits had potent hemagglutination activity for sheep erythrocytes. The hemagglutination activity of the lectin was specifically inhibited by N-acetylgalactosamine, Tn antigen, and blood group A trisaccharide, but not by N-acetylgalactosamine, galactose, galactosamine, or blood group B trisaccharide. The specificity of the lectin was further examined using various glycosphingolipids and biotin-labeled
The lectin was found to bind to Gb5Cer, but not Gb4Cer, Gb3Cer, GM1a, GM2, or asialo-GM2, indicating that the lectin specifically binds to the terminal \(\alpha\)-GalNAc at the nonreducing end. The hemagglutination activity of the lectin was completely abolished by chelation with EDTA or EGTA and completely restored by the addition of CaCl2. cDNA cloning of the lectin showed that the protein is composed of 168 amino acids, including a signal sequence of 18 residues, and possesses the typical C-type lectin motif. These findings indicate that the protein is a C-type lectin. The recombinant lectin, produced in a soluble form by Escherichia coli, showed binding activity for asialomucin in the presence of Ca2+ but no hemagglutination.

Growth Hormone & IGF Research (2)


http://www.sciencedirect.com/science/article/B6WG5-474D6N3-4/2/747507edd94b499d2c6bfa39448f9884


http://www.sciencedirect.com/science/article/B6WG5-46HDNYV-4/2/53036f4d85e06916e61e6f68f771e52a

Gut (20)


http://gut.bmjournals.com/cgi/content/abstract/53/4/501

Background: Stressful events in the early period of life (for example, maternal deprivation) have been shown to modify adult immune and gastrointestinal tract functions. The present study aimed to establish whether maternal deprivation affects colonic epithelial barrier and the development of an experimental colitis in adult rats. Methods: Male Wistar rat pups were separated during postnatal days 2-14 or left undisturbed with their dam. At 12 weeks of age, we assessed colonic paracellular permeability, bacterial translocation, myeloperoxidase (MPO) activity, mucosal mast cell density, cytokine (interleukin (IL)-1{beta}, IL-2, IL-4, IL-10, and interferon (gamma) (IFN-(gamma)) mRNA expression, and macroscopic damage. Total gut permeability, MPO activity, and macroscopic damage were also assessed four days after intracolonic administration of 2,4,6-trinitrobenzenesulphonic acid (TNBS). Results: Maternal deprivation triggered a significant
increase in colonic permeability associated with bacterial translocation into the mesenteric lymph nodes, liver, and spleen. These alterations were associated with some macroscopic damage and an increase in colonic MPO activity, mucosal mast cell density, and cytokine mRNA expression. Intracolonic infusion of TNBS induced a significantly higher inflammatory reaction in separated animals, as judged by enhanced MPO colonic levels, total gut permeability, and macroscopic lesions. Conclusions: Maternal deprivation promotes long term alterations in the colonic epithelial barrier associated with an exaggerated immune response to an external immune stimulus. This suggests a role for early psychological factors in the regulation of colonic mucosal barrier in later life.


http://gut.bmjournals.com/cgi/content/abstract/53/12/1751

Background: Endogenous cyclooxygenase (COX) activity is required to maintain a relatively alkaline surface pH at the gastric luminal surface. Aims: The purpose of this study was to determine which COX isoform, COX-1 or COX-2, is responsible for regulating the protective surface pH gradient and to test if COX inhibitors also had non-COX mediated effects in vivo. Methods: Immunofluorescence and western blot analysis showed constitutive expression of both COX isoforms in the normal mouse stomach. We used in vivo confocal microscopy to measure pH near the mucosal surface of anaesthetised COX-1 (-/-), COX-2 (-/-), or wild-type mice of the same genetic background. Results: When the gastric mucosal surface was exposed and superfused (0.2 ml/min) with a weakly buffered saline solution (pH 3) containing the pH indicator CI-NERF, the pH directly at the gastric surface and thickness of the pH gradient were similar in wild-type and COX-2 (-/-) mice, but COX-1 (-/-) mice had a significantly thinner pH gradient. Addition of indomethacin had minimal effects on the residual surface pH gradient in COX-1 (-/-) mice, suggesting no role for COX-2 in surface pH regulation. Whole stomach perfusion studies demonstrated diminished net alkali secretion in COX-1 (-/-) mice, and application of SC-560 or rofecoxib to wild-type mice and mutant mice confirmed that only COX-1 inhibition reduced alkali secretion. Conclusion: COX-1 is the dominant isoform regulating the normal thickness of the protective surface pH gradient in mouse stomach.


http://gut.bmjournals.com/cgi/content/abstract/51/5/677

Background: Germline mutations in the mismatch repair (MMR) genes hMLH1 and hMSH2 can cause hereditary non-polyposis colorectal cancer (HNPCC). However, the functional in vitro analysis of hMLH1 and hMSH2 mutations remains difficult. Aims: To establish an in vitro method for the functional characterisation of hMLH1 and hMSH2 mutations. Methods: hMLH1 and hMSH2 wild type (wt) genes and several mutated subclones were transiently transfected in mismatch repair deficient cell lines (HCT-116 and LOVO). Apoptosis, proliferation, and regulation of mRNA expression and protein expression of interacting proteins were analysed by Hoechst staining, AlamarBlue staining, real time polymerase chain reaction, and western blotting, respectively. Results: The protein expression of hMLH1 and hMSH2 mutants was significantly decreased after transfection compared with wild type transfections. The hMLH1 and hMSH2 interacting proteins hPMS2 and hMSH6 became detectable only after transfection of the respective wild type genes. In parallel, hMSH6 mRNA levels were increased in hMSH2 wt transfected cells. However, hPMS2 mRNA levels were independent of the mutation status of its interacting partner hMLH1, indicating a post-transcriptional regulating pathway. In the hMLH1
deficient HCT-116 cell line apoptosis was not affected by transfection of any mismatch repair gene, whereas complementation of hMSH2 deficiency in LOVO cells increased apoptosis. Conversely, proliferative activity of HCT-116 was decreased by complementation with hMLH1wt and unaffected in hMSH2 deficient LOVO cells. Conclusion: These data show that the cellular role of the MMR genes and its mutations are assessable in a simple transient transfection system and show the influence of MMR gene regulation on major cell growth regulating mechanisms. This method is applicable for the functional definition of mutations in hMLH1 and hMSH2 genes observed in patients with suspected HNPCC.


http://gut.bmjournals.com/cgi/content/abstract/53/12/1772

Background and aims: The cellular and molecular events involved in ischaemia reperfusion (IR) injury are complex and not fully understood. Previous studies have implicated polymorphonuclear neutrophils (PMN) as major inflammatory cells in IR injury. However, anti-PMN antiserum treatment offers only limited protection, indicating that other inflammatory cells are involved. We have therefore investigated the contribution of resident macrophages in IR injury using an IR gut injury model. Methods: DA rats were divided into sham operation and IR groups. The superior mesenteric artery was clamped for 30, 45, or 60 minutes (ischaemia) followed by 60 minutes of reperfusion. IR injuries were evaluated by histological staining. Expression of early growth response factor 1 (Egr-1), myeloperoxidase (MPO), and proinflammatory cytokines was analysed by immunohistochemistry, reverse transcription-polymerase chain reaction, and western blotting analysis. The specific role of macrophages in IR gut injury was also evaluated in resident macrophage depleted rats. Results: Mucosal sloughing and villi destruction were seen in 45/60 minute and 60/60 minute IR guts. PMN infiltration at the damaged mucosal area was undetectable in 45/60 minute and 60/60 minute IR guts. PMN were localised around the capillaries at the base of the crypts in 60/60 minute IR gut. Obvious PMN infiltration was only observed in damaged villi after three hours of reperfusion. Elevated nuclear Egr-1 immunostaining was localised in resident macrophages at the damaged villi before histological appearance of mucosal damage. Furthermore, resident macrophages at the damaged site expressed MPO. Protein levels of the proinflammatory cytokines RANTES and MCP-1 were increased in IR gut. Depletion of resident macrophages by dichloromethylene bisphosphonate significantly reduced mucosal damage in rat guts after IR. Conclusion: Our findings indicate that resident macrophages play a role in early mucosal damage in IR gut injury. Therefore, macrophages should be treated as a prime target for therapeutic intervention for IR damage.


http://gut.bmjournals.com/cgi/content/abstract/53/5/710

Background: Several animal models for human ulcerative colitis (UC) associated neoplasia have been reported. However, most neoplasias developed in these models have morphological and genetic characteristics different from UC associated neoplasia. Aims: To establish a new colitis associated neoplasia model in p53 deficient mice by treatment with dextran sulphate sodium (DSS). Methods: DSS colitis was induced in homozygous p53 deficient mice (p53/−/−DSS), heterozygous p53 deficient mice (p53+/−DSS) and wild-type mice (p53+/+DSS) by treatment with 4% DSS. Numbers of developed neoplasias were compared among the experimental groups, and macroscopic and microscopic features of the neoplasias were analysed. Furthermore, K-ras mutation and beta-catenin expression were assessed. Results: p53/−/−DSS
Mice showed 100% incidence of neoplasias whereas the incidences in p53/+--DSS and p53+/+-DSS mice were 46.2% and 13.3%, respectively. No neoplasias were observed in the control groups. The mean numbers of total neoplasias per mouse were 5.0 (p53/+--DSS), 0.62 (p53/+--DSS), and 0.2 (p53/++DSS). The number of neoplasias per mouse in the p53/+--DSS group was significantly higher than that in the other DSS groups. The incidences of superficial type neoplasias were 91.7% in p53/+--DSS mice, 75.0% in p53/+--DSS mice, and 33.3% in p53/++DSS mice. The K-ras mutation was not detected in any of the neoplasias tested. Translocation of beta-catenin from the cell membrane to the cytoplasm or nucleus was observed in 19 of 23 (82.6%) neoplasias. Conclusions: The p53/+--DSS mice is an excellent animal model of UC associated neoplasia because the morphological features and molecular genetics are similar to those of UC associated neoplasia. Therefore, this model will contribute to the analysis of tumorigenesis related to human UC associated neoplasia and the development of chemopreventive agents.


http://gut.bmjournals.com/cgi/content/abstract/gut.2004.062059v1

Background: Surveillance colonoscopy is widely recommended in patients with long-standing and extensive ulcerative colitis (UC), in order to detect colorectal neoplasia at an early stage. However, it still remains questionable whether surveillance colonoscopy effectively enables early detection of UC-associated neoplasia. There is a great need for sensitive markers to identify individuals at increased risk of neoplasia. The estrogen receptor (ER) gene shows age-related methylation in the colorectal epithelium and is methylated frequently in sporadic colorectal neoplasia, suggesting that ER methylation might predispose to colorectal neoplasia. Aim: To clarify whether analysis of methylation of the ER gene in non-neoplastic epithelium can contribute to the prediction of increased neoplasia risk in UC patients. Materials and Methods: A total of 165 non-neoplastic colorectal epithelia from 30 patients with long-standing and extensive UC, including 13 UC patients with neoplasia and 17 patients without, were evaluated. Methylation-specific PCR was performed to determine the methylation status of the ER gene. Results: Methylation of the ER gene was detected in 54 of 70 (77.1%) non-neoplastic colorectal epithelia in UC with neoplasia, whereas in only 23 of 95 (24.2%) without neoplasia. Methylation of the ER gene was significantly more frequent in non-neoplastic epithelium from UC with neoplasia than in chronic colitic epithelium from UC without neoplasia. In UC with neoplasia, furthermore, the ER gene was extensively methylated in non-neoplastic epithelia throughout the colorectum as compared with those in UC without neoplasia. Conclusion: These results suggest that analysis of ER gene methylation may have potential to be useful marker for identifying individuals at increased risk of neoplasia among patients with long-standing and extensive UC.


http://gut.bmjournals.com/cgi/content/abstract/52/4/563

Background: Hirschsprung's disease (HSCR) is a congenital disorder characterised by an absence of ganglion cells in the nerve plexuses of the lower digestive tract. Manifestation of the disease has been linked to mutations in genes that encode the crucial signals for the development of the enteric nervous system—the RET and EDNRB signalling pathways. The Phox2b gene is involved in neurogenesis and regulates Ret expression in mice, in which disruption of the Phox2b results in a HSCR-like phenotype. Aims: To investigate the contribution
of PHOX2B to the HSCR phenotype. Methods: Using polymerase chain reaction amplification and direct sequencing, we screened PHOX2B coding regions and intron/exon boundaries for mutations and polymorphisms in 91 patients with HSCR and 71 ethnically matched controls. Seventy five HSCR patients with no RET mutations were independently considered. Haplotype and genotype frequencies were compared using the standard case control statistic. Results: Sequence analysis revealed three new polymorphisms: two novel single nucleotide polymorphisms (A[-&gt;G]1364; A[-&gt;C]2607) and a 15 base pair deletion (DEL2609). Statistically significant differences were found for A[-&gt;G]1364. Genotypes comprising allele G were underrepresented in patients (19% v 36%; \( \chi^2 = 9.30; p = 0.0095 \) and 22% v 36%; \( \chi^2 = 7.38; p = 0.024 \) for patients with no RET mutations). Pairwise linkage disequilibrium (LD) analysis revealed no LD between physically close polymorphisms indicating a hot spot for recombination in exon 3. Conclusion: The PHOX2B A[-&gt;G]1364 polymorphism is associated with HSCR. Whether it directly contributes to disease susceptibility or represents a marker for a locus in LD with PHOX2B needs further investigation. Our findings are in accordance with the involvement of PHOX2B in the signalling pathways governing the development of enteric neurones.


http://gut.bmjournals.com/cgi/content/abstract/52/1/47

Background: It is well established that the wheat protein gliadin triggers inflammation in coeliac patients. However, the potential toxicity of avenin, the equivalent protein in oats, is debated. Aim: To investigate the immunogenicity of avenin using the cytokines interferon (gamma) (IFN-(gamma)) and interleukin (IL)-2 as markers of immunological activity. Methods: Duodenal biopsies from coeliac patients were cultured with 5 mg/ml of peptic tryptic (PT) gliadin (n=9) or 5 mg/ml of PT avenin (n=8) for four hours. Biopsies cultured with RPMI 1640 alone served as controls. Non-coeliac biopsies were also cultured with PT gliadin (n=8) and PT avenin (n=8). Total RNA was extracted from the tissue after culture. Cytokine mRNA was quantified by TaqMan polymerase chain reaction. Secreted cytokine protein was measured in the culture supernatant by enzyme linked immunosorbent assay. Results: After culture with PT gliadin, an increase in IFN-(gamma) mRNA was observed in all nine patients with coeliac disease. Increased IFN-(gamma) protein was also found in four of these patients. Smaller increases in IL-2 mRNA were detected in six subjects with increased IL-2 protein found in two patients. In contrast with PT gliadin, there was no significant IFN-(gamma) or IL-2 response when coeliac biopsies were cultured with PT avenin. Similarly, biopsies from normal controls did not respond to PT gliadin or PT avenin stimulation. Conclusions: The findings of this study suggest that the immunogenic sequences in gliadin are not present in avenin. Moreover, they are in keeping with in vivo studies which report that oats are safe for consumption by coeliac patients.


http://gut.bmjournals.com/cgi/content/abstract/52/8/1148

Background and aims: Colorectal epithelial cells are prone to malignant transformation. Therefore, identification of differences in gene expression in the process from normal colonic crypts to adenomas with low grade dysplasia is essential for further insights into early tumorigenesis. To achieve this goal, a novel gene expression analysis strategy, screening for expressed transcripts in small histologically defined tissue samples, was performed. Methods: First, laser mediated microdissection was used to isolate normal and adenomatous crypts from
colonic cryosections. Then, nested RNA arbitrarily primed polymerase chain reaction (RAP-PCR) for differential display was performed to screen mRNA populations and to generate hybridisation probes for cDNA expression arrays. After evaluation of cDNA expression arrays, differential expression was confirmed at the protein level by immunohistochemistry. Results: Evaluation of gene expression profiles of normal versus adenomatous colonic crypts of six different patients revealed, in general, dysregulation of up to 11% of all analysed genes (total number n=588): specifically, p21-rac1 was upregulated in four of six patients, mitogen activated protein kinase (MAPK) p38(.alpha) in three of six patients, and interferon (gamma) receptor in three of six patients. Conversely, FAST kinase was found to be downregulated in three of six patients, p53 in three of six patients, and thrombospondin 2 in three of six patients. Conclusions: For the first time, distinct gene expression profiles of dysplastic areas within colonic adenomas, using the combination of laser mediated microdissection with RAP-PCR and cDNA expression array, were shown. In these samples, upregulation of proliferation associated genes (ras-oncogene related p21-rac1 and MAPK p38(alpha)) as well as downregulation of apoptosis related genes (FAST kinase and p53) most likely reflects specific alterations in adenomas with low grade dysplasia. Based on upregulation of p21-rac1 and MAPK p38(alfa), activation of the MAPK pathway appears to be an early event in colonic carcinogenesis.


http://gut.bmjournals.com/cgi/content/abstract/53/3/331

Background and aims: Although peroxisome proliferator activated receptor (gamma) (PPAR(gamma)) agonists have been implicated in differentiation and growth inhibition of cancer cells, the potential therapeutic and chemopreventive effects on gastric cancer are poorly defined. We examined the in vitro and in vivo effects of PPAR(gamma) ligands on growth of gastric cancer, and the effect of PPAR(gamma) activation on expression of cyclooxygenase 2 (COX-2) and cancer related genes. Methods: Gastric cell lines (MKN28 and MKN45) were treated with two specific PPAR(gamma) ligands: ciglitazone and 15-deoxy-(Delta)12,14-prostaglandin J2. Cell growth was determined by bromodeoxyuridine incorporation assay and apoptosis was measured by DNA fragmentation. Expression of COX-2 was determined by western blot and real time quantitative polymerase chain reaction (PCR). Expression profiles of cancer related genes were screened with cDNA array. In vivo growth of implanted MKN45 cells in nude mice was monitored after oral treatment with rosiglitazone. Results: PPAR(gamma) ligands suppressed the in vitro growth of MKN45 cells in a dose dependent manner whereas prostacyclin, a PPAR(delta) agonist, had no growth inhibitory effect. Growth inhibition was more pronounced in MKN45 cells, which was accompanied by DNA fragmentation and downregulation of COX-2. Screening by cDNA microarray showed that PPAR(gamma) ligand treatment was associated with upregulation of bad and p53, and downregulation of bcl-2, bcl-xl, and cyclin E1 in MKN45 cells, which was confirmed by quantitative real time PCR. In contrast, MKN28 cells with lower PPAR(gamma) and COX-2 expression levels had lower growth inhibitory responses to PPAR(gamma) ligands. Microarray experiments only showed induction of the bad gene in MKN28 cells. In vivo growth of MKN45 cells in nude mice was retarded by rosiglitazone. Mean tumour volume in rosiglitazone treated mice was significantly lower than controls at six weeks (p = 0.019) and seven weeks (p = 0.001) after treatment. Conclusions: PPAR(gamma) ligands suppress both in vitro and in vivo growth of gastric cancer and may play a major role in cancer therapy and prevention.


http://gut.bmjournals.com/cgi/content/abstract/52/4/541
Background and aims: Genetic variation in the chromosome 5q31 cytokine cluster (IBD5 risk haplotype) has been associated with Crohn's disease (CD) in a Canadian population. We studied the IBD5 risk haplotype in both British and Japanese cohorts. Disease associations have also been reported for CARD15/NOD2 and TNF variants. Complex interactions between susceptibility loci have been shown in animal models, and we tested for potential gene-gene interactions between the three CD associated loci. Methods: Family based association analyses were performed in 457 British families (252 ulcerative colitis, 282 CD trios) genotyped for the IBD5 haplotype, common CARD15, and TNF-857 variants. To test for possible epistatic interactions between variants, transmission disequilibrium test analyses were further stratified by genotype at other loci, and novel log linear analyses were performed using the haplotype relative risk model. Case control association analyses were performed in 178 Japanese CD patients and 156 healthy controls genotyped for the IBD5 haplotype. Results: The IBD5 haplotype was associated with CD (p=0.007), but not with UC, in the British Caucasian population. The CARD15 variants and IBD5 haplotype showed additive main effects, and in particular no evidence for epistatic interactions was found. Variants from the IBD5 haplotype were extremely rare in the Japanese. Conclusions: The IBD5 risk haplotype is associated with British CD. Genetic variants predisposing to CD show heterogeneity and population specific differences.


http://gut.bmjjournals.com/cgi/content/abstract/53/5/685

Background and aims: The intestinal bacterial microflora plays an important role in the aetiology of inflammatory bowel disease (IBD). As most of the colonic bacteria cannot be identified by culture techniques, genomic technology can be used for analysis of the composition of the microflora. Patients and methods: The mucosa associated colonic microflora of 57 patients with active inflammatory bowel disease and 46 controls was investigated using 16S rDNA based single strand conformation polymorphism (SSCP) fingerprint, cloning experiments, and real time polymerase chain reaction (PCR). Results: Full length sequencing of 1019 clones from 16S rDNA libraries (n = 3) revealed an overall bacterial diversity of 83 non-redundant sequences—among them, only 49 known bacterial species. Molecular epidemiology of the composition of the colonic microflora was investigated by SSCP. Diversity of the microflora in Crohn's disease was reduced to 50% compared with controls (21.7 v 50.4; p<0.0001) and to 30% in ulcerative colitis (17.2 v 50.4; p<0.0001). The reduction in diversity in inflammatory bowel disease was due to loss of normal anaerobic bacteria such as Bacteroides species, Eubacterium species, and Lactobacillus species, as revealed by direct sequencing of variable bands and confirmed by real time PCR. Bacterial diversity in the Crohn's group showed no association with CARD15/NOD2 status. Conclusions: Mucosal inflammation in inflammatory bowel disease is associated with loss of normal anaerobic bacteria. This effect is independent of NOD2/CARD15 status of patients.


http://gut.bmjjournals.com/cgi/content/abstract/52/2/231

Background: Superoxide (O2-) generation through the activity of reduced nicotinamide dinucleotide (NADH) or reduced nicotinamide dinucleotide phosphate (NADPH) oxidases has been demonstrated in a variety of cell types, but not in human colonic epithelial cells. Aims: To measure O2- production and effects of modulators of NAD(P)H oxidase activity and inhibitors of potential O2- generating enzymes in cultures of human colonic epithelial cells. Expression of the catalytic subunits of NAD(P)H oxidase, Nox1 and gp91phox (phox, phagocytic oxidase), and the
membrane bound subunit p22phox was assessed. Methods: The transformed colonic epithelial cell lines (DLD-1, HT-29, and Caco-2) were studied at subconfluence, confluence, and after differentiation. Primary colonic epithelial cells were isolated from mucosal biopsies from the normal human colon. Extracellular O2- production was measured by the cytochrome c reduction assay or luminol enhanced luminescence. Nox1, gp91phox, and p22phox mRNA expression was assessed in colonic epithelial cells and blood neutrophils by reverse transcriptase-polymerase chain reaction. Results: Production rates of O2- were higher in subconfluent transformed cells (mean (SEM) 35.8 (4.2) nmol/mg of protein/h) and primary cells (40.4 (5.9)) than in confluent transformed cells (6.0 (0.9); p<0.01). The oxidoreductase inhibitor diphenylene iodonium significantly inhibited O2- production whereas NADPH and NADH increased production rates. In contrast, O2- was unaffected by phorbol myristate ester, NG-nitro-L-arginine methyl ester, indomethacin, or allopurinol. Nox1 mRNA was expressed in all colonic epithelial cells whereas gp91phox was detected only in HT-29 cells and neutrophils. p22phox was expressed in all cell types. Conclusions: Cultures of transformed and primary epithelial cells from human colon may produce extracellular O2- through an NAD(P)H oxidase expressing Nox1 and p22phox.


http://gut.bmjjournals.com/cgi/content/abstract/52/7/1060

Background and aim: Liver regeneration after severe liver damage depends in part on proliferation and differentiation of hepatic progenitor cells (HPCs). Under these conditions they must be able to withstand the toxic milieu of the damaged liver. ATP binding cassette (ABC) transporters are cytoprotective efflux pumps that may contribute to the preservation of these cells. The aim of this study was to determine the ABC transporter phenotype of HPCs. Methods: HPC activation was studied in rats treated with 2- acetylaminofluorene (2-AAF) followed by partial heptatectomy (PHx). ABC transporter gene expression was determined by real time detection reverse transcription-polymerase chain reaction in isolated HPCs, hepatocytes, cholangiocytes, and cultured progenitor cell-like RLF {phi} 13 cells and by immunohistochemistry of total liver samples. ABC transporter efflux activity was studied in RLF {phi} 13 cells by flow cytometry. Results: 2-AAF/PHx treated animals showed increased hepatic mRNA levels of the genes encoding multidrug resistance proteins Mdr1b, Mrp1, and Mrp3. Immunohistochemistry demonstrated expression of Mrp1 and Mrp3 proteins in periportal progenitor cells and of the Mdr1b protein in periportal hepatocytes. Freshly isolated Thy-1 positive cells and cultured RLF {phi} 13 progenitor cells highly expressed Mrp1 and Mrp3 mRNA while the hepatocyte specific transporters Mdr2, Bsep, Mrp2, and Mrp6 were only minimally expressed. Blocking Mrp activity by MK-571 resulted in accumulation of the Mrp specific substrate carboxyfluorescein in RLF {phi} 13 cells. Conclusion: HPCs express high levels of active Mrp1 and Mrp3. These may have a cytoprotective role in conditions of severe hepatotoxicity.


http://gut.bmjjournals.com/cgi/content/abstract/53/7/1001

Background and aim: Significant telomere shortening of hepatocytes is associated with replicative senescence and a non-dividing state in chronic liver disease, resulting in end stage liver failure and/or development of hepatocellular carcinoma. To prevent critical telomere shortening in hepatocytes, we have focused on oestrogen dependent transactivation of the human telomerase reverse transcriptase (hTERT) gene as a form of telomerase therapy in chronic liver disease.
Methods: We examined expression of hTERT mRNA and its protein, and telomerase activity (TA) in three human normal hepatic cell lines (Hc-cells, h-Nheps, and WRL-68) before and after treatment with 17\(^\beta\)-oestradiol. The effects of exogenous oestradiol administration were examined in a carbon tetrachloride (CCl4) induced model of liver fibrosis in rats. Results: Expression of hTERT mRNA and its protein was upregulated by oestradiol treatment. Telomere length decreased in Hc-cells and h-Nheps with accumulated passages whereas with long term oestradiol exposure it was greater than without oestradiol. The incidence of \(\beta\)-galactosidase positive cells, indicating a state of senescence, decreased significantly in oestradiol treated cells in comparison with non-treated cells (p<0.05). TA in both male and female rats with CCl4 induced liver fibrosis was significantly higher with oestradiol administration than without (p<0.05). Long term oestradiol administration markedly rescued the hepatic telomere from extensive shortening in both male and female rats. Conclusion: These results suggest that oestradiol acts as a positive modulator of the hTERT gene in the liver. Oestrogen dependent transactivation of the hTERT gene is a new strategy for slowing the progression of chronic liver disease.


http://gut.bmjournals.com/cgi/content/abstract/50/4/530

Background: Although circulating tumour DNA has been detected in patients with different types of cancer, little is known of free RNA in cancer patients. Aims: We investigated the presence of RNA from epithelial tumours in plasma from patients with colorectal carcinomas, and its correlation with tumour characteristics and circulating tumour cells. Methods: \(\beta\)-actin mRNA was analysed to assess the viability of plasma RNA in samples from 53 patients with colonic cancer and 25 controls. Subsequently, nested primers were used to detect the presence of cytokeratin 19 (CK19) and carcinoembryonic antigen (CEA) RNA in the same samples. Nine clinicopathological parameters were studied to correlate the molecular and clinical parameters. Additionally, we investigated for micrometastases in blood in 18 of these patients and in 10 of the controls samples. Results: All samples had detectable quantities of \(\beta\)-actin RNA. In the controls, one case (4%) was positive for CEA and five (20%) for CK19 RNA; of the 53 patients, 17 cases (32%) were positive for CEA and 39 (73.6%) for CK19 RNA. This was statistically significant (p=0.000001). Advanced stages (p=0.03) and soluble CEA status (p=0.03) were associated with the presence of CEA, CK19, or both RNAs in plasma. Lymph node metastases (p=0.06) and vascular invasion (p=0.07) were almost significant. On the basis of these results, we examined the possible presence of micrometastases in blood in several of these patients. The presence of plasma tumour RNA was found to be associated with circulating tumour cells in blood (p=0.04). Conclusions: Epithelial tumour RNA is detectable in plasma from colon cancer patients. This molecular event is associated with advanced stages and circulating tumour cells. Our results could offer new approaches in the diagnosis and monitoring of colon cancer.


http://gut.bmjournals.com/cgi/content/abstract/52/5/706

Backgrounds: The Raf/MEK/ERK (mitogen activated protein kinase--MAPK) signal transduction cascade is an important mediator of a number of cellular fates, including growth, proliferation, and survival. The BRAF gene, one of the human isoforms of RAF, is activated by oncogenic Ras, leading to cooperative effects in cells responding to growth factor signals. Aims: The aim of this study was to elucidate a possible function of BRAF in liver tumours. Methods: Mutations of BRAF and KRAS were evaluated in 25 hepatocellular carcinomas (HCC) and in 69
cholangiocarcinomas (CC) by direct DNA sequencing analyses after microdissection. The presence of active intermediates of the MAPK pathway was assessed immunohistochemically. The results obtained were correlated with histopathological variables and patient survival.

Results: Activating BRAF missense mutations were identified in 15/69 CC (22%) and in one case of tumour surrounding liver. KRAS mutations were found in 31 of 69 (45%) CC examined and in two cases of tumour surrounding non-neoplastic liver tissue. In HCC, neither BRAF nor KRAS mutations were detected. All 31 CC with KRAS mutations had an intact BRAF gene. We failed to observe a correlation between BRAF or KRAS mutations and histopathological factors or prognosis of patients. Conclusions: Our data indicate that BRAF gene mutations are a relatively common event in CC but not in HCC. Disruption of the Raf/MEK/ERK (MAPK) kinase pathway, either by RAS or BRAF mutation, was detected in approximately 62% of all CC and is therefore one of the most frequent defects in cholangiocellular carcinogenesis.

Waidmann, M., Y. Allemand, et al. (2002). "Microflora reactive IL-10 producing regulatory T cells are present in the colon of IL-2 deficient mice but lack efficacious inhibition of IFN-({gamma}) and TNF-({alpha}) production." Gut 50(2): 170-179.

http://gut.bmjournals.com/cgi/content/abstract/50/2/170

Background: Inflammatory bowel disease in interleukin 2 (IL-2) deficient (IL-2-/-) mice is triggered by the intestinal microflora and mediated by CD4+ T cells. Aims: To determine the characteristics of microflora specific intestinal T cells, including migration and cytokine production. Methods: Intestinal T cell populations and cytokine mRNA expression of specific pathogen free (SPF) and germ free (GF) IL-2-/- and IL-2+/+ mice were compared by flow cytometry and reverse transcription-polymerase chain reaction. Cytokine production of intestinal mononuclear cells on stimulation with microflora antigens was assessed by ELISA. In vivo migration of T cells was assessed by adoptive transfer of 51Cr labelled CD4+CD25-{alpha}+ T cells. The ability of intestinal T cell lines to promote colitis was determined by adoptive transfer experiments. Results: SPF IL-2-/- mice produced higher interferon {gamma} (IFN-({gamma})) and tumour necrosis factor {alpha} mRNA levels than GF IL-2-/- mice, which was accompanied by an increased number of CD4+{alpha}+ T cells in the colon. Tracking of 51Cr labelled and adoptively transferred T cells revealed an increased MAdCAM-1 dependent but VCAM-1 independent recruitment of these cells into the colon of SPF IL-2-/- mice. Colon lamina propria lymphocytes (LPL) from SPF IL-2-/- mice showed increased spontaneous IFN-({gamma}) production in vitro. On stimulation with bacterial microflora antigens, intraepithelial lymphocytes and LPL did not produce IFN-({gamma}), but high quantities of IL-10, which did not suppress IFN-({gamma}) production. Bacterial antigen specific cell lines established from colon LPL of SPF IL-2-/- mice with colitis showed a regulatory T cell-like cytokine profile and only marginally modulated the course of colitis and survival of IL-2-/- mice. Conclusions: Our results suggest that microflora reactive regulatory T cells are present in the colon of SPF IL-2-/- mice. However, IL-10 produced by these cells did not significantly modulate a possible secondary proinflammatory CD4 Th1 cell population to produce IFN-({gamma}).


http://gut.bmjournals.com/cgi/content/abstract/53/10/1452

Background and aims: Serotonin (5-hydroxtryptamine, 5-HT) is an important factor in gut function, playing key roles in intestinal peristalsis and secretion, and in sensory signalling in the brain-gut axis. Removal from its sites of action is mediated by a specific protein called the serotonin
reuptake transporter (SERT or 5-HTT). Polymorphisms in the promoter region of the SERT gene have effects on transcriptional activity, resulting in altered 5-HT reuptake efficiency. It has been speculated that such functional polymorphisms may underlie disturbance in gut function in individuals suffering with disorders such as irritable bowel syndrome (IBS). The aim of this study was to assess the potential association between SERT polymorphisms and the diarrhoea predominant IBS (dIBS) phenotype. Subjects: A total of 194 North American Caucasian female dIBS patients and 448 female Caucasian controls were subjected to genotyping. Methods: Leucocyte DNA of all subjects was analysed by polymerase chain reaction based technologies for nine SERT polymorphisms, including the insertion/deletion polymorphism in the promoter (SERT-P) and the variable tandem repeat in intron 2. Statistical analysis was performed to assess association of any SERT polymorphism allele with the dIBS phenotype. Results: A strong genotypic association was observed between the SERT-P deletion/deletion genotype and the dIBS phenotype (p = 3.07x10-5; n = 194). None of the other polymorphisms analysed was significantly associated with the presence of disease. Conclusions: Significant association was observed between dIBS and the SERT-P deletion/deletion genotype, suggesting that the serotonin transporter is a potential candidate gene for dIBS in women.


http://gut.bmjournals.com/cgi/content/abstract/51/4/480

Background: Helicobacter pylori blood group antigen binding adhesin (BabA) mediates bacterial adherence to human blood group antigens on gastric epithelium. Although strains harbouring babA2 were recently found to be associated with peptic ulcer and gastric cancer, the role of babA2 in cellular turnover, severity of gastritis, and premalignant changes is poorly understood. Aim: We correlated H pylori babA2, vacuolating toxin (vacA), and cytotoxin associated gene A (cagA) genotypes with the severity of gastric inflammation and epithelial cell turnover in a group of Chinese patients from an area with a high incidence of gastric cancer. Patients and methods: H pylori isolates were obtained from 104 Chinese patients who participated in a gastric cancer prevention programme. Genotype variants of babA2, vacA, and cagA were determined by polymerase chain reaction. Antrum and corpus histopathology was examined according to the updated Sydney classification. Apoptosis was scored by terminal uridine deoxynucleotidyl nick end labeling (TUNEL) and proliferation by Ki-67 immunostaining. Results: Of the 104 patients, 102 (98.1%) harboured cagA+ strains and all had vacA s1 genotype. The babA2+ strains were found in 83 (79.8%) patients and were associated with higher lymphocytic infiltration (p=0.028), presence of glandular atrophy (odds ratio (OR) 7.5, 95% confidence interval (CI) 2.3-24.3), and intestinal metaplasia (OR 7.4, 95% CI 2.2-25.3) in the antrum. Increased epithelial proliferation was also noted in individuals infected with babA2+ strains (p=0.025). Strains harbouring cagA+/vacA s1 genotypes lacked this association in the absence of babA2. Conclusions: The presence of babA2+ H pylori strains alone or in combination with cagA+ and vacA s1 was associated with the presence of preneoplastic gastric lesions.


Gynecologic Oncology (28)

Objective(s)

To compare the type-specific human papillomavirus (HPV) recovery from physician and patient-collected samples.

Methods

Three hundred thirty-four (334) women attending colposcopy clinics in three countries were enrolled in this cross-sectional study. Cervicovaginal samples were collected by patients and physicians and processed with polymerase chain reaction and reverse line blot genotyping. McNemar's Chi-squared tests and Kappa statistics were utilized to determine statistical associations between physician- versus patient-collected samples.

Results

Oncogenic HPV infection was identified in 23.2% of patient-collected specimens compared to 34.9% of physician-collected specimens. Physician sampling detected significantly more infections with type 16 and 52 than did self-sampling and significantly more oncogenic HPV infection overall. For non-oncogenic HPV detection, there was no statistical difference between physician- and patient-collected samples.

Conclusion(s)

Patient sampling for HPV using a single vaginal brush does not identify all oncogenic HPV subtypes.


Objective

Osteopontin (OPN) is a glycoprotein of the extracellular matrix that can bind to different types of receptors including integrins and CD44 receptors. Multiple binding affinity enables OPN to play a role in many physiological and pathological processes. OPN contributes to tumorigenesis in several types of cancers. OPN is also expressed by the endometrium and by trophoblast cells of the chorionic villus in human placenta, where OPN may regulate implantation and placentation in early pregnancies by promoting cell-cell interactions, adhesion, spreading, and migration of trophoblast. Our purpose was to determine the expression of OPN mRNA and protein in hydatidiform mole and in normal placenta of comparable gestational age.

Methods

A total of 13 fresh tissues from complete hydatidiform moles, 2 from partial hydatidiform moles, and 9 from normal placentas were analyzed by performing quantitative real-time PCR on microdissected trophoblast cells and immunohistochemistry on frozen sections of tissue.

Results

Our results showed significantly lower expression of OPN mRNA and protein in hydatidiform mole, and in particular complete mole (P = 0.001 by real-time PCR and P Conclusion

Although precise molecular mechanisms of gestational trophoblastic diseases have not yet been determined, down-regulation of osteopontin may play an important role in the pathogenesis of molar pregnancy.


Objective

A deletion variant in the CHEK2 gene (del1100C) has been implicated as a low-penetrance risk factor for breast cancer. We sought to determine contribution of CHEK2 mutations to the etiology of ovarian cancer (OvCa). Methods

We used cases ascertained from the United States through Gynecologic Oncology Group (GOG) protocols 172, 182, and 144, the University of Hawaii Cancer Research Center, and Creighton University. Control women were recruited from Pittsburgh and Hawaii. Denaturing high-performance liquid chromatography,
sequence analysis, and single nucleotide polymorphism genotyping by Pyrosequencing were employed to analyze the CHEK2 gene. Results: Mutation screening of the CHEK2 gene in 48 cases who had a first-degree relative with OvCa uncovered only del1100C and A252G variants. Altogether, the del1100C variant was detected in none of 751 unselected cases, in 1 of 52 (1.9%) cases who had a first-degree relative with OvCa, and in 3 of 521 (0.6%) unselected controls. The frequencies of del1100C and A252G variants did not show statistically significant differences between the cases and the controls. Conclusions: These results suggest that variations in CHEK2 do not make a significant contribution to the pathogenesis of OvCa in the U.S. population.


http://www.sciencedirect.com/science/article/B6WG6-4C5HR81-4/2/e36759276f9b89c1a20d680c942e5b0

Objectives. We studied the role of epigenetic and genetic changes of PTEN in the development of squamous cell carcinoma (SCC) of the uterine cervix and their value as a prognostic factor. Methods. Ten high-grade cervical intraepithelial neoplasia (CIN-H) and 62 SCC tissues were used in this study. Microdissection was performed before loss of PTEN function through methylation of promoter CpG islands, deletion and mutation were studied. The findings were verified with PTEN protein expression and correlated with clinico-pathologic information. Results. PTEN mutation assessed by single-strand conformation polymorphism (PCR-SSCP) was not noted in any of the 62 SCC. Loss of heterozygosity (LOH) was only seen in eight SCC. PTEN promoter methylation was detected in 40% (4/10) of CIN-H and 58% (36/62) of SCC specimens. Loss of PTEN protein expression was associated with methylation of PTEN. PTEN methylation was not related to patient age, tumor grade or stage. Patients with persistent disease or who died of disease had a significantly higher percentage of PTEN methylation than those without evidence of recurrence. Multivariate Cox regression models confirmed PTEN was an important significant predictor both for total and disease-free survival after controlling age, pathologic grade and clinical stage. Conclusions. PTEN methylation and loss of PTEN expression are early events in the development of cervical cancer and may have prognostic significance.


http://www.sciencedirect.com/science/article/B6WG6-4F05G1K-F/2/4aa71d09ecd5602c57b6ffe025e05cb0

Objectives. We studied the loss of heterozygosity (LOH) in chromosome 1 in squamous cell carcinoma (SCC) of the uterine cervix and evaluated its clinical and pathological significance. Methods. Sixty-three highly polymorphic markers were used to study the LOH in 84 SCC. Microdissection was performed to enrich the tumor cells population before the alleotyping study. The findings were correlated with clinicopathologic findings. Results. LOH was detected in all but one SCC. The number of loci showing LOH in each case ranged from 0 to 41. Five loci showed LOH in greater-than-or-equal to 30% SCC and 28 other loci had an LOH frequency between 20% and 30%. Six of the eight markers located at 1p36.21 to 1p36.33 had a frequency of LOH >20%. Shortened total survival was associated with LOH at 14 loci and shortened disease-free survival was associated with LOH at 11 loci while LOH at nine loci were associated with both. A high frequency of LOH was associated with stage as well as shortened total and disease-free survival. Conclusions. LOH is a common and early event in the development of cervical SCC. Tumor suppressor genes may be present at 1p36. The incidence of LOH increases as the tumor progresses but a high frequency of LOH is not an independent prognostic factor.

http://www.sciencedirect.com/science/article/B6WG6-48N2PVH-9/2/b7d6a509fb3a4b5d78ed702b9fa1302

Introduction

We aimed to verify not only whether homozygous Arg at codon 72 of the p53 apoptotic domain is a possible risk factor for cervical human papillomavirus (HPV)-related cancer, but whether degraded p53 may have an effect on a G2 checkpoint of the cell cycle. The implication of the codon 72 polymorphism of p53 in cervical tumor remains controversial. Furthermore, G2 checkpoint alteration and its relationship with p53, the codon 72 allotype, according to HPV infection in cervical tumors, has not been studied.

Materials and methods

The purified genomic DNA from 252 archival cervical tissues [102 cervical intraepithelial neoplasias (CINs) and 46 squamous cell carcinomas of the uterine cervix (SCCs), and 104 normal] were amplified by nested polymerase chain reaction (PCR) for HPV-16/HPV-18. In addition, all of them were amplified by PCR for exon 4 of p53, where the codon 72 resides. The amplified PCR products were then sequenced using the forward primer. A polymorphism analysis was done by SnaPshot ddNTP primer extension and following direct sequencing. The reaction mixture was treated with 0.25 unit of shrimp alkaline phosphatase (Amersham) at 37°C for 1 h, subsequently performed in an ABI Prism 310 Genetic Analyzer (Perkin-Elmer). The archival slides were incubated overnight at 4°C using mouse anti-human recombinant cyclin B1 polyclonal antibody or mouse anti-Xenopus p34cdc2 monoclonal antibody for immunohistochemistry (Santa Cruz Biotech, Santa Cruz, CA).

Results

The frequency of Arg allelic homozygosity was high in both cases (89.1%) and the control (80.8%) group (P = 0.4703). All groups except CIN were in Hardy-Weinberg equilibrium. There was no significant difference in the frequency of p53 polymorphism between the HPV-positive (Arg, 88.0%) and the negative (Arg, 88.8%) groups, or between CIN (Arg, 88.2%) and SCC (Arg, 89.1%). Both immunoreactivities to cyclin B and p34cdc2 were strongly correlated with the HPV infection (P = 0.0001) and the histological types (P = 0.0001) between CIN and SCC, being strongly correlated with each other ([alpha]:0.62954, P = 0.0001). Conclusion

The particular type of the p53 polymorphism does not bear relation to the progression of cervical cancer, HPV infection, or to the p53 codon 72 polymorphism. However, the G2 checkpoint appears to be altered in the case of a HPV-positive SCC.


http://www.sciencedirect.com/science/article/B6WG6-4DM29PK-1/2/65dfd06ab81791947f9a2e96455578e1

Objectives

Microarray expression analysis of cervical tumors has revealed differential expression of genes that may be useful as markers or targets for treatment. We question the application of array findings across the major categories of cervical cancer. We sought to identify differences between normal squamous epithelium (NSQ) and glandular epithelium (NGL) of the uterine cervix and their malignant variants: squamous cell cancer (SCC) and adenocarcinoma (ACA).

Methods

Eight genes were selected: 12-lipoxygenase (12-LOX), keratin 4, trypsinogen 2 (TRY2), Rh glycoprotein C (RhGC), collagen type V alpha 2, integrin alpha 5, integrin alpha 6, and c-myc. Ten cases each of SCC and ACA of the cervix were selected from our tumor bank. NSQ and NGL epithelia were obtained from consecutive patients undergoing surgery for benign disease. RNA extraction, cDNA synthesis, and DNA amplification of all samples were performed according to an established protocol. Electrophoresis of the multiplexed polymerase chain reaction (PCR) products was performed under standard conditions, followed by digital image
capture. A ratio of target to control gene (β-actin) was obtained for each sample. Analysis of variance was applied to the mean ratios for each tissue to establish significant differences. Individual pairwise comparisons were made by Student t tests and verified with the Tukey-Kramer test.

Results. Clinically valid comparisons are NSQ to NGL, NSQ to SCC, NGL to ACA, and SCC to ACA. Various expression patterns were observed between the epithelia and their malignant phenotypes. Significant differences in gene expression were observed between benign squamous and glandular epithelium in four of the eight genes and between malignant squamous and glandular epithelium in three of the eight genes. Significant differences in gene expression between benign and malignant tissues were demonstrated in four of the eight genes.

Conclusions. We have defined significant differential expression changes between the two principal cervical tumor types. Differences in genes are demonstrated and must be considered if array technology is applied to the study of the biologic behavior of these tumors as well as their screening and management. The observed differential expression should be a compelling argument to perform type-specific expression analysis for other tumors with histological variants.


http://www.sciencedirect.com/science/article/B6WG6-4DS947X-5/2/25a74b647ce928b9796f1da165622cb8

Background. Cervical cancer represents a major health problem in Venezuela as well as in other Latin American countries. High-risk human papillomavirus (HR-HPV) infection is known as the major risk factor of cervical cancer. However, whether or not a HR-HPV-infected woman progresses to cervical cancer may depend on the immune system effectors induced by viral antigens presented by her specific human leukocyte antigens (HLA) alleles. The role of the HLA system in presenting peptides to antigen-specific T-cells may be critical for genetic susceptibility and genetic resistance to cervical carcinoma.

Objective. We aimed to investigate the relationship between HLA-DQB1, HPV infection, and cervical cancer in Venezuelan women.

Methods. Blood samples and cervical swabs were obtained from 36 patients and 79 healthy controls; additional cervical biopsies were obtained from all the patients. HPV DNA was detected by PCR and HLA-DQB1 genotyping was performed using a PCR-SSP protocol.

Results. A positive association with cervical cancer was observed for HLA-DQB1*0201-0202 and *0402 alleles, however after Bonferroni correction only HLA-DQB1*0402 remained statistically significant (P value = 0.004, RR = 5.067).

Conclusion. This is the first report of HLA-DQB1 alleles associated with cervical carcinoma in Venezuelan women. Larger studies are needed to assess whether these HLA-DQB1*0201-0202 and *0402 alleles have a direct effect on disease susceptibility.


http://www.sciencedirect.com/science/article/B6WG6-4DTSJH7-7/2/4f43c33ead14b22b9a699c2c500df7

Objective. We have shown that HER2/Neu may activate the Smad7 promoter in endometrial, ovarian, and breast cancer cell lines. Elevated Smad7 levels could then antagonize the TGF-[beta] pathway, leading to a reduction in tumor surveillance and potential cancer formation. Our aim was to determine if Smad7 was in fact overexpressed in endometrial cancers and whether Smad7 RNA levels correlated with tumor grade or clinical endpoints.

Methods. Snap-frozen endometrial cancer specimens from 16 patients with grade 1 disease and 23 patients with grade 3 disease were obtained. Additionally, the endometrium from 18 patients who underwent hysterectomy for benign indications was collected as a control. RNA was extracted and subjected
to quantitative real-time PCR to determine the degree of Smad7 RNA expression. Clinical outcomes including time to recurrence were recorded through retrospective chart review.

Results Smad7 transcripts in the tumors were over 11-fold elevated on average than in controls (P<Smad7 RNA between grades 1 and 3 tumors. For the 19 patients who recurred, median time to recurrence was 56.3 months for those with low Smad7 expression versus 30 months for those with high Smad7 expression (P<0.001). Conclusion Smad7 appears to be upregulated in endometrial cancers compared to normal endometrium. Furthermore, high Smad7 gene expression was associated with a shorter time to recurrence. Given that many endometrial cancers have been shown to be TGF-[beta]-unresponsive, Smad7 should be investigated as a potential target to restore TGF-[beta] responsiveness and limit tumor growth.


Objective Human ovarian carcinoma samples were orthotopically implanted into SCID mice to investigate the contribution of matrix metalloproteases (MMPs) to the spread of ovarian tumors. Methods Mice were inoculated with patient tumor samples, and developed ovarian tumors over a 16-week period with metastasis occurring in some mice. Species-specific quantitative RT-PCR was used to identify the source of tumor-associated MMPs. Results Membrane-type (MT)1-MMP mRNA was significantly increased in high-grade tumors, tumors with evidence of serosal involvement, and tumors in which distant metastases were detected. The increase in MT1-MMP expression was predominantly from the human tumor cells, with a minor contribution from the mouse ovarian stroma. Neither human nor mouse MT2-MMP were correlated with tumor progression and MT3-MMP levels were negligible. While tumor cells did not produce significant amounts of MMP-2 or MMP-9, the presence of tumor was associated with increased levels of MMP-2 expression by mouse ovarian stroma. Stromal-derived MT1-MMP was greater in large tumors and was associated with stromal MMP-2 expression but neither was significantly linked with metastasis. Conclusions These studies indicate that tumor-derived MT1-MMP, more so than other gelatinolytic MMPs, is strongly linked to aggressive tumor behavior. This orthotopic model of human ovarian carcinoma is appropriate for studying ovarian tumor progression, and will be valuable in the further investigation of the metastatic process.


http://www.sciencedirect.com/science/article/B6WG6-4DFBSV4-7/2/77a1e2e577385cc23f4a56f75b27bd57

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tumors and was associated with stromal MMP-2 expression but neither was significantly linked with metastasis. Conclusions These studies indicate that tumor-derived MT1-MMP, more so than other gelatinolytic MMPs, is strongly linked to aggressive tumor behavior. This orthotopic model of human ovarian carcinoma is appropriate for studying ovarian tumor progression, and will be valuable in the further investigation of the metastatic process.


http://www.sciencedirect.com/science/article/B6WG6-4BG8T8V-7/2/079d7315a426a1c5b9d7f69575389661

Objective. To determine whether squamous cervical cancers exhibit mutations or deletions in MHC class I genes or transport-associated protein (TAP) genes. Methods. Polymerase chain reaction based protocols were used to examine HLA class I and TAP genes in a panel of cervical tumours, using DNA from corresponding blood samples as controls. SSP-PCR protocols were similarly used for examination of all TAP alleles in tumour and blood samples. Results. In a series of cervical carcinomas, 7 of 27 (26%) exhibited mutations in HLA-A genes, while 12 of 23 (52%) exhibited mutations in TAP genes. HLA gene mutations were detected in 2 of 14 CIN2-3 lesions, and TAP gene mutations in none of 14, a frequency significantly less than observed in the cervical carcinoma samples (P < 0.05). Conclusion. These data suggest that TAP genes may be relevant to evolution of cervical cancer from precursor lesions.


http://www.sciencedirect.com/science/article/B6WG6-4C2R20C-2/2/5c97a5ada80b403eb0d955eff7866d13

Objective. Data regarding signal transduction pathways in human tumors are largely confined to cell line studies to date. We have recently reported on the activation and prognostic role of mitogen-activated protein kinases (MAPK) in ovarian carcinoma in effusions. The objective of the present study was to investigate the expression and clinical role of dual-specificity phosphatases (DUSP), inhibitors of MAPK signaling, in ovarian cancer cells at this site. Methods. Thirty-nine fresh frozen malignant effusions from patients diagnosed with serous ovarian carcinoma were studied for mRNA expression of the DUSP MKP-1, MKP-4, MKP-5, and PAC-1 using RT-PCR. DUSP expression was analyzed for possible correlation with patient age, disease stage, tumor grade, histological grade, chemotherapy status, and survival. Results. MKP-1 and PAC-1 mRNA were found in 36 and 37 effusions, respectively, with expression levels showing considerable variation. MKP-4 and MKP-5 were uniformly absent. MKP-1 showed no association with clinicopathologic parameters. However, PAC-1 expression was significantly higher in effusions obtained before the institution of treatment with both platinum compounds (P = 0.029) and paclitaxel (P = 0.036). In univariate survival analysis, high level of expression of PAC-1 mRNA predicted significantly worse overall survival compared to low expression (mean = 30 vs. 52 months, MEDIAN = 25 vs. 46 months) (P = 0.007). Conclusions. Despite the limited size of this cohort, our results present the first evidence supporting a clinical role for PAC-1 in ovarian carcinoma. In view of the improved outcome associated with activation of all three MAPK families, as well as their elevated expression and activation in post-chemotherapy specimens presented in our previous work, they also suggest that PAC-1 is a true negative regulator of MAPK in ovarian carcinoma cells in effusions.
Objective The likelihood of developing cervical cancer has been shown to be increased in persons with certain HLA alleles. We evaluated immune response genes in the HLA region of chromosome 6 to see if individual or interactive associations with cervical cancer risk could be identified.

Methods Tissue was obtained from 127 women undergoing surgical treatment for cervical cancer. Blood samples were obtained from 175 control subjects. A combination of polymerase chain reaction (PCR), sequence-specific PCR, and DNA sequencing was used to evaluate polymorphic alleles, including HLA class I B7, TNF [alpha], HLA class II DR2, TAP1, and TAP2 genes. Fisher's exact test and logistic regression modeling were used for statistical analysis.

Results A significantly greater proportion of the patients with cervical cancer were found to have the HLA class II DR2 1501 allele (P = 0.023) and the TAP2 A/B heterozygous pattern of alleles (P = 0.0006) than were women without cervical cancer. A proportion of patients with cervical cancer significantly smaller than that of the control women had a polymorphism at the -238 position of the TNF promoter and the TAP1 C/C homozygous pattern of alleles. With logistic modeling, the markers that showed consistent association with the occurrence of cervical cancer were TAP2 A/B, HLA-DR2 1501, and TAP1 C/C.

Conclusions We demonstrated a significant association between immune response genes and the risk of cervical cancer. Our data create a compelling argument for a gene or a cluster of genes in the HLA region of chromosome 6 that regulates host immune responses to human papillomavirus infection in a manner that results in inherited susceptibility or resistance to the transforming properties of oncogenic papillomaviruses.


Vulvar intraepithelial neoplasia (VIN) is becoming more widespread and the patients are becoming still younger. Although progression to invasive vulvar carcinoma is uncommon, local recurrences are frequent and about one-quarter of the patients have multicentric genital disease. The aim of the present study was to search for a possible significant association of human papillomavirus (HPV) infection with vulvar carcinoma, recurrences, and multicentric disease. We used the polymerase chain reaction to examine vulvar and cervical biopsies from 43 patients with vulvar neoplasia for HPV type 16, which is the subtype most often detected in genital malignant or premalignant lesions. HPV 16 DNA sequences were found in 14 of 24 (58%) vulvar squamous carcinomas and in 15 of 19 (79%) VIN lesions. Nine patients (21%) had associated cervical neoplasia and six of these harbored HPV 16 in both lesions. Patients with recurrent intraepithelial neoplasia had a significantly higher incidence of HPV 16-positive lesions. No association was found with regard to the occurrence of multicentric disease or risk of malignant progression.

Fifty cervical adenocarcinomas and 50 squamous cell carcinomas from age-matched patients were examined for human papillomavirus (HPV) types 16 and 18. The polymerase chain reaction was used to examine formalin-fixed, paraffin-embedded carcinoma tissues for 120 and 113 bp sequences, respectively, of the highly conserved E6/E7 regions of the viral genomes. HPV type 16 was detected more often in squamous cell carcinomas than in adenocarcinomas (60% vs 18%, P < 0.001). These differences may reflect the fact that different virus receptors exist in cervical cells with different morphologic potential, or they may indicate that the specific HPV infection actually plays a role in directing carcinogenesis.

Objective. Topotecan, a novel topoisomerase-I inhibitor, is an active agent of second-line chemotherapy for extending the platinum-free interval (PFI) and improving the chances of a response to platinum in recurrent ovarian cancer patients. The aim of this study was to understand the molecular mechanism of topotecan-based second-line chemotherapy through an in vitro cell culture model and to gain clinical insight into sequencing issues for second-line treatment with novel agents versus retreatment with platinum. Study design. The human ovarian cancer cell line A2780 and the cisplatin resistance cell line A2780-CR were separately seeded in 6-well cell culture plates and then exposed to multiple concentrations of cisplatin plus paclitaxel or topotecan for 7 days. Surviving cells were recovered and cultured in drug-free media for 3 weeks and then replated in a 96-well microtiter plate. The LD50 for these cells was determined by a cytotoxic MTT assay after exposure to multiple clinically relevant concentrations of cisplatin or topotecan. Surviving cells were cultured in drug-free media for an additional 4 weeks at which time the LD50 was reassessed for each cell population by a second MTT assay. Using RT-PCR and Northern blot hybridization to measure mRNA expression, the molecular profile of these cells in terms of resistance was evaluated for the multidrug-resistant gene (MDR-1), multidrug-resistant protein (MRP), Topoisomerase-I, and [beta]-Actin. Results. The LD50 to cisplatin was unchanged in A2780-CR cells treated by topotecan. Those A2780-CR cell lines originally exposed to higher concentrations of cisplatin became more resistant to cisplatin in the MTT assays, while those A2780-CR cell lines treated with a combination of lower cisplatin concentrations and paclitaxel became more sensitive to cisplatin in the MTT assay (P 50 for cisplatin in every cell line decreased significantly after a 4-week drug-free interval (P Conclusions. The acquired resistance to cisplatin in A2780 is potentially due to P-glycoprotein-mediated multidrug resistance. This acquired resistance to cisplatin is an unstable phenotype in that some cell populations become sensitive after a drug-free interval and topotecan treatment. This reversal of resistance, however, does not appear to be simply due to loss of MDR-1 expression. While in vivo confirmation is required, agents with novel mechanisms of action offer a strategy to extend the platinum-free interval and thereby improve survival in patients with recurrent ovarian cancer.


http://www.sciencedirect.com/science/article/B6WG6-4DTSJH7-1/2/b674a7f0cb825cb1990a8a6f1b07dcb5
Objective. (1) To identify and (2) validate genes that are up-regulated in ovarian cancer, and (3) to investigate whether the activity of a candidate gene, creatine kinase B (CKB) is elevated in pre-operative sera from ovarian cancer patients compared to patients with benign pelvic masses and normal controls.

Methods. MICROMAX cDNA microarray system and RNA derived from pooled ovarian cancer cell lines and normal ovary surface epithelial cells (HOSE) were used to identify differentially expressed genes. Using RNA from both cell lines and from tissue obtained through laser capture microdissection (LCM), we performed quantitative PCR in order to validate up-regulation of one of these genes, creatine kinase B (CKB). Using a commercially available enzyme assay, CKB activity was measured in pre-operative serum samples obtained from 45 ovarian cancer patients, 49 patients with a benign pelvic mass, as well as 37 normal controls. Statistical analysis was performed using an unpaired Student's t test.

Results. Microarray technology revealed that CKB gene expression had a cancer to HOSE ratio of 18. RNA levels of CKB, measured by real-time PCR, were elevated a mean (and standard error) of 36-fold (8.4) in cancer cell lines compared with HOSE cells and 22.75-fold (10.45) in microdissected ovarian cancer epithelial cells compared with normal ovarian epithelial cells. In serum, the mean ([plus-or-minus sign]standard error) of CKB enzyme activity in cancer cases was 24.7 U/L units ([plus-or-minus sign]5.1) compared to 9.6 U/L ([plus-or-minus sign]1.6) for benign mass cases (P = 0.0088) and to 8.5 U/L (1.7) for normal controls (P = 0.0096).

Conclusions. Microarray technology offers a method to identify tumor biomarkers with potential clinical usefulness. Our data indicated that CKB gene expression is up-regulated in ovarian cancer cells in vitro and in vivo and that CKB enzyme activity is significantly elevated in sera from ovarian cancer patients, including those with stage I disease. These findings suggest a potential role for CKB as a marker for early diagnosis.


http://www.sciencedirect.com/science/article/B6WG6-4D09FKH-1/2/fa109c8877bcb2bbaa3296c3fb538840

Objective. Methylation of p16 promoter was evaluated in ovarian cancer to determine the role of p16 methylation in ovarian cancer prognosis.

Methods. Two hundred and forty-nine patients with primary epithelial ovarian cancer were selected for the study; these patients were followed for a median of 31 months. Genomic DNA extracted from fresh frozen tumor tissues were treated with sodium bisulfite and were analyzed for p16 methylation using methylation-specific PCR (MSP). Cox regression survival analysis was performed to examine the associations of p16 methylation with progression-free and overall survivals.

Results. Of the 249 patients, 100 (40%) were tested positive for p16 promoter methylation. The status of p16 methylation did not change significantly with patient age, disease stage, histological grade, residual tumor size, and debulking results, although p16 methylation seemed to occur more often in patients with advanced diseases or aggressive tumors. Compared to those without p16 methylation, patients with p16 methylation had significantly higher risk for disease progression (P = 0.01). The relative risk for progression was 1.69 (95% CI: 1.12-2.54), and the association remained statistically significant (RR = 1.54, 95% CI: 1.01-2.34) after adjusting for clinical and pathological variables. The risk for death was also higher in methylation positive patients than in methylation negative patients (RR = 1.33, 95% CI: 0.88-2.00), but the difference was not statistically significant.

Conclusion. The study suggests that promoter methylation in the p16 gene is associated with ovarian cancer progression, and evaluation of p16 methylation may have values in predicting ovarian cancer prognosis.


http://www.sciencedirect.com/science/article/B6WG6-4B2CB8B-
Objectives. The aim of this study was to detect and identify human papillomavirus (HPV) genotypes on a population of women infected by the human immunodeficiency virus (HIV) and to investigate the role of multiple infections on cervical dysplasia. Methods. Two hundred and fifty-five HIV-infected women were enrolled on a study to evaluate the prevalence of HPV and cervical intra-epithelial neoplasia (CIN). A group of HIV-negative women with confirmed CIN diagnosis was included for comparison. A polymerase chain reaction (PCR)-reverse hybridization method was applied to detect and precisely identify HPV types, specifically multiple infections. Results. On HIV patients, an altered Pap smear confirmed by biopsy was observed on 45 (18%); HPV-DNA prevalence was 87% (223/255), with 45% (116/255) infected by more than two types. In contrast, HPV-DNA was detected in all 36 women of the control group but only 3 were infected by more than two types. Cervical dysplasia was associated with low CD4 counts and elevated high-risk HPV viral load. However, the presence of multiple HPV types did not correlate with the degree of immune suppression or the presence of cervical lesions. Conclusions. Infection with multiple HPV types is a rather frequent finding on Brazilian HIV-infected women. On this population, concomitant infection with three or more HPV types does not seem to confer an additional risk of cervical dysplasia in comparison to single/double infections, nor to be related to more severe immune suppression.


http://www.sciencedirect.com/science/article/B6WG6-4DTTCJ1-1/2/c95f71cad86409bd60fcae94ea07b66a

Objectives. Functional assays of tumor suppression and loss of heterozygosity point to a tumor suppressor gene (TSG) for cervical cancer (CC) on chromosome 11q23. We evaluated IGSF4, a putative TSG located in the region, for promoter methylation and gene silencing in CC cell lines and cervical tissues. Methods. IGSF4 expression was detected by both RT-PCR and Northern blot analysis. Methylation maps of the IGSF4 promoter region were generated for 11 CC cell lines based upon bisulfite-genomic sequencing, using seven nested-PCR primer sets covering 97 CpG sites. Methylation fingerprints in primary cervical tissues were evaluated by denaturing high performance liquid chromatography. Results. A 4.4-kb mRNA was seen in cell lines, consistent with the RT-PCR results for both cell lines and primary cervical tissue. IGSF4 was expressed in 6/11 cell lines, 6/8 CC tissues and in all seven normal cervical epithelia. In the cell lines, IGSF4 silencing was associated with promoter hypermethylation. The methylation status in the region covering the -18 to -2 CpG sites correlated most strongly with expression, pointing to the existence of an unmethylated core in the IGSF4 promoter in cell lines expressing IGSF4. This unmethylated core spans approximately 180 bp and is immediately upstream of the ATG site. In primary tissues, methylation was detected in 15/23 (65%) CC specimens but in none of seven normal cervical epithelia. Conclusions. Our data strongly suggest that IGSF4 is a TSG and that gene silencing by aberrant hypermethylation may contribute to the development of CC.


http://www.sciencedirect.com/science/article/B6WG6-48R1T3V-5/2/e07e9f5812a9bd6312079a3e8d916651
Objective

Despite the high prevalence of uterine leiomyoma in women, little is known about the pathophysiology of this tumor. This study intends to define the epigenetic modulation of this tumor.

Methods

Twenty-three pairs of leiomyomas and their adjacent myometria were collected. Status of DNA global methylation was determined by using DNA methyl acceptance assay and immunohistochemistry staining with 5-methylcytidine antibody. mRNA level of DNA methyltransferases (DNMT1, 3A, and 3B) was assessed by quantitative real time PCR.

Results

DNA global hypomethylation was detected in the leiomyoma tissues as compared with the adjacent myometria. DNMT1 expression was increased in 47.5% and was equal in 47.5% in leiomyomas compared to the adjacent myometria. On the other hand, over 74% of cases showed decreased expression of DNMT3A and 3B in leiomyomas compared to the adjacent myometria.

Conclusion

Global hypomethylation and imbalanced expression of DNMTs in uterine leiomyoma suggested a potential mechanism of epigenetic modulation in the development of this tumor.


http://www.sciencedirect.com/science/article/B6WG6-4DR1NPV-1/2/4670b8c4ca9022d47f08cf84c8370c68

Background

To develop a simple and cost-effective method for the detection and genotyping of high-risk human papillomaviruses (HPV) using seminested polymerase chain reaction (PCR) and reverse hybridization.

Methods

Cervical swabs for HPV testing were collected from 127 women with normal cervical cytology and 57 patients with cervical lesions of various degrees. After DNA isolation, PCR amplification was first carried out using MY11 and MY09/HMB01 primers, then labeled by seminested PCR using the first PCR products and MY11/bioGP6+ primers. One fifth of the second PCR products were resolved by gel electrophoresis. Genotyping for high-risk HPV was done separately, using the remaining products, by a high-risk HPV chip, which contained 13 type-specific oligonucleotides on a nylon membrane. The final result was detected by colorimetric change on the chip under direct visualization.

Results

High-risk HPV DNA was detected in 19 (15%) of 127 women with normal cervical smear cytology, in 26 (89.7%) of 29 patients with cervical intraepithelial neoplasia (CIN), and in 27 (96.4%) of 28 patients with invasive cervical carcinoma. Multiple high-risk HPV infections were detected in five cases. HPV type 16 was the most frequent type of infection, comprising 34.5% and 53.6% of the patients with CIN and invasive carcinoma, respectively. The samples without a visible 190-bp band on electrophoresis exclusively showed negative hybridization results. This method could detect one to two copies of the HPV-16 genome derived from one SiHa cell. The overall sensitivity of HPV detection was 25 to 50 copies of HPV genome for each specimen. Thirteen high-risk types and twenty-four different types of HPV DNA showed specific hybridization without any cross-reaction.

Conclusions

Our results demonstrated the feasibility and optimistic prospects for this simple and cheap method of high-risk HPV genotyping. This technology can be easily set up in a routine molecular laboratory and would probably be of great value in cervical cancer prevention programs.


http://www.sciencedirect.com/science/article/B6WG6-4DVBGSG-4/2/02960d1d4b0f86daa5526cd692042d4a

Objective

In this study, genetic polymorphisms, NQO1 C609T, GSTM1 positive/null, and GSTT1
positive/null, were examined with reference to cervical cancer risk in a population-based incident case-control study in Japanese. Methods The cases comprised 131 cervical cancer patients: 87 cases with squamous cell carcinoma (SCC) and 44 with adenocarcinoma (ADC) or adenosquamous carcinoma (ADSC). Controls were sampled from 320 healthy women who underwent a health checkup. Results The cervical cancer risk was substantially elevated with smoking for all cases, SCC cases, and ADC/ADSC cases (OR = 4.50, 95% CI = 2.48-8.17, P = 0.032; respectively). The frequency of the NQO1 609TT genotype, reported to be associated with null enzyme activity, was higher in individuals with all cases and SCC than in the healthy controls (OR = 1.97, 95% CI = 1.06-3.66, P = 0.032; and OR = 2.42, 95% CI = 1.21-4.82, P = 0.012; respectively), but not in ADC/ADSC cases. Analysis of polymorphisms for GSTM1 and GSTT1 showed no significant differences between cervical cancer patients and controls. In stratification analysis, significant elevated risk of all cases and SCC was associated with the NQO1 609TT genotype among nonsmokers (OR = 2.15, 95% CI = 1.08-4.30, P = 0.030; and OR = 2.83, 95% CI = 1.21-6.31, P = 0.011; respectively), but not smokers. No gene-gene interaction was observed in our case subjects. Conclusion This is the first report that the NQO1 gene might be important in relation to the risk of squamous cell carcinoma of the cervix.


http://www.sciencedirect.com/science/article/B6WG6-49BY0WV-F/2/06b021cdd0ebeb9f9af0e59537de2efa

Objective Several tumors express the protein product of the protooncogene c-KIT. Some of these respond to imatinib mesylate, a tyrosine kinase inhibitor. The tumors that respond frequently have mutation(s) in exon 11 of c-KIT that encodes for the regulatory juxtamembrane helix. Some tumors that express KIT protein have mutation(s) in exon 17 of c-KIT; however, these do not respond to imatinib mesylate. This investigation was performed to determine the expression of KIT protein and mutational status of exons 11 and 17 of c-KIT in uterine sarcomas. Methods Twenty-five uterine sarcomas treated from 1990 to 2002 were evaluated. These included 14 malignant mullerian mixed tumors (MMMT), 7 leiomyosarcomas (LMS), 2 endometrial stromal sarcomas (ESS), and 2 high-grade heterologous sarcomas (HGHS). Formalin-fixed, paraffin-embedded tissue sections were immunostained with anti-KIT antibody (Santa Cruz Biotechnology, Santa Cruz, CA) with a semiquantitative assessment. Normal myometrium when present in the section was used as an internal negative control. Areas of tumor were microdissected followed by DNA extraction, polymerase chain reaction (PCR) amplification of exons 11 and 17, single-strand conformational polymorphism (SSCP), and DNA sequencing to detect the presence of mutation(s). Results All 25 tumors expressed KIT protein at varying levels as assessed by immunohistochemistry. The staining was diffuse and of moderate to strong intensity in 22 tumors. In three tumors (one of each type except MMMT) the staining intensity was weak. In MMMT the epithelial and sarcomatous foci stained similarly. No mutation(s) in exons 11 or 17 of c-KIT were identified in 24/25 tumors. One LMS had deletion of both exons 11 and 17. Conclusion Although uterine sarcomas express KIT protein, they lack KIT-activating mutation(s) in exon 11 or 17 of c-KIT. Therefore, these tumors are unlikely to respond to imatinib mesylate.


http://www.sciencedirect.com/science/article/B6WG6-4DF46J8-H/2/23fa37f4dad317ee63e37e239bd9608d
Objectives: Methylenetetrahydrofolate reductase (MTHFR) gene is associated with DNA hypomethylation, an established hallmark of human cancer cells. The aim of this study was to examine the effect of MTHFR polymorphism C677T on cervical carcinogenesis in the context of other environmental factors, such as smoking, parity, and age at the first intercourse.

Methods: The study subjects were patients who were pathologically diagnosed with cervical neoplasia and who had a positive result for human papillomavirus (N = 462), and they were compared to normal healthy women as normal controls (N = 454). Genotypes of the patients and control samples were assayed by single base primer extension assay using SNapShot assay kit.

Results: Compared with MTHFR C/C, the odds ratio (95% confidence interval) for MTHFR T/T was 1.4 (0.9-2.3) for invasive cervical cancer and 1.3 (0.8-2.3) for cervical intraepithelial neoplasia (CIN) II or III. The risks for invasive cervical cancer were higher with less than 40 years old at diagnosis (2.1, 1.0-4.3), than with over 40 years old at diagnosis (1.2, 0.7-2.2). Current smoking women with early onset with MTHFR T/T had a 4.7 (0.6-36.2) times higher risk of cervical cancer. The risks of MTHFR T/T or C/T also increased for women with an early age of first intercourse or for women with two or more children, as compared with MTHFR C/C.

Conclusion: Polymorphisms of MTHFR are associated with a higher risk of developing cervical cancer, and in particular for an early onset of cervical carcinogenesis.


http://www.sciencedirect.com/science/article/B6WG6-4CN9MNB-2/2/33e1a3748f17602a76e8beb29dceaf4e

Objective: Human [alpha]-catenin gene (CTNNA1) on chromosome 5q31 is aberrantly expressed in various types of cancer including epithelial ovarian tumors. Allelic imbalance on this region has also been described in several malignant diseases. In the present work, the role of CTNNA1 as a candidate tumor suppressor gene was studied by comparing protein expression with allelic imbalance in human epithelial ovarian tumors.

Methods: [alpha]-Catenin protein expression was determined from two areas of 41 tumors, and tissues from these areas were microdissected. After DNA extraction, AI analysis was carried out with eight microsatellite markers.

Results: Altogether, 93% of the tumors (38 of 41) showed allelic imbalance at one or more loci. Two distinct common regions of allelic imbalance were identified, one comprising markers DSS2002 and DSS1995 and the other markers DSS393 and DSS476. Loss of the CTNNA1 gene did not appear to be involved in down-regulation of [alpha]-catenin in ovarian tumors, since allelic imbalance with a variety of markers, including CTNNA1 associated marker DSS476, was found in tumor samples independently of [alpha]-catenin expression. Furthermore, allelic imbalance analyses of two different samples from the same tumor revealed genetic heterogeneity.

Conclusions: High allelic imbalance frequency indicates that chromosomal region 5q31 is functionally important in epithelial ovarian cancer. Allelic imbalance occurs at two distinct regions of which one includes the CTNNA1 gene. However, this gene is likely to be inactivated by mechanisms other than allelic imbalance. In addition, genetic heterogeneity observed in these tumors demonstrates the multiclonal nature of epithelial ovarian tumors.


http://www.sciencedirect.com/science/article/B6WG6-4BG8T8V-B/2/5f018e2f2ac3bd039aad6de2f83e80a

Objective: Leptin and its receptor are the key players in the regulation of energy balance and body weight control. However, their roles in gynecological malignancies are mostly unclear. In
In this study, we analyzed the expression and possible involvement of leptin and the leptin receptor in the pathogenesis of endometrial cancer. Methods. Radioimmunoassay was performed to analyze the serum leptin levels in the endometrial cancer patients, while RT-PCR, immunoblotting, and immunohistochemistry techniques were applied to study the expression of leptin receptor in the endometrioid-type endometrial cancer tissues. Furthermore, BrdU labeling followed by immunofluorescent analysis was used to analyze the effect of leptin receptor overexpression on endometrial cancer cell proliferation. Results. Serum leptin levels are elevated in endometrial cancer patients, but show no significant difference to those of normal controls when normalized by body mass index. On the other hand, lower expression levels of leptin receptor short form (Ob-Ra) were observed in most endometrial cancer tissues, especially in the poorly differentiated ones, and the forced expression of Ob-Ra in RL95-2 endometrial cancer cells prevented them from entering the S-phase. Conclusion. In summary, our data demonstrates for the first time that the leptin receptor is aberrantly expressed in endometrial cancer tissues and is possibly involved in the pathogenesis of endometrial cancer.

**Hearing Research** (15)


http://www.sciencedirect.com/science/article/B6T73-49H73CH-1/2/03986cb3a52659a07b899f6054fa84d3

Hair cells are specialized mechanoreceptors common to auditory and vestibular sensory organs of mammalian and non-mammalian species. Different hair cells are believed to share common features related to their mechanosensory function. It has been shown that hair cells possess various forms of motile properties that enhance their receptor function. Membrane-based electromotility is a form of hair cell motility observed in isolated outer hair cells (OHCs) of the cochlea. A novel membrane protein, prestin, recently cloned from gerbil and rat tissues, is presumably responsible for electromotility. We cloned prestin from mouse organ of Corti and confirmed strong homology of this protein among different rodent species. We explored whether or not prestin is present in hair cells of the vestibular system. Using reverse transcription-polymerase chain reaction, we demonstrated that prestin is expressed in mouse and rat auditory and vestibular organs, but not in chicken auditory periphery. In situ hybridization and immunolocalization studies confirmed the presence of prestin in OHCs as well as in vestibular hair cells (VHCs) of rodent sacculus, utricle and crista ampullaris. However, in the VHCs, staining of varying intensity with anti-prestin antibodies was observed in the cytoplasm, but not in the lateral plasma membrane or in the stereociliary membrane. Whole-cell patch-clamp recordings showed that VHCs do not possess the voltage-dependent capacitance associated with membrane-based electromotility. We conclude that although prestin is expressed in VHCs, it is unlikely that it supports the form of somatic motility observed in OHCs.


http://www.sciencedirect.com/science/article/B6T73-478RSJ4-5/2/dbd40922ee3a3a3b8cbf342f118382d9
The type 1 vanilloid receptor (VR1) is a non-specific cation channel activated by capsaicin, lipoxygenase (LOX) products, heat and acid. This study demonstrates VR1 and 5-LOX expression by inner ear ganglion cells. A PCR product (210 bp) was amplified from both oligo(dT)- and random primer-generated cDNAs of rat spiral ganglion cells using VR1 gene-specific primers constructed from the 3' non-homologous region. This PCR product shared 100% sequence homology to a rat VR1 cDNA (GenBank accession no. AF029310) and a rat vanilloid receptor splice variant mRNA (GenBank accession no. AF158248). Frozen sections of PLP-fixed, decalcified Long-Evans rat temporal bones were stained immunohistochemically for VR1. Neurons and satellite cells in both the vestibular and spiral ganglia were VR1-immunopositive. Neurons and supporting cells in adjacent sections of these ganglia were immunopositive for 5-LOX. These findings raise the hypothesis that activation of VR1 by endogenous ligands may contribute to hypersensitivity of the eighth nerve to hair cell inputs in a variety of pathologic conditions, such as tinnitus, Meniere's disease and migraine. In particular, these data suggest that LOX activation during inflammatory processes or during cyclo-oxygenase inhibition (e.g. by aspirin) is a potential intrinsic source of VR1 activation in inner ear ganglia.


http://www.sciencedirect.com/science/article/B6T73-44MFJR7-3/2/4dc6b6b315651dc3b0ef9344339bd2bb

The recently manifested important role of the Ca2+-activated K+ channels, especially of the Slo gene-coded channels, for the cochlea function of the chicken raised the question of homolog expression in mammalian inner ear tissue. Molecular biological methods were used to demonstrate the expression of Ca2+-activated K+ channel subunits and splice variants of the Slo gene in the rat organ of Corti. RT-PCR experiments for the detection of rat Slo [alpha] subunit mRNA revealed the presence of several already known splice variants including variants which appeared to be typical for the organ of Corti (+58 aa) and for the brain (+61 aa). To detect the accessory [beta] subunit we used Southern blot hybridization. Our data support the hypothesis that Ca2+-activated K+ channel subunits (i.e. Slo variants) are also involved in the hearing of mammals in the organ of Corti.


http://www.sciencedirect.com/science/article/B6T73-411X9NB-3/2/4a5d722c1aab33daa0300a9f111050be

The pattern of expression of potassium (K+) channel subunits is thought to contribute to the establishment of the unique discharge characteristics exhibited by cochlear nucleus (CN) neurons. This study describes the developmental distribution of mRNA for the three Shal channel subunits Kv4.1, Kv4.2 and Kv4.3 within the mouse CN, as assessed with in situ hybridization and RT-PCR techniques. Kv4.1 was not present in CN at any age. Kv4.2 mRNA was detectable as early as postnatal day 2 (P2) in all CN subdivisions, and continued to be constitutively expressed throughout development. Kv4.2 was abundantly expressed in a variety of CN cell types, including all of the major projection neuron classes (i.e., octopus, bushy, stellate, fusiform, and giant cells). In contrast, Kv4.3 was expressed at lower levels and by fewer cell types. Kv4.3-labeled cells were more prevalent in ventral subdivisions than in the dorsal CN. Kv4.3 expression was significantly delayed developmentally in comparison to Kv4.2, as it was detectable only after P14. Although the techniques employed in this study detect mRNA and not protein, it can be inferred from the differential distribution of Kv4 transcripts that CN neurons selectively regulate the expression of
Shal K+ channels among individual neurons throughout development.


http://www.sciencedirect.com/science/article/B6T73-485PD2X-6N/2/dd8db1dac9c127230d5dce006c370795

Expression of mineralocorticoid type I receptor (MR) gene in the rat cochlea was determined using molecular biological techniques. We synthesized complementary DNA (cDNA) from rat cochlear total RNA and then amplified MR cDNA fragments by polymerase chain reaction (PCR). The amplified cDNA fragments were subcloned into an expression vector and the nucleotide sequence was analyzed to confirm the expression of mRNA encoding MR in the cochlea. We then synthesized digoxigenin-labeled riboprobes with this cloned DNA template and examined the localization of MR mRNA in the cochlea by in situ hybridization. The amino acid sequence of MR cDNA expressed in the cochlea was identical to that of the MR first cloned in the rat hippocampus. In situ hybridization showed the expression of MR mRNA in marginal cells of the stria vascularis, suggesting that aldosterone may regulate microhomeostasis of the endolymph, presumably by modulating Na, K-ATPase activity. Intense MR signal was also identified in spiral ganglion cells, the function of which remains to be determined.


http://www.sciencedirect.com/science/article/B6T73-3W0NHBW-3/2/4fa3c41313d5b743058b124471348469

We used differential display of mRNA, a method based on reverse transcriptase-PCR, to identify genes whose expression increases in response to acoustic trauma in the chick basilar papilla. Identifying these genes would provide insight into processes involved in repair of the damaged epithelium or in hair cell regeneration. We compared mRNA from the basilar papilla of normal chicks, from chicks exposed to an octave band noise (center frequency: 1.5 kHz) presented at 118 dB for 6 h, and from chicks exposed to noise and allowed to recover for 2 days. Thus far, we have identified 70 bands that appear to be differentially displayed on DNA sequencing gels; approximately 40 of these bands have been subcloned and sequenced. DNA sequences were compared with sequences in the GenBank database to identify genes with significant (70-85%) sequence identity to known genes. Chick cDNAs identified included: the parathyroid hormone-related protein, an immediate early gene; the [delta]-subunit of the neuronal-specific Ca2+/calmodulin-regulated protein kinase II; and the GTP-binding protein CDC42, a member of the ras superfamily of G proteins. A fourth cDNA had 84% sequence identity to an uncharacterized human cDNA (expressed sequence tag), indicating that this is a novel gene. Slot-blot hybridization analysis of these cDNAs probed with labeled DNA generated from mRNA from each experimental group indicated higher levels of mRNA for each of these four genes after noise exposure. These results indicate the potential involvement of both Ca2+/calmodulin-mediated signaling and GTPase cascades in the response to noise damage and during hair cell regeneration in the chick basilar papilla.

A recessive deafness mutation in the mouse arose spontaneously and was identified in a colony segregating a null allele of the gastrin-releasing peptide receptor (Grpr) locus. Auditory-evoked brain stem response measurements revealed deafness in 7-week-old affected mice. By linkage analyses, the mutant phenotype was mapped near marker D10Mit186 and the protocadherin gene Pcdh15. As shown by complementation testing, the new mutation is allelic with Ames waltzer (Pcdh15av). Sequencing mutant-derived brain Pcdh15 cDNAs identified the insertion of a cytosine residue at nucleotide position c2099 (2099insC), which results in a frame-shift and premature stop codon. Abnormal stereocilia on inner and outer hair cells of the organ of Corti were identified by scanning electron microscopy as early as postnatal day 0 and cross-sectional histology revealed severe neuroepithelial degeneration in cochleas of 30-50-day-old mutants. The new allele of Ames waltzer, designated Pcdh15av-Jfb, may aid in studying the histopathology associated with Usher syndrome type 1F, which is caused by a functional null allele of PCDH15.


Biotinidase deficiency is an autosomal recessively inherited disorder characterized by neurological and cutaneous features, including sensorineural hearing loss. Although many of the features of the disorder are reversible following treatment with biotin, the hearing loss appears to be irreversible. To better characterize the nature of the hearing loss in this disorder, location of the expression and presence of biotinidase within the brain was examined using Northern blot analysis, in vitro hybridization of a cDNA panel, and immunohistochemical staining. Results indicate low, but detectable expression of biotinidase throughout the brain, but increased concentrations of biotinidase within the dorsal cochlear nucleus, ventral cochlear nucleus, and superior olivary complex of the brainstem, as well as, in the hair cells and spiral ganglion of the cochlea. These findings suggest that biotinidase and possibly biotin plays an important role in hearing.


The transient receptor potential cation channel subfamily V (TRPV) is a non-specific cation ion channel receptor family that is gated by heat, protons, low extracellular osmolarity and arachidonic acid derivatives. Since some of these endogenous agonists of TRPV receptors are reactive oxygen intermediates produced by lipoxygenases, it has been hypothesized that some members of the TRPV family may respond to challenges by reactive oxygen species. This study used real-time PCR to quantitatively track changes in TRPV1-4 mRNA expression in the spiral, vestibular, and trigeminal ganglia and the kidney from kanamycin (KM)-treated mice. TRPV1, TRPV2, TRPV3 and TRPV4 mRNAs were expressed in spiral and vestibular ganglia, and TRPV2 and TRPV1 mRNAs were most predominant in control mice. After KM (700 mg/kg s.c. b.i.d., 14 days), TRPV1 mRNA and protein expression were significantly up-regulated both in the spiral and
vestibular ganglia, but expression was unaffected in the trigeminal ganglion and kidney. Real-time PCR also demonstrated a significant down-regulation in TRPV4 mRNA expression in the inner ear ganglia and kidney after KM treatment. All these mRNA and protein expression changes were eliminated by simultaneous administration of dihydroxybenzoate (300 mg/kg s.c. b.i.d., 14 days), an anti-oxidant that blocks KM ototoxicity. It is proposed that up-regulated TRPV1 expression during KM exposure may promote ganglion cell survival by contributing to neuronal depolarization, with KM-induced tinnitus and dizziness as consequences.


http://www.sciencedirect.com/science/article/B6T73-4CTD19J-1/2/87fe07b77fbb058cb31504a2ab0129bb

Uncoupling proteins (UCPs) are a proton transporter family located in the mitochondrial inner membrane. The molecular expression and activity of UCPs in brown adipose tissue and skeletal muscle are regulated by factors as diverse as chronic overeating and cold exposure, suggesting roles in energy expenditure and heat production. Although UCP2, UCP4 and brain mitochondrial carrier protein-1 (BMCP-1, i.e. UCP5) mRNAs are expressed in the central nervous system, their central function is unknown. This study presents the first evidence on localization and quantitative expression of UCPs in the rat inner ear by real-time PCR and immunohistochemistry. Real-time PCR studies revealed that UCP2 mRNA was expressed in the vestibular and spiral ganglia more abundantly than any other UCP. Neocortex, by contrast, contained UCP2 and UCP4 equally. Notably, UCP3 and UCP4 mRNAs were expressed in inner ear ganglia, but brain UCP3 mRNA expression level was undetectable by simple PCR. Immunohistochemical studies confirmed that both UCP2- and UCP3-like immunoreactivities were detected in vestibular and spiral ganglion cells and co-localized with a mitochondrial marker, MitoFluorGreen. According to previous reports, UCP2 and UCP3 are thermogenic in yeast and brain UCP2 has been suggested to modulate pre- and post-synaptic events by axonal thermogenesis. It has also been reported recently that UCP2 and UCP3 responses to superoxide application may be an antioxidant protective mechanism. Therefore, it is suggested that mitochondrial UCPs (UCP2, UCP3, UCP4) may play both a protective role against oxidative damage and a thermal signaling role in the eighth nerve.


http://www.sciencedirect.com/science/article/B6T73-3T3MDP-C/2/3e716af9a6f56cd7fb0fcb2eec7e8538

Although mechanisms regulating inner ear fluid have not been yet elucidated, control of blood flow has been thought to be of great importance. Vasoactive intestinal polypeptide (VIP) was the first neuropeptide demonstrated in cerebrovascular nerves. To study the possible role of VIP in regulation of inner ear fluid, we investigated the presence of mRNA for VIP and VIP receptor in the rat inner ear using a reverse transcription-polymerase chain reaction (RT-PCR) method. A single band of the size expected for VIP and its receptor was detected in mRNA from the rat inner ear by using primers specific for VIP and the receptor. The nucleotide sequences of the subcloned RT-PCR products were identical to those of rat VIP and the rat lung VIP receptor. These results indicate that both VIP and VIP receptor are expressed in the inner ear of the rat and suggest that VIP may be implicated in regulation of fluid in the inner ear.

Mutations in the Connexin 26 (Cx26) gene (GJB2) are a common cause of hereditary hearing impairment. We report the identification of a novel point mutation in the Cx26 gene, Leu205Pro(L205P), linked to familial, autosomal recessive sensorineural hearing loss. This missense mutation, causing amino acid leucine at position 205 to be substituted by proline, is located in the highly conserved sequence of the fourth transmembrane domain (TM4) of Cx26. Hearing loss with this mutation occurred in a Georgian Jewish family, was congenital, moderate to profound and nonprogressive. We have shown that the new mutation L205P in Cx26 is strongly associated with congenital NSHL. Multiple-sample screening for this mutation can be easily performed with a mismatch PCR that creates a restriction site.

http://www.sciencedirect.com/science/article/B6T73-485V0XS-2/2/933a07d5c5b0f346914e86b27f7ddfc9

Levels of expression of mRNAs encoding the different Ephs and ephrins were measured by semi-quantitative reverse-transcription polymerase chain reaction in developing mouse whole inner ears, and in dissected fractions of the neonatal mouse inner ear. Nineteen of the 24 known Ephs and ephrins were surveyed. The results showed that between embryonic age (E) 11.5 days and E12.5, levels increased 10-300 times per unit of tissue. In neonatal mice, the fraction containing combined organ of Corti and spiral ganglion showed relatively strong expression of EphA4, EphB3, ephrin-A3, ephrin-B2 and ephrin-B3. In the lateral wall, EphA4, ephrin-A3 and ephrin-B2 were strongly expressed, while ephrin-A3 was particularly strongly expressed in utricular and saccular sensory epithelia. The results suggest that the Ephs and ephrins are likely to play a part in the differentiation of the structures of the inner ear, and show which Ephs and ephrins are most likely to play important roles in the different structures.

http://www.sciencedirect.com/science/article/B6T73-42WX3VB-6/2/e1dc4770c512ad277bf3b92a8cadab97

Four different fibroblast growth factor receptors (FGFR) are known, three of which have splice variants (known as the b and c variants) in the FGF-binding domain, to give different patterns of sensitivity to the different FGFs. The expression of the b and c variants of the FGF receptors, together with the expression of the ligands FGF1, FGF2, FGF3, FGF7, FGF8b and FGF8c, was determined by quantitative reverse transcription-polymerase chain reaction in developing whole mouse inner ears, and in dissected components of the postnatal mouse inner ear. At embryonic age (E) 10.5 days, when the otocyst is a simple closed sac, the receptor most heavily expressed was FGFR2b, relative to the postnatal day 0 level. Over the period E10.5-E12.5, during which the structures of the inner ear start to form, the expression of the different FGF receptors increased 102-104 fold per unit of tissue, and there was a gradual switch towards expression of the 'c' splice
variants of FGFR2 and FGFR3 rather than the 'b' variants. At E10.5, the ligands most heavily expressed, relative to the postnatal day 0 level, were FGF3, FGF8b and FGF8c. In the postnatal inner ear, the patterns of expression of receptors and ligands tended to be correlated, such that receptor variants were expressed in the same regions as the ligands that are known to activate them effectively. The neural/sensory region expressed high levels of FGFR3c, and high levels of the ligand FGF8b. The same area also expressed high levels of FGFR1b and FGFR2b, and high levels of FGF3. The lateral wall of the cochlea (including the stria vascularis and the spiral ligament) expressed high levels of FGFR1c and FGF2. It is suggested that the different FGF receptors and ligands are expressed in a spatially coordinated pattern, to selectively program cochlear development.


http://www.sciencedirect.com/science/article/B6T73-42WX3VB-G/2/42550fd61174a8af615f222720019925

Glial cell line-derived neurotrophic factor (GDNF) is a survival factor for many neuronal cell types which signals through a heterodimer receptor consisting of GDNF-family receptor [alpha] 1 (GFR[alpha]-1) and Ret (arranged during ransformation). GDNF expression has previously been reported in the inner hair cells of the rat cochlea, with expression of GFR[alpha]-1 but not Ret in the cell bodies of the auditory nerve (spiral ganglion cells), using in situ hybridization. The present study used reverse transcription-polymerase chain reaction (RT-PCR), and immunocytochemistry to examine GDNF, GFR[alpha]-1 and Ret in the adult rat auditory nerve. Semi-quantitative RT-PCR showed expression of GDNF and the two receptor components, GFR[alpha]-1 and Ret, in the modiolar subfraction of the cochlea containing spiral ganglion cells. A shorter mRNA splice variant for GDNF was also detected. Immunocytochemistry showed immunostaining in the modiolus for GDNF, GFR[alpha]-1 and Ret that was confined to spiral ganglion cells. When RT-PCR expression levels were compared to the expression in the substantia nigra, GFR[alpha]-1 expression levels were similar, Ret mRNA was lower in the modiolus and GDNF expression was higher in the modiolus. However, when GDNF was further assessed using Western blot, while GDNF protein was found in the modiolus it was at lower levels than in substantia nigra tissue. These results demonstrate that GDNF and both of its receptor components are found in spiral ganglion cells of the adult rat cochlea. Along with the previous report of GDNF in inner hair cells, these new results provide a basis for the role of GDNF as a survival factor for the auditory nerve, as suggested by previous studies.


http://heart.bmjjournals.com
Intrauterine and neonatal manifestations of congenital long QT syndrome are associated with a high cardiac risk, particularly when atrioventricular block and excessive QT prolongation (> 600 ms1/2) are present. In a female newborn with these features, treatment with propranolol and mexiletine led to complete reduction of arrhythmia that was maintained 1.5 years later. High throughput genetic analysis found a sodium channel gene (LQT3) mutation. Disappearance of the 2:1 atrioventricular block and QTc shortening (from 740 ms1/2 to 480 ms1/2), however, was achieved when mexiletine was added to propranolol. This effect was considered to be possibly genotype related. Early onset forms of long QT syndrome may benefit from advanced genotyping.

Hepatology (3)


The p53 gene is frequently mutated in human tumors; in hepatocellular carcinomas, there is a high frequency of a specific mutation at codon 249 in regions with significant aflatoxin exposure. To assess the role of this p53 mutation in the development of hepatocellular carcinoma, a mutant murine p53 gene, p53ser246, which corresponds to human codon 249, was transfected into a differentiated, nontransformed hepatocyte cell line AML12. Expression of p53ser246 in this line resulted in a growth advantage when compared with either a control vector (which contains a large p53 deletion) or with a different p53 mutant, val135, not found in hepatocellular carcinoma. Overall, there was a threefold increase in colony formation after transfection with p53ser246 as compared with the control or p53val135 vectors, and the p53ser246 plates developed consistently larger colonies. Whereas clones expressing the control or p53val135 constructs showed no significant morphological changes, clones expressing p53ser246 showed increased heterogeneity (large multinucleated cells and areas of small crowded cells) without focus formation. In addition, the ser246 mutation imparted a growth advantage in serum free media, suggesting less dependence on specific factors present in serum. None of the mutant p53 or control lines were capable of growth in soft agar or tumor formation in nude mice. Thus in this model, in which endogenous wild-type p53 expression is retained, a high level of mutant p53 expression is not sufficient to transform hepatocytes. Our findings indicate that p53ser246 has effects on hepatocytes that may result in a clonal growth advantage and suggest that additional factors are required for the development of hepatocellular carcinoma.

One hypothesis is that postnatal liver growth involves replication of mature hepatocytes, which have an unlimited proliferative potential. An alternative viewpoint is that only certain periportal cells can replicate extensively and that daughter cells stream slowly from the periportal to the pericentral region of the liver. Transgenic mice expressing the beta-galactosidase ([beta]-gal) gene from the human al antitrypsin promoter were used to examine the proliferative potential of hepatocytes. Surprisingly, only 10% of hepatocytes in two different transgenic lines stain blue with X-gal. In neonatal animals, singlets or doublets of expressing cells are randomly scattered throughout the liver. Although the overall frequency of blue cells is similar in older animals, these cells are present in much larger clusters, suggesting that individual expressing cells have replicated to form a clonally derived cluster. Expression patterns are not altered by the administration of an acute phase stimulus or by the performance a partial hepatectomy, suggesting that the expression state cannot be easily altered, and making it more likely that the expression state is indeed fixed. These results suggest that the clusters of blue cells are clonally derived in the transgenic mice. They argue that the parenchymal hepatocyte is responsible for growth in the postnatal liver and that streaming of liver cells does not occur.


http://www.sciencedirect.com/science/article/B6WG8-4CNT2X9-27/2/3031a328a32718be6096b1d4c98aebf9

Reovirus type 3 has been implicated in the origin and pathogenesis of extrahepatic biliary atresia and idiopathic neonatal hepatitis, but routine detection of this virus in hepatobiliary tissues from affected infants by culture and histological techniques has been unsuccessful. In this study, oligonucleotide primers specific to the M3 genome segment of reovirus 3 (Dearing) were used in a reverse transcriptase--mediated polymerase chain reaction technique to develop a sensitive and specific assay for the detection of reovirus 3 RNA in formalinfixed, paraffin-embedded patient samples. Optimal reaction conditions were determined by testing infected murine tissues and preserved human liver tissue supplemented with reovirus 3. Archival specimens from 50 infants, including 14 with extrahepatic biliary atresia, 20 with idiopathic neonatal hepatitis, and 16 age-matched controls, were evaluated. Successful amplification of human albumin complementary DNA from the preserved tissues confirmed the presence of intact RNA in every patient specimen tested. Analysis of the amplification reactions by agarose gel electrophoresis and Southern blot hybridization detected the presence of reoviral RNA only once in a single patient sample. These results do not support a strong role for reovirus 3 in the development of neonatal cholestatic liver disease. The recent association of other RNA viruses of the Reoviridae family with murine liver disease and human extrahepatic biliary atresia indicates that continued investigation into a viral cause for idiopathic neonatal hepatobiliary disease is warranted.

Hepatology Research (19)

Although hepatitis C virus (HCV) detection by polymerase chain reaction (PCR) assay is now the standard, extensive clinical application has been thwarted by the troublesome procedure, long reaction time and potential for contamination. To overcome these problems, we carried out a PCR assay for the detection of HCV, hepatitis G virus (HGV) and hepatitis B virus (HBV) genomes directly from serum samples without any nucleic acid extraction (direct PCR). The sensitivity of this assay was one chimpanzee-infectious dose of HCV and a 10-1 chimpanzee-infectious dose of HBV. This result was similar to the sensitivity determined by the conventional PCR. Furthermore, when the detection rate of these genomes in serum samples from chimpanzees and humans is compared, the results matched completely between two different PCR assays. The whole process, including the reverse transcriptase reaction and second round PCR, can be completed within 6 h by the combination of the direct PCR and one-step PCR assay. Our findings indicate that this method is simple, rapid and highly sensitive and could be useful for the screening of blood-borne hepatitis virus infections using serum samples.


During the follow-up of 19 patients with self-limited acute hepatitis B for more than 2 years, clearance of hepatitis B surface antigen from the sera was observed in all patients within 6 months after disease onset, and the corresponding antibody (anti-HBs) appeared in 17 of the 19 patients within 12 months. However, upon performing nested polymerase chain reaction with the estimated sensitivity of 120-200 copies/ml, using two independent pairs of primers derived from the well-conserved sequences in the S gene or C gene region of the hepatitis B virus (HBV) genomes of all seven genotypes, HBV DNA was detected over a period of at least 12 months in serum samples obtained from five (26%) of the 19 patients, although it became undetectable in all five patients at 2-3 years after disease onset. The titer of antibody against hepatitis B core antigen (anti-HBc), assayed by the hemagglutination inhibition (HI, 2N) test, was significantly lower at the initial examination in the five patients who remained viremic for at least 12 months, than in the remaining 14 patients who cleared HBV DNA from their sera within 12 months after disease onset (10.6+/−2.7 vs. 13.6+/−0.7, PP<0.01). These results indicate that the initial titer and dynamics of anti-HBc may reflect the evolution of HBV viremia after clinical recovery from acute hepatitis B.


To elucidate the role of hepatitis viruses in the pathogenesis of Behcet's disease (BD), we measured hepatitis viral markers (anti-hepatitis A (anti-HA), HBsAg, anti-HBs, anti-HBc) and viral nucleic acids (hepatitis B virus (HBV)-DNA, hepatitis C virus (HCV)-RNA, GB virus C (GBV-C)-
RNA, TT virus (TTV)-DNA) in the sera of 68 BD patients along with 76 blood donors matched for age and sex. Positivity of anti-HA in patients with BD (36.8%) was lower than that in blood donors (68.0%). Both anti-HCV and HCV-RNA were detected in only one (1.5%) patient with BD and in none of the blood donors. The prevalence ratios of HBsAg, anti-HBs, anti-HBc in both groups were similar (2.9:0, 16.2:15.8 and 17.7:19.7%, respectively). However, serum HBV-DNA was detected more frequently in BD patients (8/68; 11.8%) than in blood donors (2/76; 2.6%) (P<0.05). The prevalence of GBV-C-RNA was also higher in patients with BD (4/68; 5.9%) compared with blood donors (0%). However, characteristics and clinical features are similar between GBV-C-RNA-positive and -negative groups. With respect to the prevalence of TTV-DNA, there was no significant difference between BD patients (23.5%) and blood donors (30.3%). Our study indicates that HBV and GBV-C infection might be related to BD, although the role of these viruses remains to be investigated.


http://www.sciencedirect.com/science/article/B6T74-485PJ0B-1/2/e3f761f50d067ade285bda0f2042c2c

The aim of this work was to study the induction and secretion of interleukin 8 (IL-8) and some oxidative stress parameters after ethanol (EtOH), acetaldehyde (Ac) or lipopolysaccharide (LPS) treatment on HepG2 cells. Cells were treated with 50 mM EtOH, 175 [mu]M Ac or 1 [mu]g/ml of LPS. IL-8 induction and secretion were determined in the presence of the toxics, and the effect of antioxidants N-acetyl--cysteine and 1,1,3,3-tetramethyl-2-thiourea was evaluated. Further, the effect of adding polyclonal anti-human tumor necrosis factor [alpha] (TNF-[alpha]) and H2O2 was studied, and catalase, superoxide dismutase and glutathione peroxidase activities were determined. Lipid peroxidation increased significantly only in Ac-treated cells. All toxics failed to decrease significantly the intracellular levels of reduced GSH. Catalase activity was diminished in all treatments, while other enzyme activities did not present changes. No change in peroxide production was found with any treatment. IL-8 secretion increased in Ac (41%) and in LPS (38%)-treated cells. Antioxidant and anti-TNF-[alpha] treatments decreased IL-8 secretion. H2O2 (0.25 mM)-treated cells increased IL-8 secretion. IL-8 reverse transcriptase-polymerase chain reaction results correlated with secretion values. Our results show that Ac and LPS treatment produced an increased IL-8 induction and secretion. Oxidative stress and TNF-[alpha] are mediators in IL-8 response. This observation suggests that in the in vivo liver, the mechanism of ethanol-induced IL-8 production requires ethanol metabolism, and hepatocytes do not require the interaction among different populations of liver cells to respond.


http://www.sciencedirect.com/science/article/B6T74-47GYK8P-5/2/3e4fc9f100a5e052217993600dac8ec5

Recently, we identified TTV isolates from nonhuman primates and named them simian TTV (s-TTV). To investigate the prevalence of s-TTV in humans, we examined sera from healthy individuals and patients with liver diseases in Japan for the presence of s-TTV DNA by PCR assay. s-TTV DNA was determined by nested PCR using s-TTV-specific primers designed from untranslated region of s-TTV genome. s-TTV DNA sequence was detected in three of 200 (1.5%) healthy adults but none of 48 infants without liver disease. On the other hand, s-TTV DNA was detected in 30 of 287 (10.5%) Japanese patients with liver disease. s-TTV co-infection with hepatitis B virus and hepatitis C virus were present in 16.7 and 30% of these patients,
respectively, while 53.3% of patients were positive for s-TTV alone. Nucleotide sequence analyses in 20 patients confirmed that these PCR products were derived from s-TTV genome sequences and nearly 85% identical to those of s-TTV prototypes from chimpanzees. Phylogenetic analysis demonstrated that all s-TTV isolates from humans were distinguished clearly from the human TTV isolates. Furthermore, s-TTV in humans was classified into two different genotypes as well as simians. Our results indicate that generally 10.5% of Japanese patients with liver diseases were infected with s-TTV. The routes of s-TTV transmission from animal to human require clarification.


A transfusion-transmissible agent, designated hepatitis G virus (HGV), was recently identified. We have cloned the full-length nucleotide sequence of the HGV genome (denoted HGV-IM71) recovered from a Japanese patient with liver cirrhosis. The HGV-IM71 isolate was composed of 9387 nucleotides (nt) with 5' and 3' untranslated regions of 458 and 310 nt, respectively. It had a single open reading frame, encoding 2873 amino acid (aa) residues. This isolate differed from previously reported HGV/GBV-C isolates by 8-15% of the nucleotide sequence and 3-5% of the amino acid sequence. The isolate lacked a clearly identifiable core gene that had only 47 aa residues. Based on phylogenetic analysis of the full-genome sequence, it was concluded that HGV-IM71 belonged to the Asia type (type 3) of HGV genotypes.


Background:Hepatitis B virus (HBV) has been classified into seven genotypes (A-G). HBV genotypes have a geographically characteristic distribution. Since HBV genotype G (HBV/G) was identified recently, little is known about the distribution of HBV/G in Japan. The aim of this study was to clarify this issue. Patients and methods: Seven hundred and twenty-one serum samples obtained from patients with HBV in Japan were investigated. The patients included 149 asymptomatic carriers, 325 with chronic hepatitis, 129 with liver cirrhosis, and 118 with hepatocellular carcinoma. Six HBV genotypes (A-F) were determined by restriction fragment length polymorphism targeting to the S region of the HBV genome. Furthermore, HBV/G was investigated by polymerase chain reaction with hemi-nested primers derived from an HBV/G-specific nucleotide sequence. Results: Of the 721 serum samples investigated, 12 subjects were classified as having HBV/A, 88 HBV/B, 610 HBV/C, 3 HBV/D, and 1 HBV/F. Seven subjects had a mixed infection with distinct genotypes, two with HBV/A and HBV/D, and five with HBV/B and HBV/C. HBV/G was not identified among the 721 samples. Conclusion: HBV/G was not identified in a large cohort of patients with HBV, either single or dual infection. HBV/G seems to be an extremely rare genotype in Japan.

Accumulating evidence suggests the presence of latent hepatitis B virus (HBV) infection in the liver of individuals negative for HBV surface antigen (HBsAg) but positive for antibodies to HBV core antigen (anti-HBc) at low titer. It remains unclear, however, whether positive anti-HBc in the serum invariably reflects latent HBV infection. In this study, we examined the presence of HBV genomes in the liver tissue of 33 donors and 30 recipients of living-related liver transplantation with positive for anti-HBc together with time course changes in their anti-HBc titer. None of these anti-HBc-positive healthy donors or recipients was positive for HBV-DNA nor anti-HBc at high titer (200 dilution) in their sera. However, HBV-DNA was detected in the liver tissue of 24 of 33 healthy anti-HBc-positive donors (72.7%) and five of 30 anti-HBc-positive recipients (16.7%). Interestingly, anti-HBc was continuously positive in all healthy donors tested. In contrast, anti-HBc titers in 75% of recipients, who were positive for anti-HBc at the time of liver transplantation, gradually decreased after the operation, and finally became negative after the mean follow up period of 9.0 months (range 1.2-45.1). Notably, HBV-DNA was never detected in the liver of those recipients who were transiently positive for anti-HBc. In conclusion, our findings suggested the possibility that presence of circulating anti-HBc does not always reflect the presence of HBV genomes in the liver tissue of anti-HBc-positive patients.


Inactivation of the p53 tumor suppressor gene is considered to occur as a late event involved in hepatocarcinogenesis. Its detection is thought to provide a useful information for the clinical management of hepatocellular carcinomas (HCCs). A rapid screening test was devised for allelic loss of the p53 gene in samples obtained by needle biopsy using non-radioisotopic (RI) microsatellite analysis combined with the microdissection method for clinical laboratory. Biopsy materials from 23 HCCs were examined to detect loss of heterozygosity (LOH) of the p53 gene after use for a histopathological diagnosis. Two microsatellite loci in the p53 gene were amplified by the polymerase chain reaction (PCR) using DNA extracted from microdissected cells, and amplified DNA fragments were subjected to non-RI detection using silver staining. More than 40-50 microdissected cells from a formalin-fixed paraffin-embedded tissue are enough to amplify both alleles of the p53 gene during the PCR. The combination of the two polymorphic microsatellite markers encompassed 60% of Japanese patients as informative. This method has detected LOH of the p53 locus in 54% of informative primary HCCs. Furthermore, LOH of the p53 gene was frequently detected in more advanced HCC as to the histological grade and clinical stage as shown in the previous report. The system for rapid and safe detection of the p53 gene allelic loss should provide useful information on the strategy for the treatment of HCC in the clinical laboratory.

Information about M6P/IGF2R and p53 genes in hepatocarcinogenesis is limited and controversial. We tested the loss of heterozygosity (LOH) of M6P/IGF2R and p53 genes in cirrhotic and neoplastic foci in surgically resected livers of 30 patients with hepatocellular carcinoma (HCC). The DNAs extracted from microdissected specimens were used for polymerase-chain-reaction (PCR)-based assay. LOH of the M6P/IGF2R gene in the primary HCCs was detected in 10 of 22 informative cases (45%). In five of these 10 cases (50%), LOH was detected in cirrhotic lesions adjacent to the HCCs. The allelic loss patterns of M6P/IGF2R in liver cirrhosis (LC) were identical to those in the corresponding HCC, suggesting that HCC could develop from one of the cells in which M6P/IGF2R had been lost. Furthermore, LOH of the p53 gene in HCC was detected in 10 (43%) of 23 informative cases, and p53 loss in cirrhotic foci adjacent to HCC was shown in one of the 10 cases (10%). The pattern of allelic loss of the p53 gene in the cirrhotic foci was identical with that in the corresponding tumor. The LOH of the M6P/IGF2R and p53 genes occurred independently in HCCs. LOH of the M6P/IGF2R locus was a relatively frequent and possibly early event in hepatocarcinogenesis, and LOH of the M6P/IGF2R gene and LOH of the p53 gene occurred independently.


TT virus (TTV) has been reported to occur in association with elevated alanine aminotransferase (ALT) levels in patients with posttransfusion hepatitis of unknown etiology. We examined whether the presence, change of DNA titer, or variation in sequence of this virus is associated with acute or chronic liver dysfunction in Japanese. We detected TTV by polymerase chain reaction (PCR) using primers generated from the conserved region of the TTV genome. Direct DNA sequencing of the original N22 region was used to characterize TTV isolates. We detected TTV DNA in 15 (25%) of 60 patients with liver dysfunction. Variants recovered from infected patients formed four genotypes/subtypes, corresponding to G1a, G1b, G2, and G4. Although TTV DNA titers in patients with G2 and G4 were lower than those with G1, TTV was consistently detected regardless of genotype/subtype. TTV infection continued for at least 1 year after normalization of ALT level in patients with acute liver dysfunction. Changes in DNA titer, substitutions of deduced amino acids, and variety of quasispecies of TTV were detected during the observation period, but no significant fluctuation in ALT level was found. We conclude that persistent infection, changes in DNA titer, and variation in sequence of this novel virus are not significantly related to hepatic disorders.


Acute ethanol administration temporarily decreases the sensitivity to endotoxin (lipopolysaccharide, LPS) in the liver. The purpose of this study was to investigate the changes of toll-like receptor (TLR)-4, a newly identified LPS receptor in macrophages, in the liver following acute ethanol administration. Male C57BL/6N mice were given a bolus intragastric administration
of ethanol (5 mg/g BW) through a gastric canula, and liver samples were obtained 2-48 h later. RAW264.7 macrophages were cultured in the presence of ethanol (100 mM) or LPS (10 ng/ml) for up to 4 h. TLR-4 mRNA in the liver and RAW264.7 cells was detected by RNase protection assay. As expected, TLR-4 mRNA was clearly detected in the control liver; however, it was barely detectable in the liver 2-6 h after ethanol administration, followed by the gradual increase to the basal levels 48 h later. Interestingly, LPS (10 ng/ml), but not ethanol (100 mM), decreased TLR-4 mRNA in RAW264.7 macrophages in 4 h. Indeed, gut-sterilization by oral antibiotics pretreatment prevented the decrease in TLR-4 mRNA caused by acute ethanol administration, supporting the hypothesis that gut-derived endotoxin is involved in the mechanism. These findings clearly indicated that acute ethanol administration in vivo down-regulates TLR-4 expression in the liver. This phenomenon most likely explains the mechanism by which acute ethanol blunts the response of Kupffer cells to LPS transiently.


http://www.sciencedirect.com/science/article/B6T74-3W3N8M6-7/2/3a30996f8ff6c2227d14061f14904fda

Prevalence and disease association of the TT virus (TTV) were studied in Japanese patients with various types of viral hepatitis. A total of 317 patients with viral hepatitis were analyzed, and the results were compared to those of 100 apparently healthy controls. TTV DNA in serum was measured by semi-nested polymerase chain reaction. Prevalence of TTV DNA was significantly higher in patients with hepatitis A (36%, 5/14), hepatitis B (35%, 35/101), hepatitis C (61%, 90/148), and non-A-E hepatitis (41%, 22/54) than in healthy controls (12%, 12/100), respectively. In each type of hepatitis, the prevalence did not differ between acute and chronic liver diseases, and did not increase with the complication of hepatocellular carcinoma. The clinical backgrounds did not differ between TTV DNA positive and negative patients, in patients with acute hepatitis or in those with chronic liver diseases. Similarly, no liver function test showed a significantly higher level of in TTV DNA positive patients than in negative ones. In conclusion, TTV infection was highly prevalent in patients with viral hepatitis, especially in those with hepatitis C. TTV was suggested to have a weak pathogenicity (or no pathogenicity), at least when co-infecting with an established hepatitis virus.


http://www.sciencedirect.com/science/article/B6T74-4C4X4K6-1/2/7a436120b263a92b728652af50c7041c

The aim of this study was to estimate the correlation between telomerase activity and the expression of human telomerase RNA component (hTERC), human telomerase reverse transcriptase (hTERT) in patients with hepatocellular carcinoma (HCC), and to analyze the influence of the nucleotide homology of the hTERC template region on telomerase activity. Six HCC patients and two chronic hepatitis patients were enrolled in this study. Telomerase activity was determined using the fluorescence-based telomeric repeat amplification protocol (TRAP) method. Quantification of hTERC and hTERTmRNA was performed using a real-time PCR method. Furthermore, a portion of the hTERC gene was amplified using nested RT-PCR methods. After sub-cloning, the nucleotide sequence of the cloned hTERC that contained the template region was determined. Telomerase activity and hTERTmRNA was detected in all cancerous tissues, while hTERC was present in both tumorous and non-tumorous lesions. The
level of telomerase activity correlated with expression of hTERTmRNA, but not that of hTERC. The nucleotide sequence of cloned hTERC was similar in both tumorous and non-tumorous lesions. The expression of hTERT may be a definitive factor in the activation of telomerase in hepatocarcinogenesis.


http://www.sciencedirect.com/science/article/B6T74-3YJYCPK-2/2/2a6f347739de553784a6d693ea1513bc

We employed a PCR assay system TaKaRa Ex Taq(TM) (heat-resistant DNA polymerase), which has 3'-5' exonuclease activity to increase the sensitivity for TT virus (TTV) DNA detection. Sera obtained from 95 hepatitis B virus carriers without hepatitis C virus coinfection were tested for TTV DNA and the sensitivity of this assay system was compared with the PCR systems reported previously. Of the 95 individuals, TTV DNA was identified in 14 (14.7%) with the PCR reported by Nishizawa et al., in 66 (69.5%) with the PCR reported by Okamoto et al., in 80 (84.2%) by our assay system, and in 86 (90.5%) with the PCR reported by Takahashi et al. Phylogenetic analysis of nucleotide sequences amplified by the PCR revealed that genotypes 1a, 1b, 2, 3, 4, 5 were amplified efficiently by our assay system, while only a part of TTV DNA clone of genotype 1a was amplified by the PCR reported by Nishizawa et al. The prevalence of circulating TTV DNA became higher with age. These results indicate that our assay system with TaKaRa Ex Taq(TM) has confirmed high prevalence of TTV infection and that at least five genotypes prevail in Japan. In addition, acute TTV infection is supposed to cause long-standing viremia.


http://www.sciencedirect.com/science/article/B6T74-45XXF0F-4/2/998d14ead57822112b3113abe86415db

The platelet count increases after a sustained response to interferon (IFN) treatment for chronic hepatitis C (CH-C). However, the extent of the increase differs by patient. We investigated whether concurrent TT virus (TTV) infection interferes with the improvement of thrombocytopenia. Serial serum samples were obtained from 85 noncirrhotic CH-C patients who achieved a sustained virologic response for hepatitis C virus (HCV) upon IFN treatment, and tested for TTV DNA by three polymerase chain reaction (PCR) methods (UTR, N22 and TTV genotype-1). UTR PCR can detect essentially all TTV genotypes, whereas N22 PCR primarily detects four major TTV genotypes (1-4). Eighty-four patients (84/85, 99%) were positive for TTV DNA by UTR PCR, 27 (32%) by N22 PCR and 18 (21%) by TTV genotype-1 PCR just before IFN treatment was started (baseline). A sustained virologic response for TTV was observed in 6% (5/84) by UTR PCR, 52% (14/27) by N22 PCR and 56% (10/18) by TTV genotype-1 PCR. The platelet count was significantly lower in the N22 PCR-positive group than in the N22 PCR-negative group not only at baseline (14.9+/−3.8 vs. 18.1+/−6.4 x 104/μl, P<0.05), the differences also being statistically significant by TTV genotype-1 PCR, but not by UTR PCR. These results suggest that certain TTV genotypes including genotype 1 may play a role in aggravating the thrombocytopenia of CH-C patients, either alone or in concert with HCV.
A molecular epidemiological survey of various hepatitis viral infections, including hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV), was carried out in Ho Chi Minh City, Vietnam. This study included 295 patients with liver disease (234 viral related and 61 non-viral related) and 100 healthy individuals. The infection rates of HBV and HCV in 234 liver disease patients with acute hepatitis, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma, were 31.2 and 19.2%, respectively. On the other hand, detection rates of these viruses in healthy populations were 10 and 2%, respectively (P<0.005 and <0.0001, respectively). None of cases tested was positive for HDV RNA. The most common viral genotypes were type B and C of HBV (43 and 57%) and type 2a of HCV (33.3%). Surprisingly, high prevalence of HBV pre-S2 deletion mutant was found in 22 of 87 (25.3%) patients with chronic liver disease. Moreover, antibody to hepatitis E virus (HEV) immunoglobulin G (IgG) was detected in 78 of 185 (42%) and IgM in 1 of 185 (0.5%) patients. The age prevalence of anti-HEV IgG was reached 61.9% in 21-40-year-olds. These results suggest that these hepatitis viruses, except for HDV, are spreading among liver disease patients in Ho Chi Minh city, Vietnam and HBV was the most important causative agent correlated with liver disease in this area.

TT virus (TTV) is a human circovirus with a single-stranded, circular DNA genome of 3.8 kb. A method was developed to detect IgM antibodies to TTV as a serological marker for the diagnosis of acute TTV infection. IgM antibodies in test sera were captured by a monoclonal antibody against IgM[μ] on a solid support followed by binding of IgM with TTV derived from fecal extract of a TTV carrier. The presence of IgM-specific TTV particles was determined by polymerase chain reaction (PCR) using nucleic acids extracted from the solid support. Anti-TTV IgM was detected in sera from two patients with non-A to G post-transfusion hepatitis, who were positive for TTV DNA during weeks 10-21 and 12-17, respectively, following transfusion. The anti-TTV IgM was detectable after alanine transaminase levels were elevated and TTV DNA was detectable in the patients. The duration of the anti-TTV IgM was short-lived compared with anti-TTV IgG. Anti-TTV IgM was not detected in sera from any of 36 healthy individuals, with no detectable anti-TTV IgG or TTV DNA in their serum. These results indicate that anti-TTV IgM antibodies would be a useful marker to detect acute TTV infection.
pathogenesis. Heparanase mRNA expression was measured by real-time quantitative RT-PCR and localized at the cellular level by in situ hybridization. Heparanase protein levels and its localization were determined by Western blotting and immunohistochemistry. Expression of heparanase mRNA in primary HCC was increased 2-fold compared with liver cirrhosis and 2.2-fold in comparison with normal liver tissues, and this overexpression was even more pronounced in advanced stage HCC. In contrast, heparanase expression levels between cirrhotic tissues and normal liver tissues were not significantly different. In HCC the increased heparanase expression was localized in hepatic tumor cells and was only weakly present or absent in normal hepatocytes, bile duct epithelial cells and the connective tissues. These results suggest that increased heparanase expression is involved in the pathogenesis and progression of HCC. Its specific up-regulation in HCC but not in liver cirrhosis indicates that it might be used as a molecular marker for the differentiation of these disorders.

**Hum. Mol. Genet.** (50)


http://hmg.oupjournals.org/cgi/content/abstract/12/20/2693

Deficiencies in the activity of cytochrome c oxidase (COX) are an important cause of autosomal recessive respiratory chain disorders. Patients with isolated COX deficiency are clinically and genetically heterogeneous, and mutations in several different assembly factors have been found to cause specific clinical phenotypes. Two of the most common clinical presentations, Leigh Syndrome and hypertrophic cardiomyopathy, have so far only been associated with mutations in SURF1 or SCO2 and COX15, respectively. Here we show that expression of COX10 from a retroviral vector complements the COX deficiency in a patient with anemia and Leigh Syndrome, and in a patient with anemia, sensorineural deafness and fatal infantile hypertrophic cardiomyopathy. A partial rescue was also obtained following microcell-mediated transfer of mouse chromosomes into patient fibroblasts. COX10 functions in the first step of the mitochondrial heme A biosynthetic pathway, catalyzing the conversion of protoheme (heme B) to heme O via the farnesylation of a vinyl group at position C2. Heme A content was reduced in mitochondria from patient muscle and fibroblasts in proportion to the reduction in COX enzyme activity and the amount of fully assembled enzyme. Mutation analysis of COX10 identified four different missense alleles, predicting amino acid substitutions at evolutionarily conserved residues. A topological model places these residues in regions of the protein shown to have important catalytic functions by mutation analysis of a prokaryotic ortholog. Mutations in COX10 have previously been reported in a single family with tubulopathy and leukodystrophy. This study shows that mutations in this gene can cause nearly the full range of clinical phenotypes associated with early onset isolated COX deficiency.


http://hmg.oupjournals.org/cgi/content/abstract/12/16/2031
Sequences in exons can play an important role in constitutive and regulated pre-mRNA splicing. Since exonic splicing regulatory sequences are generally poorly conserved and their mechanism of action is not well understood, the consequence of exonic mutations on splicing can only be determined empirically. In this study, we have investigated the consequence of two cystic fibrosis (CF) disease-causing mutations, E656X and 2108delA, on the function of a putative exonic splicing enhancer (ESE) in exon 13 of the CFTR gene. We have also determined whether five other CF mutations D648V, D651N, G654S, E664X and T665S located near this putative ESE could lead to aberrant splicing of exon 13. Using minigene constructs, we have demonstrated that the E656X and 2108delA mutations could indeed cause aberrant splicing in a predicted manner, supporting a role for the putative ESE sequence in pre-mRNA splicing. In addition, we have shown that D648V, E664X and T665S mutations could cause aberrant splicing of exon 13 by improving the polypyrimidine tracts of two cryptic 3’ splice sites. We also provide evidence that the relative levels of two splicing factors, hTra2{alpha} and SF2/ASF, could alter the effect on splicing of some of the exon 13 disease mutations. Taken together, our results suggest that the severity of CF disease could be modulated by changes in the fidelity of CFTR pre-mRNA splicing.


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Mutations resulting in a premature termination codon (PTC) are a major cause of inherited disorders, and the majority of these mutant RNA transcripts are subjected to nonsense-mediated mRNA decay (NMD). This RNA surveillance results in reduced mutant allele expression, the extent of which can impact on the clinical severity. The molecular mechanisms of NMD in mammalian cells, its relationship to splicing and translation, downstream sequence elements and binding factors remains only partially understood. Currently there is little information on whether the extent of NMD is gene- or tissue-specific, although nonsense mutation inhibition of RNA splicing has been shown to exhibit some tissue and gene specificity in vitro. Schmid metaphyseal chondrodysplasia results from heterozygous mutations in the gene for collagen X (COL10A1), expressed by the hypertrophic chondrocytes of growth plate cartilage. In one patient a PTC mutation has been shown to result in complete NMD and collagen X haploinsufficiency in cartilage. Here we show that, in this patient, and in another with a different collagen X PTC mutation also leading to complete NMD in cartilage, the mutant mRNAs were not subjected to NMD in non-cartilage cells (lymphoblasts and bone cells). These data suggest that novel RNA surveillance mechanisms may exist in cartilage and that tissue specificity of NMD could be of importance in understanding the molecular pathology of nonsense mutations. Furthermore, the demonstration of collagen X haploinsufficiency in the second patient to be studied at the level of tissue expression, confirms that nonsense mutations leading to complete mutant collagen X mRNA degradation in cartilage is an important molecular cause of SMCD.


http://hmg.oupjournals.org/cgi/content/abstract/14/1/59

Despite being the second most frequent type of polymorphism in the genome, diallelic insertion-deletion polymorphisms (indels) have received far less attention in the study of sequence variation. In this report, we describe an approach that can detect indels in the heterozygous state and can comprehensively identify indels in the target sequence. Using this approach, we
identified 2393 indels in a set of 330 candidate genes, i.e. an average of seven indels per gene with about two indels per gene being common (minor allele frequency $\geq 0.1$). We compared the population genetic characteristics of indels with substitutions in this data. Our data supported the findings that deletions occur more frequently in the human genome. 5'-UTR and coding regions of the genes showed a significantly lower diversity for indels compared with other regions, suggesting differences in effects of selection on indels and substitutions. Sequence diversity and pairwise linkage disequilibrium (LD) findings of the different populations were similar to earlier results and included a greater skew towards low-frequency variants and a faster rate of LD decay in the African-descent population compared with the non-African populations. Within populations, the allele frequency spectra and LD-decay profiles for indels were similar to substitutions. Overall, the findings suggest that, although the mechanisms giving rise to indels may be different from those causing substitutions, the evolutionary histories of indels and substitutions are similar, and that indels can play a valuable role in association studies and marker selection strategies.


http://hmg.oupjournals.org/cgi/content/abstract/11/8/971

Benign hereditary chorea (BHC) (MIM 118700) is an autosomal dominant movement disorder. The early onset of symptoms (usually before the age of 5 years) and the observation that in some BHC families the symptoms tend to decrease in adulthood suggests that the disorder results from a developmental disturbance of the brain. In contrast to Huntington disease (MIM 143100), BHC is non-progressive and patients have normal or slightly below normal intelligence. There is considerable inter- and intrafamilial variability, including dystaxia, axial dystonia and gait disturbances. Previously, we identified a locus for BHC on chromosome 14 and subsequently identified additional independent families linked to the same locus. Recombination analysis of all chromosome 14-linked families resulted initially in a reduction of the critical interval for the BHC gene to 8.4 cM between markers D14S49 and D14S278. More detailed analysis of the critical region in a small BHC family revealed a de novo deletion of 1.2 Mb harboring the TITF-1 gene, a homeodomain-containing transcription factor essential for the organogenesis of the lung, thyroid and the basal ganglia. Here we report evidence that mutations in TITF-1 are associated with BHC.


http://hmg.oupjournals.org/cgi/content/abstract/11/2/125

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is a small conductance chloride ion channel that may interact directly with other channels including the epithelial sodium channel (ENaC). CFTR is known to be more abundant in the airway epithelium during the second trimester of human development than after birth. This could be a consequence of the change in function of the respiratory epithelium from chloride secretion to sodium absorption near term. Alternatively it might reflect an additional role for CFTR in the developing airway epithelium. Though the lung epithelia of CF fetuses and infants rarely show gross histological abnormalities, there is often evidence of inflammation. Our aim was to establish whether CFTR expression levels correlated with specific developmental stages or differentiated functions in the ovine fetal lung. We evaluated CFTR expression using a quantitative assay of mRNA at 14 time points through gestation and showed highest levels at the start of the second trimester followed by a gradual decline through to term. In contrast, ENaC expression increased from the start of the third trimester. These results support a role for CFTR in differentiation of the respiratory epithelium and
suggest that its expression levels are not merely reflecting major changes in the sodium/chloride bulk flow close to term. These observations may have significant implications for the likely success of CF gene therapy in the postnatal lung.


http://hmg.oupjournals.org/cgi/content/abstract/13/20/2399

Multiple endocrine neoplasia type I (MEN1) is an autosomal dominant cancer predisposition syndrome, the gene for which encodes a nuclear protein, menin. The biochemical function of this protein has not been completely elucidated, but several studies have shown a role in transcriptional modulation through recruitment of histone deacetylase. The mechanism by which MEN1 mutations cause tumorigenesis is unknown. The Drosophila homolog of MEN1, Mnn1, encodes a protein 50% identical to human menin. In order to further elucidate the function of MEN1, we generated a null allele of this gene in Drosophila and showed that homozygous inactivation results in morphologically normal flies that are hypersensitive to ionizing radiation and two DNA cross-linking agents, nitrogen mustard and cisplatinum. The spectrum of agents to which mutant flies are sensitive and analysis of the molecular mechanisms of this sensitivity suggest a defect in nucleotide excision repair. Drosophila Mnn1 mutants have an elevated rate of both sporadic and DNA damage-induced mutations. In a genetic background heterozygous for lats, a Drosophila and vertebrate tumor suppressor gene, homozygous inactivation of Mnn1 enhanced somatic mutation of the second allele of lats and formation of multiple primary tumors. Our data indicate that Mnn1 is a novel member of the class of autosomal dominant cancer genes that function in maintenance of genomic integrity, similar to the BRCA and HNPCC genes.


http://hmg.oupjournals.org/cgi/content/abstract/11/17/2051

Thyroid dysgenesis is the most common cause of congenital hypothyroidism (CH) and its genetic basis is largely unknown. Here, we describe the second homozygous missense mutation in TTF-2 (or FOXE1), a transcription factor that has been implicated in thyroid development. Two male siblings, born to consanguineous parents, presented with CH, athyreosis and cleft palate and were found to be homozygous for a mutation corresponding to a serine to asparagine substitution at codon 57 (S57N) in the forkhead DNA binding domain of TTF-2. Their heterozygous parents were unaffected and this mutation was not found in 31 unrelated cases of athyreosis or normal controls. Consistent with its location, the S57N TTF-2 mutant protein showed impaired DNA binding and partial loss of transcriptional function. Such incomplete loss of TTF-2 function may account for the absence of choanal atresia and bifid epiglottis in our patients, anomalies which were present together with CH and cleft palate in two other individuals with the only other, more deleterious, TTF-2 mutation (A65V) described previously. Our observations support the role of TTF-2 in both thyroid and palate development but suggest phenotypic heterogeneity of this syndromic form of CH.

Gene expression analyses of postmortem cerebral cortex suggest that transcription of the regulator of G-protein signaling 4 (RGS4) is decreased in a diagnosis-specific manner in subjects with schizophrenia. To evaluate the possible role of RGS4 in the pathogenesis of schizophrenia, we conducted genetic association and linkage studies using samples ascertained independently in Pittsburgh and New Delhi and by the NIMH Collaborative Genetics Initiative. Using the transmission disequilibrium test, significant transmission distortion was observed in the Pittsburgh and NIMH samples. Among single-nucleotide polymorphisms (SNPs) spanning approximately 300 kb, significant associations involved four SNPs localized to a 10 kb region at RGS4, but the associated haplotypes differed. A trend for transmission distortion was also present in the Indian sample for haplotypes incorporating the same SNPs. Consistent with the linkage/association observed from the family-based tests, samples with affected siblings (NIMH, India) showed higher levels of allele sharing, identical by descent, at RGS4. When the US patients were contrasted to two population-based control samples, however, no significant differences were observed. To check the specificity of the transmission bias, we therefore examined US families with bipolar I disorder (BD1) probands. This sample also showed a trend for transmission distortion, and differed significantly from the population-based controls for the four-SNP haplotypes tested in the other samples. The transmission distortion is unlikely to be due to chance, but its mechanism and specificity require further study. Our results illustrate the potential power of combining gene expression profiling and genomic analyses to identify susceptibility genes for genetically complex disorders.


The majority of cystic fibrosis patients produce a mutant form of CFTR ((Delta)F508) which has been shown to be mislocalized in both humans and mice. G480C, another clinically severe mutation, has also been demonstrated to be defective in its intracellular processing, but when allowed to traffic in Xenopus oocytes showed similar channel characteristics to that of wild-type CFTR. We have replicated the G480C mutation in the murine Cfr gene using the hit and run double recombination procedure. As expected, the G480C cystic fibrosis mouse model expresses the G480C mutant transcript at a level comparable to that of wild-type Cfr. The homozygous mutant mice were fertile, had normal survival, weight, tooth colour and no evidence of caecal blockage, despite mild goblet cell hypertrophy in the intestine. Analysis of the mutant protein revealed that the majority of G480C CFTR was abnormally processed and no G480C CFTR-specific immunostaining in the apical membranes of intestinal cells was detected. The bioelectric phenotype of these mice revealed organ-specific electrophysiological effects. In contrast to (Delta)F508 hit and run homozygotes, the classic defect of forskolin-induced chloride ion transport is not replicated in the caecum, but the response to low chloride in the nose is clearly defective in the G480C mutant animals. The mild phenotype of these G480C mutant animals combined with the defective chloride transport in the nose uniquely provides a valuable resource to test novel pharmacological agents aimed at improving trafficking and correcting the electrophysiological defect in the respiratory tract.

Increased susceptibility to gastric cancer has been associated with a wide range of host genetic and environmental factors, including Helicobacter pylori infection. Helicobacter pylori infection is postulated to initiate a progression through atrophic gastritis, metaplasia and dysplasia to cancer, and has been associated with reduction of acid output and dysregulation of stomach mucins. Here, we present the characterization of two mouse lines carrying mutant alleles of the gene encoding the Kcnq1 potassium channel, which very rapidly establish chronic gastritis in a pathogen-exposed environment. These mice develop gastric hyperplasia, hypochlorhydria and mucin dysregulation independent of infection. Metaplasia, dysplasia and pre-malignant adenomatous hyperplasia of the stomach have been observed in these Kcnq1 mutant mice, also independent of infection. The data presented here suggest that Kcnq1 mutant mice can be used both as an efficient model for the development of atrophic gastritis after infection and to determine the processes during the later stages of progression to gastric cancer independent of infection. Thus, Kcnq1 mutant mice are a powerful new tool for investigating the connection between acid balance, Helicobacter infection and mucin disruption in the progression to gastric cancer.


Cytogenetic evidence, in the form of deletions and balanced translocations, points to the existence of a locus on 2q32-q33, for which haploinsufficiency results in isolated cleft palate (CPO). Here we show by high-resolution FISH mapping of two de novo CPO-associated translocations involving 2q32-q33 that one breakpoint interrupts the transcription unit of the gene encoding the DNA-binding protein SATB2 (formerly KIAA1034). The breakpoint in the other translocation is located 130 kb 3' to the SATB2 polyadenylation signal, within a conserved region of non-coding DNA. The SATB2 gene is transcribed in a telomeric to centromeric direction and lies in a gene-poor region of 2q32-q33; the nearest confirmed gene is 1.26 Mb centromeric to the SATB2 polyadenylation signal. SATB2-encoding transcripts are assembled from 11 exons that span 191 kb of genomic DNA. They encode a protein of 733 amino acids that has two CUT domains and a homeodomain and shows a remarkable degree of evolutionary conservation, with only three amino acid substitutions between mouse and human. This protein belongs to the same family as SATB1, a nuclear matrix-attachment region binding protein implicated in transcriptional control and control of chromatin remodelling. There are also sequence similarities to the Drosophila protein DVE. Whole mount in situ hybridization to mouse embryos shows site- and stage-specific expression of SATB2 in the developing palate. Despite the strong evidence supporting an important role for SATB2 in palate development, mutation analysis of 70 unrelated patients with CPO did not reveal any coding region variants.


Sveinsson's chorioretinal atrophy (SCRA), also referred to as helicoid peripapillary chorioretinal degeneration or atrophia areata, is an autosomal dominant eye disease, characterized by symmetrical lesions radiating from the optic disc involving the retina and the choroid. Genome-wide linkage analysis mapped the SCRA gene to chromosome 11p15 in 81 patients from a large founder pedigree in Iceland. The parametric LOD score obtained was 18.9 using an autosomal dominant model with high penetrance. Crossover analysis of the linkage region with 51 markers
identified a 593 kb segment shared by all patients. Sequencing exons of the only gene in this interval, the transcriptional enhancer TEAD1, revealed a novel missense mutation (Y421H) carried by all patients and none of the 502 controls. The mutation is in a conserved amino acid sequence in the C terminal of the protein, a potential binding site for YAP65 one of TEAD1’s cofactors that is expressed in human retina as well as TEAD1 based on RT-PCR experiments. Therefore, we conclude that the mutation in the TEAD1 gene is the cause of Sveinsson’s chorioretinal atrophy.


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Imprinting is an epigenetic mechanism leading to mono-allelic expression of imprinted genes. In order to inherit the differential epigenetic imprints from one generation to the next, these imprints have to be erased in the primordial germ cells and re-established in a sex-specific manner during gametogenesis. The exact timing of the imprint resetting is not yet known and the use of immature gametes in assisted reproductive technologies may therefore lead to abnormal imprinting and related diseases. Imprinting is associated with differential allelic methylation in a CpG-context. We studied the methylation patterns of the imprint control (IC) region of the human SNRPN-gene in human spermatozoa, oocytes in different developmental stages [germinal vesicle (GV), metaphase I and metaphase II oocytes] and in preimplantation embryos using the bisulphite sequencing technique. In the spermatozoa, almost all potential methylation sites were unmethylated whereas mainly methylated patterns were found in the oocytes at different developmental stages. In the embryos, an average methylation pattern of 53% was found indicating that the imprints, which have been set during gametogenesis, are stably maintained in the preimplantation embryo. Our results indicate that the maternal imprints for the IC-region of the human SNRPN-gene are already re-established at the GV stage and that they are not re-established in a late oocyte stage or after fertilization as previously reported. Recent advances in assisted reproductive technologies raise questions concerning safety and the epigenetic risks involved. Our study was the first to check the methylation imprints in human pre-implantation embryos and oocytes at different developmental stages.


http://hmg.oupjournals.org/cgi/content/abstract/11/19/2309

In humans, failure of testicular descent (cryptorchidism) is one of the most frequent congenital malformations, affecting 1-3% of newborn boys. The clinical consequences of this abnormality are infertility in adulthood and a significantly increased risk of testicular malignancy. Recently, we described a mouse transgene insertional mutation, crsp, causing high intraabdominal cryptorchidism in homozygous males. A candidate gene Great (G-protein-coupled receptor affecting testis descent), was identified within the transgene integration site. Great encodes a seven-transmembrane receptor with a close similarity to the glycoprotein hormone receptors. The Great gene is highly expressed in the gubernaculum, the ligament that controls testicular movement during development, and therefore may be responsible for mediating hormonal signals that affect testicular descent. Here we show that genetic targeting of the Great gene in mice causes infertile bilateral intraabdominal cryptorchidism. The mutant gubernaculae fail to differentiate, indicating that the Great gene controls their development. Mutation screening of the human GREAT gene was performed using DHPLC analysis of the genomic DNA from 60 cryptorchid patients. Nucleotide variations in GREAT cDNA were found in both the patient and
the control populations. A unique missense mutation (T222P) in the ectodomain of the GREAT receptor was identified in one of the patients. This mutant receptor fails to respond to ligand stimulation, implicating the GREAT gene in the etiology in some cases of cryptorchidism in humans.


We previously reported association of FCGR2B-Ile232Thr with systemic lupus erythematosus (SLE) in three Asian populations. Because polymorphism of CD72, another inhibitory receptor of B cells, was associated with murine SLE, we identified human CD72 polymorphisms, tested their association with SLE and examined genetic interaction with FCGR2B in the Japanese (160 SLE, 277 controls), Thais (87 SLE, 187 controls) and Caucasians (94 families containing SLE members). Four polymorphisms and six rare variations were detected. The former constituted two major haplotypes that contained one or two repeats of 13 nucleotides in intron 8 (designated as *1 and *2, respectively). Although association with susceptibility to SLE was not detected, the *1 allele was significantly associated with nephritis among the Japanese patients (P=0.024). RT-PCR identified a novel alternatively spliced (AS) transcript that was expressed at the protein level in COS-7 transfectants. The ratio of AS/common isoforms was strikingly increased in individuals with *2/*2 genotype when compared with *1/*1 (P=0.000038) or *1/*2 (P=0.0085) genotypes. Using the two Asian cohorts, significant association of FCGR2B-232Thr/Thr with SLE was observed only in the presence of CD72-*1/*1 genotype (OR 4.63, 95% CI 1.47-14.6, P=0.009 versus FCGR2B-232Ile/Ile plus CD72-*2/*2). Minigene assays demonstrated that the 13-nucleotide repeat and 4 bp deletion within the same haplotype of intron 8 could regulate alternative splicing. The AS isoform lacks exon 8, and is deduced to contain 49 amino acid changes in the membrane-distal portion of the extracellular domain, where considerable amino acid changes are known in CD72c allele associated with murine SLE. These results indicated that the presence of CD72-*2 allele decreases risk for human SLE conferred by FCGR2B-232Thr, possibly by increasing the AS isoform of CD72.


Limb girdle muscular dystrophy type 2B and Miyoshi myopathy are clinically distinct forms of muscular dystrophy that arise from defects in the dysferlin gene. Here, we report two novel lines of dysferlin-deficient mice obtained by (a) gene targeting and (b) identification of an inbred strain, A/J, bearing a retrotransposon insertion in the dysferlin gene. The mutations in these mice were located at the 3' and 5' ends of the dysferlin gene. Both lines of mice lacked dysferlin and developed a progressive muscular dystrophy with histopathological and ultrastructural features that closely resemble the human disease. Vital staining with Evans blue dye revealed loss of sarcolemmal integrity in both lines of mice, similar to that seen in mdx and caveolin-3 deficient mice. However, in contrast to the latter group of animals, the dysferlin-deficient mice have an intact dystrophin glycoprotein complex and normal levels of caveolin-3. Our findings indicate that muscle membrane disruption and myofiber degeneration in dysferlinopathy were directly mediated by the loss of dysferlin via a new pathogenic mechanism in muscular dystrophies. We also show that the mutation in the A/J mice arose between the late 1970s and the early 1980s, and had become fixed in the production breeding stocks. Therefore, all studies involving the A/J
mice or mice derived from A/J, including recombinant inbred, recombinant congenic and chromosome substitution strains, should take into account the dysferlin defect in these strains. These new dysferlin-deficient mice should be useful for elucidating the pathogenic pathway in dysferlinopathy and for developing therapeutic strategies.


http://hmg.oupjournals.org/cgi/content/abstract/12/6/685

Autosomal-recessive polycystic kidney disease (ARPKD) is caused by mutation to a large gene, PKHD1, encoding a putative receptor protein, fibrocystin. We have identified, through analysis of human genomic sequence, a PKHD1 homolog, PKHDL1, in chromosome region 8q23. The PKHDL1 transcript of 13081 bp was amplified as 16 fragments and sequenced; the sequence of the murine ortholog, Pkhdl1 (chromosome region 15B3) was also determined. PKHDL1 contains 78 exons, covers a genomic region of 168 kb and encodes a large protein, fibrocystin-L. Screening PKHDL1 in ARPKD patients with no PKHD1 mutations revealed several sequence variants but no clear mutations, making it unlikely that it is ARPKD-associated. Human fibrocystin-L is predicted to be a large receptor protein (4243 aa; 466 kDa) with a signal peptide, single transmembrane domain and short cytoplasmic tail. Fibrocystin-L is homologous to fibrocystin throughout most of the extracellular region with overall identity of 25.0% and similarity of 41.5%. Fibrocystin-L has extracellular domains similar to fibrocystin with 14 copies of the TIG domain and two regions of significant homology to the protein TMEM2. Genomic sequence analysis identified no other full-length fibrocystin homologs in humans, mice or other sequenced organisms. The Fugu fish has a fibrocystin-L ortholog but no fibrocystin, suggesting that the newly identified protein may be the ancestral form. PKHDL1 and Pkhdl1 are widely expressed at a low level in most tissues but only detected in blood-derived cell-lines. Low level expression was detected in many primary immune cell subtypes but up-regulated specifically in T lymphocytes, following activation signals, suggesting a role in cellular immunity.


http://hmg.oupjournals.org/cgi/content/abstract/11/1/107

We report the identification of mutations in lipoxygenase-3 (ALOXE3) and 12(R)-lipoxygenase (ALOX12B) genes in non-bullous congenital ichthyosiform erythroderma (NCIE) linked to chromosome 17. Linkage disequilibrium analysis of six families affected by NCIE permitted us to reduce a recently reported interval of 8.4 cM on chromosome 17p13.1 to a 600 kb region around the marker D17S1796, which contains LOX genes. LOX products have long been implicated in skin disorders. Two point mutations and one deletion were found in ALOXE3 and three point mutations were found in ALOX12B in these consanguineous families from the Mediterranean basin. ALOXE3 and ALOX12B are two genes which are physically linked and functionally related. They are separated by 38 kb, have one more exon than the other LOX genes and are mainly expressed in epithelial cells including keratinocytes. Although the main substrate(s) of the two enzymes is (are) still unknown, the products of ALOX12B obtained in experimental systems have been demonstrated to be of R-chirality. It seems likely that the product of one of these enzymes may be the substrate of the other, and that they belong to the same metabolic pathway.

http://hmg.oupjournals.org/cgi/content/abstract/12/23/3075

We mapped two new recessive mutations causing circling behavior and deafness to the same region on chromosome 7 and showed they are allelic by complementation analysis. One was named deaf circler' (allele symbol dfcr) and the other deaf circler 2 Jackson' (allele symbol dfcr-2J). Both were shown to be mutations of the Ush1c gene, the mouse ortholog of the gene responsible for human Usher syndrome type IC and for the non-syndromic deafness disorder DFNB18. The Ush1c gene contains 28 exons, 20 that are constitutive and eight that are alternatively spliced. The dfcr mutation is a 12.8 kb intragenic deletion that eliminates three constitutive and five alternatively spliced exons. The dfcr-2J mutation is a 1 bp deletion in an alternatively spliced exon that creates a transcriptional frame shift, changing 38 amino acid codons before introducing a premature stop codon. Both mutations cause congenital deafness and severe balance deficits due to inner ear dysfunction. The stereocilia of cochlear hair cells are disorganized and splayed in mutant mice, with subsequent degeneration of the hair cells and spiral ganglion cells. Harmonin, the protein encoded by Ush1c, has been shown to bind, by means of its PDZ-domains, with the products of other Usher syndrome genes, including Myo7a, Cdh23 and Sans. The complexes formed by these protein interactions are thought to be essential for maintaining the integrity of hair cell stereocilia. The Ush1c mutant mice described here provide a means to directly investigate these interactions in vivo and to evaluate gene structure-function relationships that affect inner ear and eye phenotypes.


http://hmg.oupjournals.org/cgi/content/abstract/12/5/453

The Jackson shaker (js) mouse carries a recessive mutation causing phenotypes such as deafness, abnormal behavior (circling and/or head-tossing) and degeneration of inner ear neuroepithelia. Two alleles have been identified so far, the original js and jsseal. A contig of three BAC clones was isolated by positional cloning. Two of the clones rescue the js phenotype by BAC transgenesis. Analysis of transcripts in an overlapping region of the two clones revealed a gene encoding a new scaffold-like protein, Sans, that showed mutations in the two js mutants. One was a guanine nucleotide insertion in the original js allele and the other a 7-base insertion in the jsseal allele. Both insertions are predicted to inactivate the Sans protein by frameshift mutations resulting in a truncated protein lacking the C-terminal SAM domain. Cochlear hair cells in the js mutants show disorganized stereocilia bundles, and Sans were highly expressed in inner and outer hair cells of cochlea. The existence of major motifs, ankyrin repeats and a SAM domain suggests that Sans may have an important role in the development and maintenance of the stereocilia bundles through protein-protein interaction.


http://hmg.oupjournals.org/cgi/content/abstract/12/14/1661
The MITF/TFE subfamily of basic helix-loop-helix leucine-zipper (bHLH-LZ) transcription factors consists of four closely related members, TFE3, TFEB, TFEC and MITF, which can form both homo- and heterodimers. Previously, we demonstrated that in t(X;1)(p11;q21)-positive renal cell carcinomas (RCCs), the TFE3 gene on the X chromosome is disrupted and fused to the PRCC gene on chromosome 1. Here we show that in t(6;11)(p21;q13)-positive RCCs the TFEB gene on chromosome 6 is fused to the Alpha gene on chromosome 11. The AlphaTFEB fusion gene appears to contain all coding exons of the TFEB gene linked to 5’ upstream regulatory sequences of the Alpha gene. Quantitative PCR analysis revealed that AlphaTFEB mRNA levels are up to 60-fold upregulated in primary tumor cells as compared with wild-type TFEB mRNA levels in normal kidney samples, resulting in a dramatic upregulation of TFEB protein levels. Additional transfection studies revealed that the TFEB protein encoded by the AlphaTFEB fusion gene is efficiently targeted to the nucleus. Based on these results we conclude that the RCC-associated t(6;11)(p21;q13) translocation leads to a dramatic transcriptional and translational upregulation of TFEB due to promoter substitution, thereby severely unbalancing the nuclear ratios of the MITF/TFE subfamily members. We speculate that this imbalance may lead to changes in the expression of downstream target genes, ultimately resulting in the development of RCC. Moreover, since this is the second MITF/TFE transcription factor that is involved in RCC development, our findings point towards a concept in which this bHLH-LZ subfamily may play a critical role in the regulation of (aberrant) renal cellular growth.


http://hmg.oupjournals.org/cgi/content/abstract/11/5/525

Tuberous sclerosis (TSC) is a autosomal dominant genetic disorder caused by mutations in either TSC1 or TSC2, and characterized by benign hamartoma growth. We developed a murine model of Tsc1 disease by gene targeting. Tsc1 null embryos die at mid-gestation from a failure of liver development. Tsc1 heterozygotes develop kidney cystadenomas and liver hemangiomas at high frequency, but the incidence of kidney tumors is somewhat lower than in Tsc2 heterozygote mice. Liver hemangiomas were more common, more severe and caused higher mortality in female than in male Tsc1 heterozygotes. Tsc1 null embryo fibroblast lines have persistent phosphorylation of the p70S6K (S6K) and its substrate S6, that is sensitive to treatment with rapamycin, indicating constitutive activation of the mTOR-S6K pathway due to loss of the Tsc1 protein, hamartin. Hyperphosphorylation of S6 is also seen in kidney tumors in the heterozygote mice, suggesting that inhibition of this pathway may have benefit in control of TSC hamartomas.


http://hmg.oupjournals.org/cgi/content/abstract/13/17/1913

The most common form of hereditary haemochromatosis is an adult-onset condition usually associated with the HFE C282Y/C282Y genotype. The phenotypic expression of this genotype is heterogeneous and depends on a complex interplay of genetic and non-genetic factors. The aim of the present study was to determine if mutations in the recently identified HJV gene were associated with more severe iron overload phenotypes in C282Y homozygous patients. From a cohort of 310 C282Y homozygous patients, we found nine (six males and three females) with an additional HJV missense mutation in the heterozygous state (S105L, E302K, N372D, R335Q or the previously described L101P and G320V). The iron indices of eight patients appeared to be
more severe than those observed in C282Y homozygous patients of identical sex and similar age ranges. The mean serum ferritin concentration of the six males with an HJV mutation was significantly higher than that of C282Y homozygous males without an additional mutation [2350.3 (±1429.9) versus 1227.2 (±1130.1) μg/l; P=0.0233, Student's t-test]. We have recently reported that mutations in the gene that encodes hepcidin (HAMP) could explain one part of the C282Y/C282Y-related phenotypic heterogeneity by accentuating the iron burden. Our new data reveal that mutations in the HJV gene could be associated with a similar effect. Taken together, these results emphasize that a search for modifier genes could enable us to more precisely distinguish those C282Y homozygous patients with a higher risk to develop a severe iron overload and, consequently, clinical complications.


http://hmg.oupjournals.org/cgi/content/abstract/13/16/1715

Crohn's disease (CD) is a chronic inflammation affecting the gastrointestinal tract. Three mutations (Arg702Trp, Gly908Arg and Leu1007fsinsC) within the NOD2/CARD15 gene increase CD susceptibility. Here, we define cytokine regulation in primary human mononuclear cells, with muramyl dipeptide (MDP), the minimal NOD2/CARD15 activating component of peptidoglycan. By microarray, MDP induces a broad array of transcripts, including interleukin 1β (IL-1β) and interleukin 8 (IL-8). Leu1007fsinsC homozygotes demonstrated decreased transcriptional response to MDP. Electromobility shift assay demonstrated that MDP-induced NF-κB activation is mediated via p50 and p65 subunits, but not RelB or c-Rel. In wild-type individuals, MDP-induced IL-8 protein expression with a greater response to high dose (1 μg/ml) compared with low-dose (10 ng/ml) MDP. At low MDP doses, in all homozygotes, we observed no induction of IL-8 protein. With high doses of MDP, Leu1007fsinsC homozygotes showed no induction. Modest induction of IL-8 protein was observed in Gly908Arg and Arg702Trp homozygotes, indicating varying MDP sensitivity of the CD-associated mutations. In wild-type healthy control, CD and ulcerative colitis individuals, low-dose MDP and TNF(α) alone results in only modest IL-1β protein induction. With MDP plus TNF(α), there is a synergistic induction of IL-1β secretion. In Leu1007fsinsC homozygotes, there is a profound defect in IL-1β secretion, despite marked induction of IL-1β mRNA. These findings demonstrate post-transcriptional dependency on the NOD2/CARD15 pathway for IL-1β secretion with MDP and TNF(α) treatment. Taken together, these studies suggest that a signaling defect of innate immunity to MDP may be an essential underlying defect in the pathogenesis of some CD patients.


http://hmg.oupjournals.org/cgi/content/abstract/13/2/171

Systemic lupus erythematosus (SLE), a complex multigenic disease, is a typical antibody-mediated autoimmune disease characterized by production of autoantibodies against a variety of autoantigens and immune complex-type tissue inflammation, most prominently in the kidney. Evidence suggests that genetic factors predisposing to aberrant proliferation/maturation of self-reactive B cells initiate and propagate the disease. In SLE-prone New Zealand Black (NZB) mice and their F1 cross with New Zealand White (NZW) mice, B cell abnormalities can be ascribed mainly to self-reactive CD5+ B1 cells. Our genome-wide scans to search for susceptibility genes for aberrant activation of B1 cells in these mice showed evidence that the gene, Ltk, encoding leukocyte tyrosine kinase (LTK), is a possible candidate. LTK is a receptor-type protein tyrosine
kinase, belonging to the insulin receptor superfamily, and is mainly expressed in B lymphocyte precursors and neuronal tissues. Sequence and functional analyses of the gene revealed that NZB has a gain-of-function polymorphism in the LTK kinase domain near YXXM, a binding motif of the p85 subunit of phosphatidylinositol 3-kinase (PI3K). SLE patients also had this type of Ltk polymorphism with a significantly higher frequency compared with the healthy controls. Our findings suggest that these polymorphic LTKs cause up-regulation of the PI3K pathway and possibly form one genetic component of susceptibility to abnormal proliferation of self-reactive B cells in SLE.


http://hmg.oupjournals.org/cgi/content/abstract/12/1/41

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant cerebellar ataxia caused by a CAG repeat expansion in the ataxin-7 gene. In humans, SCA7 is characterized by marked anticipation due to intergenerational repeat instability with a bias toward expansion, and is thus regarded as the most unstable of the polyglutamine diseases. To study the molecular basis of CAG/CTG repeat instability and its pathological significance, we generated lines of transgenic mice carrying either a SCA7 cDNA construct or a 13.5 kb SCA7 genomic fragment with 92 CAG repeats. While the cDNA transgenic mice showed little intergenerational repeat instability, the genomic fragment transgenic mice displayed marked intergenerational instability with an obvious expansion bias. We then went on to generate additional lines of genomic fragment transgenic mice, and observed that deletion of the 3’ genomic region significantly stabilized intergenerational transmission of the SCA7 CAG92 repeat. These results suggest that cis-information present on the genomic fragment is driving the instability process. As the SCA7 genomic fragment contains a large number of replication-associated motifs, the presence of such sequence elements may make the SCA7 CAG repeat region more susceptible to instability. Small-pool and standard PCR analysis of tissues from genomic fragment mice revealed large repeat expansions in their brains and livers, but no such changes were found in any tissues from cDNA transgenic mice that have been shown to undergo neurodegeneration. As large somatic repeat expansions are absent from the brains of SCA7 cDNA mice, our results indicate that neurodegeneration can occur without marked somatic mosaicism, at least in these mice.


http://hmg.oupjournals.org/cgi/content/abstract/ddi148v1

The vesicular monoamine transporter 2 (VMAT2, SLC18A2) takes up cytosolic monoamines into intracellular secretory vesicles, preventing their neurotoxicity in the cytosol and discharging them into extracellular space by exocytosis. It has been shown that one-copy deletion of the VMAT2 gene increases locomotion activity significantly in response to drug treatments and dopamine neuron death rate in response to neurotoxin treatments in knockout mice. Little is known about promoter polymorphisms and their influence on SLC18A2 promoter activity. We have resequenced 17.4 kb DNA in the SLC18A2 promoter region for Caucasians and revealed 47 polymorphisms that confer 13 haplotypes. One of the haplotypes reaches a frequency as high as 65%, likely due to positive selection. In vitro analysis showed a 20% difference in promoter activity between the two frequent haplotypes and identified some of the polymorphisms that influence promoter activity. Four haplotype-defining single nucleotide polymorphisms (hdSNPs) can define the frequent haplotypes and by genotyping these hdSNPs, we find that haplotypes
with -14234G and -2504C of SLC18A2 promoter region represent a protective factor against alcoholism (p=0.0038 by Fisher’s exact tests). Therefore, SLC18A2 promoter haplotypes defined here create a foundation for transcriptional characterization of individuality and for association study on monoamine-related human diseases.


http://hmg.oupjournals.org/cgi/content/abstract/12/21/2853

The congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive disorders. A new pathomechanism has recently been identified in a group of these disorders in which known or putative glycosyltransferases are defective. Common to all these conditions is the hypoglycosylation of {alpha}-dystroglycan. Fukuyama CMD, muscle-eye-brain disease and Walker-Warburg syndrome, each associated with eye abnormalities and neuronal migration defects, result from mutations in fukutin, POMGnT1 and POMT1, respectively, while mutations in the fukutin-related protein (FKRP) gene cause congenital muscular dystrophy 1C, typically lacking brain involvement. Another putative glycosyltransferase, Large, is mutated in the myodystrophy mouse. The human homologue of this gene is therefore a strong candidate for involvement in novel forms of muscular dystrophy. We studied 36 patients with muscular dystrophy and either mental retardation, structural brain changes or abnormal {alpha}-dystroglycan immunolabelling, unlinked to any reported CMD loci. Linkage analysis in seven informative families excluded involvement of LARGE but sequencing of this gene in the remaining 29 families identified one patient with a G1525A (Glu509Lys) missense mutation and a 1 bp insertion, 1999insT. This 17-year-old girl presented with congenital muscular dystrophy, profound mental retardation, white matter changes and subtle structural abnormalities on brain MRI. Her skeletal muscle biopsy showed reduced immunolabelling of {alpha}-dystroglycan. Immunoblotting with an antibody to a glycosylated epitope demonstrated a reduced molecular weight form of {alpha}-dystroglycan that retained some laminin binding activity. This is the first description of mutations in the human LARGE gene and we propose to name this new disorder MDC1D.


http://hmg.oupjournals.org/cgi/content/abstract/ddi172v1

Mutations in GJB2 (gap junction protein, beta-2) are the major cause of autosomal recessive non-syndromic hearing loss. A few allele variants of this gene also cause autosomal dominant non-syndromic hearing loss as a dominant-negative consequence of expression of the mutant protein. Allele-specific gene suppression by RNA interference (RNAi) is a potentially attractive strategy to prevent hearing loss caused by this mechanism. In this proof-of-principle study, we identified a potent GJB2-targeting short interfering RNA (siRNA) to post-transcriptionally silence expression of the R75W allele variant of GJB2 in cultured mammalian cells. In a mouse model, this siRNA duplex selectively suppressed GJB2R75W expression by more than 70% of control levels thereby preventing hearing loss. The level of endogenous murine Gjb2 expression was not affected. Our data show that RNAi can be used with specificity and efficiency in vivo to protect against hearing loss caused as a dominant-negative consequence of mutant gene expression.

http://hmg.oupjournals.org/cgi/content/abstract/12/18/2395

Laryngo-onycho-cutaneous (LOC or Shabbir) syndrome (OMIM 245660) is an autosomal recessive epithelial disorder confined to the Punjabi Muslim population. The condition is characterized by cutaneous erosions, nail dystrophy and exuberant vascular granulation tissue in certain epithelia, especially conjunctiva and larynx. Genome-wide homozygosity mapping localized the gene to a 2 Mb region on chromosome 18q11.2 with an LOD score of 19.8 at {theta}=0. This region includes the laminin {alpha}3 gene (LAMA3), in which loss-of-expression mutations cause the lethal skin blistering disorder Herlitz junctional epidermolysis bullosa. Detailed investigation showed that this gene possesses a further 38 exons (76 exons in total) spanning 318 kb of genomic DNA, and encodes three distinct proteins, designated laminin {alpha}3a, {alpha}3b1 and {alpha}3b2. The causative mutation in 15 families was a frameshift mutation 151insG predicting a stop codon 7 bp downstream in an exon that is specific to laminin {alpha}3a. This protein is secreted only by the basal keratinocytes of stratified epithelia, implying that LOC is caused by dysfunction of keratinocyte-mesenchymal communication. Surprisingly, the 151insG mutation does not result in nonsense-mediated mRNA decay due to rescue of the transcript by an alternative translation start site 6 exons downstream. The resultant N-terminal deletion of laminin {alpha}3a was confirmed by immunoprecipitation of secreted proteins from LOC keratinocytes. These studies show that the laminin {alpha}3a N-terminal domain is a key regulator of the granulation tissue response, with important implications not only in LOC but in a range of other clinical conditions associated with abnormal wound healing.


http://hmg.oupjournals.org/cgi/content/abstract/12/17/2179

Mutations within the CRB1 gene have been shown to cause human retinal diseases including retinitis pigmentosa and Leber congenital amaurosis. We have recently identified a mouse model, retinal degeneration 8 (rd8) with a single base deletion in the Crb1 gene. This mutation is predicted to cause a frame shift and premature stop codon which truncates the transmembrane and cytoplasmic domain of CRB1. Like in Drosophila crumbs (crb) mutants, staining for adherens junction proteins known to localize to the external limiting membrane, the equivalent of the zonula adherens in the mammalian retina, is discontinuous and fragmented. Shortened photoreceptor inner and outer segments are observed as early as 2 weeks after birth, suggesting a developmental defect in these structures rather than a degenerative process. Photoreceptor degeneration is observed only within regions of retinal spotting, which is seen predominantly in the inferior nasal quadrant of the eye, and is caused by retinal folds and pseudorosettes. Photoreceptor dysplasia and degeneration in Crb1 mutants strongly vary with genetic background, suggesting that the variability in phenotypes of human patients that carry mutations in CRB1 may be due to interactions with background modifiers in addition to allelic variations. The Crb1rd8 mouse model will facilitate the analysis of Crb1 function in the neural retina and the identification of interacting factors as candidate retinal disease genes.

Osteoarthritis (OA), a common skeletal disease, is a leading cause of disability among the elderly populations. OA is characterized by gradual loss of articular cartilage, but the etiology and pathogenesis of OA are largely unknown. Epidemiological and genetic studies have demonstrated that genetic factors play an important role in OA. To identify susceptibility genes for OA, we performed a large-scale, case-control association study using gene-based single nucleotide polymorphisms (SNPs). In two independent case-control populations, we found significant association (P=9.8x10-7) between hip OA and a SNP (IVS3-293C>T) located in intron 3 of the calmodulin (CaM) 1 gene (CALM1). CALM1 was expressed in cultured chondrocytes and articular cartilage, and its expression was increased in OA. Subsequent linkage-disequilibrium mapping identified five SNPs showing significant association equivalent to IVS3-293C>T. One of these (-16C>T) is located in the core promoter region of CALM1. Functional analyses indicate that the susceptibility -16T allele decreases CALM1 transcription in vitro and in vivo. Inhibition of CaM in chondrogenic cells reduced the expression of the major cartilage matrix genes Col2a1 and Agc1. These results suggest that the transcriptional level of CALM1 is associated with susceptibility for hip OA through modulation of chondrogenic activity. Our findings reveal the CALM1-mediated signaling pathway in chondrocytes as a novel potential target for treatment of OA.


Women with germline mutations in BRCA1 have a greatly elevated risk of breast and ovarian cancer. However, considerable variation in the degree of breast cancer risk associated with a BRCA1 mutation has been observed, suggesting that modifiers of BRCA1 penetrance may exist. We hypothesized that the modifier genes might be located in regions of allelic imbalance in the tumors of BRCA1 mutation carriers, as have been reported on chromosomes 4p, 4q and 5q. In order to determine whether novel genetic modifiers of BRCA1-associated breast cancer penetrance in these regions exist, we used non-parametric linkage analysis methods to determine whether allele sharing of chromosomes 4p, 4q and 5q was observed preferentially within BRCA1 mutation families in women with BRCA1 mutations and breast cancer. No significant linkage on chromosome 4p or 4q was observed associated with breast cancer risk in BRCA1 mutation carriers. However, we observed a significant linkage signal at D5S1471 on chromosome 5q (P=0.009) in all the families analyzed together. The significance of this observation increased in the subset of families with an average of breast cancer diagnosis less than 45 years (P=0.003). These results suggest the presence of one or more genes on chromosome 5q33-34 that modify breast cancer risk in BRCA1 mutation carriers. The approach described here may be utilized to identify penetrance modifiers in other autosomal dominant syndromes.


Angelman syndrome is a neurogenetic disorder caused by the loss of function of the imprinted UBE3A gene in 15q11-q13. In a small group of patients, the disease is due to an imprinting defect (ID) that silences the maternal UBE3A allele. The presence of a faint maternal band detected by
methylation-specific PCR analysis of the SNURF-SNRPN locus in approximately one-third of patients who have an ID but no imprinting center deletion suggested that these patients are mosaics of ID cells and normal cells. In two patients studied, somatic mosaicism was proven by molecular and cellular cloning, respectively. X inactivation studies of cloned fibroblasts from one patient suggest that ID occurred before the blastocyst stage. To quantify the degree of mosaicism, we developed a novel quantitative methylation assay based on real-time PCR. In 24 patients tested, the percentage of normal cells ranged from <1% to 40%. Regression analysis suggests that patients with a higher percentage of normally methylated cells tend to have milder clinical symptoms than patients with a lower percentage. In conclusion, we suggest that the role of mosaic imprinting defects in mental retardation is underestimated.


Hirschsprung disease (HSCR) is a complex disorder that exhibits incomplete penetrance and variable expressivity due to interactions among multiple susceptibility genes. Studies in HSCR families have identified RET-dependent modifiers for short-segment HSCR (S-HSCR), but epistatic effects in long-segment (L-HSCR) and syndromic cases have not been fully explained. SOX10 mutations contribute to syndromic HSCR cases and Sox10 alleles in mice exhibit aganglionosis and pigmentary anomalies typical of a subset of HSCR patients categorized as Waardenburg-Shah Syndrome (WS4, OMIM 277580). Sox10 mutant alleles in mice exhibit strain dependent variation in penetrance and expressivity of aganglionic megacolon analogous to the variation observed in patients with aganglionosis. In this study we focused on enteric ganglia deficits in Sox10Dom mice and defined aganglionosis as a quantitative trait in Sox10Dom intercross progeny to investigate the contribution of strain background to variation in enteric nervous system deficits. We observe that the phenotype of Sox10Dom/+ mutants ranges over a continuum from severe aganglionosis to no detectable phenotype in the gut. To systematically identify genes that modulate Sox10-dependent aganglionosis we performed a SNP-based genome scan in Sox10Dom/+ F1 intercross progeny. Our analysis reveals modifier loci on mouse chromosomes 3, 5, 8, 11 and 14 with distinct effects on penetrance and severity of aganglionosis. Three of these loci on chromosomes 3, 8, and 11 do not coincide with previously known aganglionosis susceptibility genes or modifier loci and offer new avenues for elucidating the genetic network that modulates this complex neurocristopathy.


The severe reduction in mRNA and protein levels of the mitochondrial protein frataxin, encoded by the X25 gene, causes Friedreich ataxia (FRDA), the most common form of recessive hereditary ataxia. Increasing evidence underlines the pathogenetic role of oxidative stress in this disease. We generated an in vitro cellular model of regulated human frataxin overexpression. We identified, by differential display technique, the mitogen activated protein kinase kinase 4 mRNA down regulation in frataxin overexpressing cells. We studied the stress kinases pathway in this cellular model and in fibroblasts from FRDA patients. Frataxin overexpression reduced c-Jun N-terminal kinase phosphorylation. Furthermore, exposure of FRDA fibroblasts to several forms of environmental stress caused an up regulation of phospho-JNK and phospho-c-Jun. To understand if this susceptibility results in cell death, we have investigated the involvement of caspases. A significantly higher activation of caspase-9 was observed in FRDA versus control
fibroblasts after serum-withdrawal. Our findings suggest the presence, in FRDA patient cells, of a hyperactive stress signaling pathway. The role of frataxin in FRDA pathogenesis could be explained, at least in part, by this hyperactivity.


http://hmg.oupjournals.org/cgi/content/abstract/13/24/3089

The distribution of linkage disequilibrium (LD) in the human genome has important consequences for the design of experiments that infer susceptibility genes for complex disease using association studies. Recent studies have shown a non-random distribution of human meiotic recombination associated with intervening tracts of LD. Little is known about the processes, patterns and frequency of reciprocal meiotic recombination in humans. However, this phenomenon can be better understood by the fine structure analysis of several genomic regions by mapping hotspots and characterizing regions with variable LD. Here, we report clustered hotspot activity with intervening blocks of LD within the human PGM1 gene (1p31) using data derived from meiotic and population studies. Earlier work has suggested a high recombination rate in two regions within the PGM1 gene, site A (exons 4-8) and site B (exons 1A-4). Sequencing of eight individuals across 6 kb of targeted regions in site B identified 18 informative SNPs. Individuals from three distinct populations, Caucasian (n=264), Chinese (n=222) and Vietnamese (n=187), were genotyped, and haplotypes were determined using estimate of haplotypes, ldmax and Arlequin. Allelic association and haplotype analysis in these samples revealed variable recombination rates across PGM1, demonstrating the presence of: (i) three hotspots and (ii) three haplotype blocks. The spatial arrangement of haplotype blocks was identical in all populations studied. The pattern of association within PGM1 represents a region decomposed into small blocks of LD, where increased recombination activity has disrupted the ancestral chromosome. Additionally, crossovers in phased data mapped preferentially to regions where LD collapses, which also overlap with sequence motifs.


http://hmg.oupjournals.org/cgi/content/abstract/12/13/1507

Xeroderma pigmentosum (XP) is a skin cancer-prone autosomal recessive disease characterized by inability to repair UV-induced DNA damage. The major form of XP is defective in nucleotide excision repair (NER) and comprises seven complementation groups (A-G). The genes defective in all groups have been identified unambiguously with the exception of group E. The cells of some XP-E patients are deficient in a protein complex (consisting of two subunits: p127/DDB1 and p48/DDB2) which binds to UV-damaged DNA (UV-DDB) and is specifically involved in the removal of photoproducts from the non-transcribed regions of the genome. However, other XP-E patients have been reported not to lack UV-damaged DNA binding activity (DDB+). Here we describe several genetically unrelated XP-E patients, not previously analyzed in depth, each carrying two mutated alleles for DDB2, causing either a single amino acid change or a protein truncation or internal deletion. These defects result in a severe decrease of detectable p48 protein, abolish interaction with the p127 subunit, and produce a deficiency in UV-DDB binding activity (DDB-). The role of p48 in the repair defect of these patients was demonstrated in vivo and in vitro. Investigation of four DDB+ cell strains from patients previously assigned to XP-E, allowed us to reclassify all of them into other groups and to show that they do not share the
molecular and biochemical features typical for XP-E. Besides confirming that the true XP-E phenotype is DDB-, resulting from defects in a single gene, DDB2, our results identify the functional domains of the corresponding p48 protein.


http://hmg.oupjournals.org/cgi/content/abstract/13/4/417

Chromosome 22q11.2 deletions are found in almost 90% of patients with DiGeorge/velocardiofacial syndrome (DGS/VCFS). Large, chromosome-specific low copy repeats (LCRs), flanking and within the deletion interval, are presumed to lead to misalignment and aberrant recombination in meiosis resulting in this frequent microdeletion syndrome. We traced the grandparental origin of regions flanking de novo 3 Mb deletions in 20 informative three-generation families. Haplotype reconstruction showed an unexpectedly high number of proximal interchromosomal exchanges between homologs, occurring in 19/20 families. Instead, the normal chromosome 22 in these probands showed interchromosomal exchanges in 2/15 informative meioses, a rate consistent with the genetic distance. Meiotic exchanges, visualized as MLH1 foci, localize to the distal long arm of chromosome 22 in 75% of human spermatocytes tested, also reflecting the genetic map. Additionally, we found no effect of proband gender or parental age on the crossover frequency. Parental origin studies in 65 de novo 3 Mb deletions (including these 20 patients) demonstrated no bias. Unlike Williams syndrome, we found no chromosomal inversions flanked by LCRs in 22 sets of parents of 22q11 deleted patients, or in eight non-deleted patients with a DGS/VCFS phenotype using FISH. Our data are consistent with significant aberrant interchromosomal exchange events during meiosis I in the proximal region of the affected chromosome 22 as the likely etiology for the deletion. This type of exchange occurs more often than is described for deletions of chromosomes 7q11, 15q11, 17p11 and 17q11, implying a difference in the meiotic behavior of chromosome 22.


http://hmg.oupjournals.org/cgi/content/abstract/14/4/483

Autism is a common neurodevelopmental disorder of complex genetic etiology. Rett syndrome, an X-linked dominant disorder caused by MECP2 mutations, and Angelman syndrome, an imprinted disorder caused by maternal 15q11-q13 or UBE3A deficiency, have phenotypic and genetic overlap with autism. MECP2 encodes methyl-CpG-binding protein 2 that acts as a transcriptional repressor for methylated gene constructs but is surprisingly not required for maintaining imprinted gene expression. Here, we test the hypothesis that MECP2 deficiency may affect the level of expression of UBE3A and neighboring autism candidate gene GABRB3 without necessarily affecting imprinted expression. Multiple quantitative methods were used including automated quantitation of immunofluorescence and in situ hybridization by laser scanning cytometry on tissue microarrays, immunoblot and TaqMan PCR. The results demonstrated significant defects in UBE3A/E6AP expression in two different Mecp2 deficient mouse strains and human Rett, Angelman and autism brains compared with controls. Although no difference was observed in the allelic expression of several imprinted transcripts in Mecp2-null brain, Ube3a sense expression was significantly reduced, consistent with the decrease in protein. A non-imprinted gene from 15q11-q13, GABRB3, encoding the (beta)3 subunit of the GABAA receptor, also showed significantly reduced expression in multiple Rett, Angelman and autism brain samples, and Mecp2 deficient mice by quantitative immunoblot. These results suggest an
overlapping pathway of gene dysregulation within 15q11-q13 in Rett, Angelman and autism and implicate MeCP2 in the regulation of UBE3A and GABRB3 expressions in the postnatal mammalian brain.


http://hmg.oupjournals.org/cgi/content/abstract/11/25/3231

Waardenburg syndrome (WS; deafness with pigmentary abnormalities) is a congenital disorder caused by defective function of the embryonic neural crest. Depending on additional symptoms, WS is classified into four types: WS1, WS2, WS3 and WS4. WS1 and WS3 are caused by mutations in PAX3, whereas WS2 is heterogenous, being caused by mutations in the microphthalmia (MITF) gene in some but not all affected families. The identification of Slug, a zinc-finger transcription factor expressed in migratory neural crest cells, as the gene responsible for pigmentary disturbances in mice prompted us to analyse the role of its human homologue SLUG in neural crest defects. Here we show that two unrelated patients with WS2 have homozygous deletions in SLUG which result in absence of the SLUG product. We further show that Mitf is present in Slug-deficient cells and transactivates the SLUG promoter, and that Slugh and Kit genetically interact in vivo. Our findings further define the locus heterogeneity of WS2 and point to an essential role of SLUG in the development of neural crest-derived human cell lineages: its absence causes the auditory-pigmentary symptoms in at least some individuals with WS2.


http://hmg.oupjournals.org/cgi/content/abstract/14/4/543

Familial amyloid polyneuropathy type I is an autosomal dominant disorder caused by mutations in the transthyretin (TTR) gene; however, carriers of the same mutation exhibit variability in penetrance and clinical expression. We analyzed alleles of candidate genes encoding non-fibrillar components of TTR amyloid deposits and a molecule metabolically interacting with TTR [retinol-binding protein (RBP)], for possible associations with age of disease onset and/or susceptibility in a Portuguese population sample with the TTR V30M mutation and unrelated controls. We show that the V30M carriers represent a distinct subset of the Portuguese population. Estimates of genetic distance indicated that the controls and the classical-onset group were furthest apart, whereas the late-onset group appeared to differ from both. Importantly, the data also indicate that genetic interactions among the multiple loci evaluated, rather than single-locus effects, are more likely to determine differences in the age of disease onset. Multifactor dimensionality reduction indicated that the best genetic model for classical onset group versus controls involved the APCS gene, whereas for late-onset cases, one APCS variant (APCSv1) and two RBP variants (RBPv1 and RBPv2) are involved. Thus, although the TTR V30M mutation is required for the disease in Portuguese patients, different genetic factors may govern the age of onset, as well as the occurrence of anticipation.

ICF syndrome (immunodeficiency, centromere instability and facial anomalies) is a recessive human genetic disorder resulting from mutations in the DNA methyltransferase 3B (DNMT3B) gene. Patients with this disease exhibit numerous chromosomal abnormalities, including anomalous decondensation, pairing, separation and breakage, primarily involving the pericentric regions of chromosomes 1 and 16. Global levels of DNA methylation in ICF cells are only slightly reduced; however, certain repetitive sequences and genes on the inactive X chromosome of female ICF patients are significantly hypomethylated. In the present report, we analyze the molecular defect of de novo methylation in ICF cells in greater detail by making use of a model Epstein-Barr virus (EBV)-based system and three members of the unique cellular cancer-testis (C-T) gene family. Results with the EBV-based system indicate that de novo methylation of newly introduced viral sequences is defective in ICF syndrome. Limited de novo methylation capacity is retained in ICF cells, indicating that the mutations in DNMT3B are not complete loss-of-function mutations or that other DNMTs cooperate with DNMT3B. Analysis of three C-T genes (two on the X chromosome and one autosomal) revealed that loss of methylation from cellular gene sequences is heterogeneous, with both autosomal and X chromosome-based genes demonstrating sensitivity to mutations in DNMT3B. Aberrant hypomethylation at a number of loci examined correlated with altered gene expression levels. Lastly, no consistent changes in the protein levels of the DNA methyltransferases were noted when normal and ICF cell lines were compared.


Mechanisms of mitochondrial DNA (mtDNA) maintenance have recently gained wide interest owing to their role in inherited diseases as well as in aging. Twinkle is a new mitochondrial 5'-3' DNA helicase, defects of which we have previously shown to underlie a mitochondrial disease, progressive external ophthalmoplegia with multiple mtDNA deletions. Mouse Twinkle is highly similar to the human counterpart, suggesting conserved function. Here, we have characterized the mouse Twinkle gene and expression profile and report that the expression patterns are not conserved between human and mouse, but are synchronized with the adjacent gene MrpL43, suggesting a shared promoter. To elucidate the in vivo role of Twinkle in mtDNA maintenance, we generated two transgenic mouse lines overexpressing wild-type Twinkle. We could demonstrate for the first time that increased expression of Twinkle in muscle and heart increases mtDNA copy number up to 3-fold higher than controls, more than any other factor reported to date. Additionally, we utilized cultured human cells and observed that reduced expression of Twinkle by RNA interference mediated a rapid drop in mtDNA copy number, further supporting the in vivo results. These data demonstrate that Twinkle helicase is essential for mtDNA maintenance, and that it may be a key regulator of mtDNA copy number in mammals.


Domesticated animal species such as dogs and cats, with their many different characteristics and breed-specific diseases, and their close relationship and shared environment with humans, are a potentially rich source for the identification of the genetic contribution to human biology and
Copper toxicosis in Bedlington terriers is a genetic disease occurring with a high prevalence worldwide and is unique to this breed. Copper homeostasis appears to be well regulated in mammals. Two copper carrier proteins have been identified in man and rodents which, when dysfunctional, cause either copper deficiency (Menkes disease) or copper accumulation in various tissues (Wilson disease). However, these proteins are not primarily involved in the biliary excretion of copper. Bedlington terriers have a high prevalence of copper toxicosis and it is well documented that their biliary excretion of copper is impaired. This disease is of direct relevance for the understanding of copper metabolism in mammals. Previously, we mapped the copper toxicosis gene to dog chromosome region 10q26. Based on DNA samples obtained from privately owned dogs, we were able to confine the localization of the copper toxicosis gene to a region of <500 kb by linkage disequilibrium mapping. While screening genes and expressed sequence tags in this region for mutations we found that exon 2 of the MURR1 gene is deleted in both alleles of all affected Bedlington terriers and in single alleles in obligate carriers. Although the function of the MURR1 gene is still unknown, the discovery of a mutated MURR1 gene in Bedlington terriers with copper toxicosis provides a new lead to disentangling the complexities of copper metabolism in mammals.


http://hmg.oupjournals.org/cgi/content/abstract/13/21/2659

Matrix metalloproteinase 8 (MMP8), an enzyme that degrades fibrillar collagens imparting strength to the fetal membranes, is expressed by leukocytes and chorionic cytotrophoblast cells. We identified three single nucleotide polymorphisms (SNPs) at -799C/T, -381A/G and +17C/G from the major transcription start site in the MMP8 gene, and determined the functional significance of these SNPs by analyzing their impact upon MMP8 promoter activity and their association with preterm premature rupture of membranes (PPROM). The minor alleles +17 (G) and -381 (G) were in complete linkage disequilibrium. A promoter fragment containing the three minor alleles had 3-fold greater activity in chorion-like trophoblast cells (BeWo, JEG-3 and HTR-8/SVneo) compared with the major allele promoter construct. Electrophoretic mobility shift assays revealed differences in BeWo nuclear protein binding to oligonucleotides representing the -381 and -799 SNPs, suggesting that the minor alleles have reduced transcription factor binding. A case-control study of African-American neonates using allele-specific primers revealed a statistically significant association between the three minor allele haplotype, which displays the highest MMP8 promoter activity in trophoblast cells, with PPROM with an odds ratio (OR) of 4.63 (P<0.0001), whereas the major allele promoter appeared to be protective (OR=0.52, P<0.0002). None of the minor alleles were individually associated with PPROM. These findings demonstrate the functional significance of SNP haplotypes in the MMP8 gene and associations with obstetrical outcomes.


http://hmg.oupjournals.org/cgi/content/abstract/11/1/13

The completion of the human genome sequence will greatly accelerate development of a new branch of bioscience and provide fundamental knowledge to biomedical research. We used the sequence information to measure replication timing of the entire lengths of human chromosomes 11q and 21q. Megabase-sized zones that replicate early or late in S phase (thus early/late transition) were defined at the sequence level. Early zones were more GC-rich and gene-rich
than were late zones, and early/late transitions occurred primarily at positions identical to or near GC% transitions. We also found the single nucleotide polymorphism (SNP) frequency was high in the late-replicating and replication-transition regions. In the early/late transition regions, concentrated occurrence of cancer-related genes that include CCND1 encoding cyclin D1 (BCL1), FGF4 (KFGF), TIAM1 and FLI1, was observed. The transition regions contained other disease-related genes including APP associated with familial Alzheimer's disease (AD1), SOD1 associated with familial amyotrophic lateral sclerosis (ALS1) and PTS associated with phenylketonuria. These findings are discussed with respect to the prediction that increased DNA damage occurs in replication-transition regions. We propose that genome-wide assessment of replication timing serves as an efficient strategy for identifying disease-related genes.

http://hmg.oupjournals.org/cgi/content/abstract/11/5/599

Single nucleotide polymorphisms occur throughout the human genome. A gene that causes one of the congenital disorders of glycosylation (CDG) has a mutation (911T→C) that changes a phenylalanine to serine at position 304 (F304S) of the (alpha)1,3 glucosyl transferase. We show that this change reduces the ability of the gene product to rescue defective glycosylation of an alg6-deficient strain of Saccharomycyes cerevisiae during rapid growth. This finding suggested that the mutation might affect glycosylation in humans. We therefore compared the frequency of this variant in 301 controls and in 101 CDG patients who carry known mutations in other genes involved in CDG, i.e. PMM2 (CDG-Ia; 91 patients) and MPI (CDG-Ib; 10 patients). The variant allele frequency is identical in both CDG patients (0.30) and controls (0.28). Importantly, the F304S genotype frequency in 55 CDG-Ia patients classified as mild/moderate (n = 28), or severe (n = 27) was significantly higher in severely affected patients (0.41) than in mild/moderately affected patients (0.21). Mortality (n = 9) was higher when F304S was present (n = 6). Severely affected patients with the PMM2 mutations F119L/R141H (n = 22) carry the F304S mutation more often (0.36) than mildly affected patients (0.18, n = 11) with this mutation. Clinical severity of mildly affected sibs with the same PMM2 mutations did not correlate with F304S genotype. Thus, the presence of the F304S allele may exacerbate the clinical outcome, especially in severely affected CDG patients. We speculate that this type of variant may be implicated in other multi-factorial disorders that involve N-glycosylation.

http://hmg.oupjournals.org/cgi/content/abstract/12/17/2121

Numerous missense mutations in human BRCA1 gene have been linked to predisposition to breast cancer. However, the functional significance of the majority of these mutations remains unknown. We have examined the molecular basis for three such cancer-causing mutations. The first mutation, a T→G transversion in codon 64, is predicted to change a conserved cysteine residue to glycine in the RING finger domain of the 1863 amino acid BRCA1 protein. Using a humanized mouse model we demonstrate that this missense mutation actually results in a functionally null protein. This striking result occurs because the single base alteration generates a new 5' splice site in exon 5 and also disrupts a putative exonic splicing enhancer motif. Consequently, the normal splice donor site is disrupted and an internal cryptic splice site is activated. This results in a 22-nucleotide deletion and the aberrant transcript is predicted to encode a severely truncated protein consisting of only 63 amino acids. To identify other missense
mutations in BRCA1 that may result in aberrant splicing, we screened various mutations using the Genscan program. We demonstrate that at least two other missense mutations in codons 1495 and 1823 result in aberrant splicing due to the possible disruption of cis-acting splicing regulatory elements. In conclusion, our study demonstrates for the first time the application of a humanized mouse model for functional analysis of human mutations in mice and also shows the need for a careful examination of the functional consequences of single base alterations and single nucleotide polymorphisms identified in human disease-causing genes.

**Hum. Reprod.** (16)


http://humrep.oupjournals.org/cgi/content/abstract/18/10/2039

BACKGROUND: We recently showed that vascular endothelial growth factor (VEGF) expression by endometrial glandular epithelial and stromal cells, and endometrial microvascular endothelial cell permeability, an early step in angiogenesis, were rapidly increased by estradiol (E2) administration to ovariectomized baboons. We proposed that estrogen promotes endometrial angiogenesis by regulating VEGF expression by glandular epithelial and stromal cells. In the present study, we developed a co-culture of human endometrial cells and microvascular endothelial cells to determine whether the regulatory role shown for estrogen on endometrial angiogenesis in vivo in the non-human primate would be demonstrable in vitro in the human.

METHODS AND RESULTS: Human endometrial glandular epithelial and stromal cells were co-cultured with human myometrial microvascular endothelial cells (HMMECs) and E2. HMMEC tube formation (means +/- SEM, % endothelial tube area/total endothelial cell area), an index of angiogenesis, was 65% (P < 0.05) and 2-fold (P < 0.01) greater in cells co-cultured with human glandular epithelial cells (54 +/- 7%) and glandular epithelial cells plus E2 (66 +/- 11%), respectively, compared with medium (33 +/- 4%). In contrast, endothelial tube formation was not altered in HMMECs incubated with endometrial stromal cells (32 +/- 2%) or E2 (39 +/- 3%). CONCLUSIONS: We propose that estrogen, by regulating expression and secretion of angiogenic factors such as VEGF by glandular epithelial cells of the endometrium, regulates endometrial angiogenesis.


http://humrep.oupjournals.org/cgi/content/abstract/deh852v1

BACKGROUND: Vascular endothelial growth factor (VEGF), a major mediator of angiogenesis and vascular permeability, is known to play a key role in the pathophysiology of endometriosis. METHODS AND RESULTS: The single nucleotide polymorphisms, -460C>T and +405G>C, in the 5'-untranslated region of the VEGF gene were tested for association in a case-control study of 215 affected women and 210 women with no evidence of disease. All the women were of South Indian origin and ascertained from the same infertility clinic. The genotype and allele frequencies of the -460C>T polymorphism did not differ significantly between cases and controls. In contrast,
the genotype (P=0.002) and allele (P=0.001) frequencies of the +405G>C polymorphism showed a significant difference between cases and controls. The +405 GG genotype was found more often in patients with an endometrioma >3 cm compared to controls. The frequency of the -460T/+405C haplotype (P=0.016) was significantly lower in affected women compared to controls. CONCLUSIONS: The -460T/+405C haplotype in the VEGF gene, which is associated with lower promoter activity, was significantly less common in women with endometriosis than in controls. These data suggest that the +405G allele may influence the likelihood of a woman developing the disease.


http://humrep.oupjournals.org/cgi/content/abstract/20/5/1235

BACKGROUND: Aneuploidies involve ~80% of chromosomal anomalies found in spontaneous miscarriages. Since cytogenetic studies show high rates of failure, we have incorporated the quantitative fluorescent polymerase chain reaction (QF-PCR) technique to the study of numerical chromosome anomalies in miscarriages. METHODS: Multiplex and simple QF-PCR assays have been performed on 160 miscarriage and 34 parental DNA samples analysing specific short tandem repeat (STR) markers for chromosomes 2, 7, 13, 15, 16, 18, 21, 22 and X. Cases successfully karyotyped were used as controls in our study. RESULTS: While maternal contamination could be detected in such cases, a molecular result was obtained for 94% of miscarriages without a cytogenetic one. Thirty-six per cent of them were diagnosed with numerical chromosome anomalies. Parental origin of the extra chromosome and the error stage of meiosis could be also determined. CONCLUSIONS: QF-PCR represents a useful and reliable tool to diagnose aneuploidies in spontaneous miscarriages. It provides information about parental and meiotic origin of anomaly, allowing an appropriate genetic counselling.


http://humrep.oupjournals.org/cgi/content/abstract/17/8/2073

BACKGROUND: The relationship between Chlamydia trachomatis tubal factor infertility (TFI) and the host's immunoregulatory genes was studied. METHODS: Cell-mediated immune responses to C. trachomatis and chlamydial heat shock protein (CHSP60) were determined by lymphocyte proliferation assay. HLA-DQ alleles and interleukin-10 (IL-10) promoter polymorphism (-1082 A/G) were analysed in 52 TFI cases and in 61 controls by PCR. RESULTS: HLA-DQB1 or DQA1 alleles did not significantly differ between the TFI group and the control group. However, DQA1*0102 and DQB1*0602 alleles together with IL-10 -1082AA genotype were found significantly more frequently in the TFI patients than in the controls (0.18 and 0.02 respectively; P = 0.005). Five (22%) of the 23 patients who had a positive lymphocyte proliferative response to CHSP60 were positive also for IL-10 -1082AA and for the HLA-DQA1*DQA1*0102 and HLA-DQB1*DQB1*0602 alleles. CONCLUSIONS: Our results reveal an association of a cellular immune response to CHSP60, HLA class II alleles and IL-10 promoter genotypes in patients with chlamydial TFI.

Kok, H. S., N. C. Onland-Moret, et al. (2005). "No association of estrogen receptor (alpha) and

http://humrep.oupjournals.org/cgi/content/abstract/20/2/536

**BACKGROUND:** Age at menopause is under strong genetic control. So far, genetic variations of only one gene, the PvuII polymorphism of the estrogen receptor \(\alpha\) (ER{\(\alpha\)}) gene, have been shown to be associated with age at onset of menopause. This study aims to investigate whether PvuII, XbaI and B-variant polymorphisms of the ER{\(\alpha\}) gene, and the MspAI polymorphism of the cytochrome P450c17(a) (CYP17) gene are associated with age at menopause in a Dutch cohort. **METHODS:** DNA was isolated from urine samples of 385 Caucasian women with natural menopause and the genotypes of the four polymorphisms were determined. A questionnaire was used for background characteristics. The genotypes of PvuII, XbaI, MspAI were obtained by PCR restriction fragment length polymorphism analysis. The B-variant was determined with an allele-specific oligonucleotide hybridization method. Two-sided t-tests were performed to assess the association between the four polymorphisms and menopausal age. The PvuII and XbaI polymorphisms were analysed separately as well as in a combined score. **RESULTS:** The results show that none of the polymorphisms independently, nor the combined genotypes for PvuII and XbaI, were associated with age at natural menopause. **CONCLUSION:** No evidence was found for a relationship between common variants of the ER{\(\alpha\}) gene and the CYP17 gene with age at natural menopause.


http://humrep.oupjournals.org/cgi/content/abstract/19/9/2084

**BACKGROUND:** Polymorphism in the CTG triplet number in the myotonic dystrophy type 1 (DM1PK) gene has been proposed as being associated with idiopathic azoospermia. The aim of this study was to investigate whether the CTG trinucleotide amplification in the DM1PK gene is associated with male subfertility. **METHODS:** We evaluated 107 subfertile patients, male partners of infertile couples, affected by non-obstructive azoospermia (n=38) and oligoasthenoteratozoospermia (OAT) (n=69), and 102 men with proven fertility. Main outcome measures were CTG repeat size in the DM1PK gene, testicular volume, sperm concentration, rapid progressive motility, normal morphology, serum FSH levels, testicular histology and Johnsen score. **RESULTS:** In subfertile males, no minimal mutation or mutation carriers were found. The difference in the number of CTG repeat lengths between the groups was not statistically significant (P=0.825). There was no correlation between the number of CTG repeats and the clinical parameters of subfertile patients: testicular volume, sperm concentration, rapid progressive motility, normal morphology, serum FSH level, testicular histology and Johnsen score. **CONCLUSIONS:** The number of CTG repeats in the normal or mutational range of DM1PK gene is associated with neither idiopathic male subfertility nor with clinical characteristics of male subfertility.


http://humrep.oupjournals.org/cgi/content/abstract/18/11/2264

**BACKGROUND:** The adverse effects of hydrosalpinx on the outcomes of IVF have been well documented, but the mechanisms of hydrosalpinx fluid formation remain unclear. This study
compares the mRNA expression of vascular endothelial growth factor (VEGF) and its receptors (KDR and flt-1) in the hydrosalpinx with that in the healthy oviduct. METHODS: Oviduct tissue was collected from 10 infertile women with hydrosalpinx and 10 parous women with healthy oviduct. The mRNA expression of VEGF and its receptors in isolated oviduct epithelial cells were analysed using semi-quantitative reverse-transcriptase PCR. RESULTS: mRNA expression of VEGF and its receptor flt-1 in the hydrosalpinx was significantly higher than that in the healthy oviduct, but no significant difference was demonstrated for the KDR receptor. CONCLUSIONS: This study supports the notion that VEGF may play an important role in the hydrosalpinx fluid formation, possibly by promoting vascular and epithelial permeability and therefore serum transudation.

http://humrep.oupjournals.org/cgi/content/abstract/19/7/1580

BACKGROUND: The zona pellucida (ZP) is an extracellular glycoprotein matrix which surrounds all mammalian oocytes. Recent data have shown the presence of four human zona genes (ZP1, ZP2, ZP3 and ZPB). The aim of the study was to determine if all four ZP proteins are expressed and present in the human. METHODS: cDNA derived from human oocytes were used to amplify by PCR the four ZP genes. In addition, isolated native human ZP were heat-solubilized, trypsin-digested and subjected to tandem mass spectrometry (MS/MS). RESULTS: All four genes were expressed and the respective proteins present in the human ZP. Moreover, a bioinformatics approach showed that the mouse ZPB gene, although present, is likely to encode a non-functional protein. CONCLUSIONS: Four ZP genes are expressed in human oocytes (ZP1, ZP2, ZP3 and ZPB) and preliminary data show that the four corresponding ZP proteins are present in the human ZP. Therefore, this is a fundamental difference with the mouse model.

http://humrep.oupjournals.org/cgi/content/abstract/deh837v1

BACKGROUND: The zona pellucida (ZP) has multiple roles in reproductive processes, including oocyte maturation, fertilization and implantation. We used, for the first time, a genetic approach to study whether human ZP genes possess structural alterations in women with unsuccessful IVF trials. In theory, this may result in gradual reduction of sperm-zona interaction and eventually in total fertilization failure (TFF). METHODS: Eighteen infertile women (TFFs) whose IVF did not result in any fertilized oocytes, whereas fertilization by ICSI was successful, were screened for mutations in ZP genes by means of conformation-sensitive gel electrophoresis. Twenty-three fertilizers in IVF (FIVFs) and 68 women with proven fertility (WPFs) constituted the two control groups. RESULTS: Altogether, 20 sequence variations were found in the ZP genes. Two variations in ZP3, one in the regulatory region (c. 1-87 T[-gt]G) and one in exon 6 [c. 894 G[-gt]A (p. K298)] existed more frequently in TFFs than in FIVF and WPF groups (P-values 0.027 and 0.008, respectively). CONCLUSIONS: Our study on ZP genes of infertile women revealed a high degree of sequence variations. This may reflect gradual reduction of fertility among TFFs, but the putative roles and influences of single variations can only be hypothesized.

BACKGROUND: We report the first attempts at preimplantation genetic diagnosis (PGD) and IVF and their accompanying difficulties for achondroplasia (ACH) patients. METHODS: A PGD test was developed using fluorescent single cell PCR on lymphoblasts from patients and controls and from blastomeres from surplus IVF embryos. A specific digestion control based on the use of two fluorochromes was elaborated. Ovarian stimulation and oocyte retrieval were carried out using conventional protocols. RESULTS: We performed 88 single cell tests for which amplification was obtained in 86 (97.7%) single lymphoblasts. Allele drop out (ADO) was observed in two out of 53 (3.7%) heterozygous lymphoblasts. If we combine the results from the blastomere testing from surplus embryos with those from PGD cycles and re-analysis after PGD, we obtained a PCR signal in 84% of cases of which 91% were correctly diagnosed at the G380 locus. A total of six cycles were performed resulting in three embryo transfers. We observed difficulties in ovarian stimulation and oocyte retrieval with affected female patients. No pregnancy was obtained. CONCLUSION: A PGD test for ACH is now available at our centre but our initial practice raises questions on the feasibility of such a test, specially with affected female patients.


BACKGROUND: Preimplantation genetic diagnosis (PGD) usually involves blastomere biopsy 3 days post-insemination (p.i.), followed by genetic analysis and transfer of unaffected embryos later on day 3 or 4. We evaluate a strategy involving embryo biopsy on day 3 p.i., genetic analysis on day 4 and, following culture in blastocyst sequential media, transfer of unaffected embryos on day 5 p.i. METHODS: PGD cycles were initiated in 15 couples at risk of transmitting {beta}-thalassaemia major. Oocyte retrieval and ICSI were performed according to standard protocols. Embryo culture used blastocyst sequential media. Embryos were biopsied on day 3 p.i. using acid Tyrode's for zona drilling, and the single blastomeres were genotyped by a protocol involving nested polymerase chain reaction and denaturing gradient gel electrophoresis analysis. RESULTS: Forty of 109 (37%) embryos biopsied on day 3 p.i. developed to blastocysts by day 5 p.i., with at least one blastocyst available for transfer in 12 cycles (80%). Genotype analysis characterized 51/109 (47%) embryos unaffected for {beta}-thalassaemia major, of which 28 were blastocysts. Transfer of 37 day 5 p.i. embryos (blastocysts and non blastocysts) initiated eight clinical pregnancies. Implantation rate per embryo transferred was 12/37 (32%). CONCLUSIONS: Embryo biopsy on day 3, followed by delayed transfer until day 5 p.i. offers a novel and effective strategy to overcome the time limit encountered when performing PGD, without compromising embryo implantation.

CYP1A1 variant allele was present at frequencies of 0.61 and 0.44 in cases and controls, respectively (odds ratio = 1.93; P=0.023, 95% confidence interval 1.10-3.38). The CYP2D6 variant allele was present at a frequency of 0.17 in females with RPL, while in the control population the frequency was 0.16. The GSTM1 and GSTT1 null genotypes were present at frequencies of 0.39 and 0.26 in RPL cases, whereas in controls the frequencies were 0.37 and 0.17, respectively. The mutant GSTP1 frequencies in case and control women were 0.38 and 0.40, respectively. We report a significant association of the CYP1A1*2A allele with RPL which is confirmed by logistic regression analysis. No association was observed for the other polymorphisms or in their combinations studied. CONCLUSIONS: The present study suggests the occurrence of the CYP1A1*2A allele as a probable risk factor in idiopathic recurrent miscarriages.


BACKGROUND: Endometriosis, an estrogen-dependent disease, is believed to be influenced by multiple genetic and environmental factors. Here, we evaluated whether the risk and severity of endometriosis are associated with polymorphisms in estradiol-synthesizing enzyme genes: the Ser312Gly polymorphism in 17-beta-hydroxysteroid dehydrogenase type 1 (HSD17B1) and the Arg264Cys polymorphism in cytochrome P450, subfamily XIX (CYP19). METHODS: All participants underwent diagnostic laparoscopy, and the stage of endometriosis was determined according to the Revised American Fertility Society classification. Of the 138 women enrolled, 59 had no endometriosis, 21 had stage I, 10 had stage II, 23 had stage III and 25 had stage IV. SNPs were discriminated by allele-specific oligonucleotide hybridization. RESULTS: Individuals having at least one A-allele (A/G or A/A genotype) of HSD17B1 showed a significantly increased risk of endometriosis (A/G genotype: adjusted OR, 3.06; 95%CI 1.21-7.74; A/A genotype: adjusted OR, 3.02; 95%CI 1.08-8.43). There was a significant trend associating A/G + A/A genotypes with severity of endometriosis (P for trend <0.01). No statistically significant association was found for the CYP19 polymorphism. CONCLUSIONS: Evidence for association between the Ser312Gly polymorphism in HSD17B1 and endometriosis was found in a Japanese population. The A-allele of HSD17B1 appears to confer higher risk for endometriosis.


BACKGROUND: Cytokines have been described to play a major role in the pathogenesis of idiopathic recurrent miscarriage (IRM). We investigated the association between IRM and a polymorphism of the interleukin-6 (IL-6) gene and IL-6 serum levels. METHODS: In a prospective case-control study, we studied 161 women with IRM and 124 healthy controls. Peripheral venous puncture, DNA extraction and PCR were employed to genotype women for the presence of a polymorphism at position -174 in the promoter region of IL-6. Serum IL-6 levels were assessed by a commercially available ELISA. RESULTS: Allele frequencies among women with IRM and controls were 63.4 and 58.1% respectively for allele G (wild type), and 36.6 and 41.9% respectively for allele C (mutant). No association between allele C and the occurrence of IRM was found (odds ratio 0.8; 95% confidence interval = 0.57-1.12; P = NS). IL-6 serum levels were not significantly different between genotypes and between the study and control groups. CONCLUSIONS: This is the first report on an IL-6 polymorphism in IRM. Although known to alter IL-6 expression, the IL-6 polymorphism investigated was not associated with IRM and alterations
in IL-6 serum levels in a Middle-European Caucasian population.


http://humrep.oupjournals.org/cgi/content/abstract/19/3/700

BACKGROUND: (beta)-Thalassaemia results from co-inheritance of two mutant (beta)-globin alleles. Allogeneic cord blood cell transplantation (CBT) from an HLA-identical sibling donor is an excellent treatment option for (beta)-thalassaemia. In families with an affected child and willing to have another child, IVF followed by preimplantation genetic diagnosis (PGD) can be applied to exclude affected embryos. Furthermore, healthy embryos could be HLA matched with the affected child so that cord blood from the future newborn can be used to transplant the affected sibling. METHODS: We developed an indirect single-cell HLA typing technique based on the use of a bank of seven microsatellite markers within the HLA locus from which four informative and evenly distributed markers were selected. RESULTS: The methodology was validated in three (beta)-thalassaemia families having six ovarian stimulation cycles in view of IVF and PGD. Six PGD cycles were performed in two families. On 58 embryos tested, the combined PCR was successful in 54 (93%). Two transfers were done and one clinical pregnancy was obtained. Using confirmatory analysis on 50 embryos, the accuracy for HLA typing was 100%. CONCLUSION: This strategy offers a new therapeutic option for patients with (beta)-thalassaemia and other monogenic diseases that can be cured with CBT.


http://humrep.oupjournals.org/cgi/content/abstract/19/11/2653

BACKGROUND: In view of evidence suggesting an immunological cause of recurrent spontaneous abortions (RSA) and the large number of maternal natural killer (NK) cells present in the pregnant uterus, we investigated the genetic polymorphism of the killer cell immunoglobulin-like receptors (KIR) in women with RSA. METHODS: KIR gene repertoire and KIR2DL4 (a receptor for HLA-G) genotyping were determined by SSP and SSCP respectively, in women experiencing RSA and controls. RESULTS: The KIR repertoire did not differ between RSA patients and controls in terms of: (i) the number of inhibitory receptors; (ii) the number of activating receptors; (iii) the ratio of inhibitory to activating receptors. KIR2DL4, a receptor for HLA-G, has different transmembrane alleles, which produce functionally different phenotypes. The frequency of KIR2DL4 transmembrane genotypes differed significantly between RSA patients and controls (P=0.03). However, although homozygosity for a membrane-bound receptor was more frequent in patients (25%) than controls (10%), other genotypes that would produce the same phenotype were not more frequent in patients than controls. CONCLUSIONS: The data provide little evidence that KIR polymorphism plays a role in predisposition to RSA.

http://www.sciencedirect.com/science/article/B6T3B-445B3YR-5/2/0c03b951bacce5c7f7efd40e50949e1c

Helicobacter pylori infection is linked to chronic gastritis, peptic ulcer and gastric carcinoma. During *H. pylori* infection, class II MHC expression by the gastric epithelium increases, as does the number of local CD4+ T cells, which appear to be important in the associated pathogenesis. These observations suggested that the epithelium might present antigens to T cells. Thus, we sought to determine whether gastric epithelial cells process antigens to establish their function as local antigen presenting cells (APC). We examined a panel of gastric epithelial cell lines for expression of the antigen processing cathepsins B (CB), L (CL), S (CS), and D (CD). The mRNA for these enzymes were detected by RT-PCR and the enzymes in the gastric epithelial cells were identified by various independent methods. We corroborated the expression of CB and CD on gastric epithelial cells from human biopsy samples. The functions of these proteases were confirmed by assessing their ability to digest ovalbumin, a conventional dietary antigen, and proteins from *H. pylori*. In summary, multiple lines of evidence suggest gastric epithelial cells process antigens for presentation to CD4+ T cells. To our knowledge, these are the first studies to document the antigen processing capacity of human gastric epithelial cells.


http://www.sciencedirect.com/science/article/B6T3B-47K22SY-3/2/5e38780a07861c76a744c5c6f1e86919

Natural killer (NK) cell-mediated cytolysis is stimulated and downregulated through the interaction of distinct human leukocyte antigen (HLA) class I molecules on target cells with specific killer cell immunoglobulinlike receptors (KIRs) on NK cells. Killer cell immunoglobulinlike receptors are highly polymorphic and are clonally distributed on NK cell populations within individuals. However, the regulation of KIR expression by individual HLA class I phenotypes is not well understood. To examine a potential influence of the HLA class I phenotype on KIR expression patterns we studied the KIR expression in individuals that were subgrouped according to the major HLA-C encoded KIR-epitopes (group C1 versus C2). In these individuals, NK cells were analyzed for KIR expression using flow cytometry and RNA-based expression analysis. Our results demonstrate that KIR genes are transmitted very heterogeneously with two main patterns of KIR genotypes as previously described; group A and group B (with 21 different genotypes). There are distinct populations exhibiting different densities of CD158a and/or CD158b positive NK cells that coexist in all individuals. A clear correlation between KIR expression and the currently known HLA class I ligands was not observed. In conclusion, the surface expression of KIRs in individuals with different HLA class I genotypes indicates that other non-HLA class I encoded factors contribute to the shaping of the KIR repertoire.


http://www.sciencedirect.com/science/article/B6T3B-3S6D0TN-7/2/54e9c1c636002b53294ea89e95253d54
ABSTRACT: Conventional matching is based on numbers of alleles shared between donor and recipient. This approach, however, ignores the degree of relationship between alleles and haplotypes, and therefore the actual degree of difference. To address this problem, we have compared family members using a block matching technique which reflects differences in genomic sequences. All parents and siblings had been genotyped using conventional MHC typing so that haplotypes could be assigned and relatives could be classified as sharing 0, 1 or 2 haplotypes. We trained an Artificial Neural Network (ANN) with subjects from 6 families (85 comparisons) to distinguish between relatives. Using the outputs of the ANN, we developed a score, the Histocompatibility Index (HI), as a measure of the degree of difference. Subjects from a further 3 families (106 profile comparisons) were tested. The HI score for each comparison was plotted. We show that the HI score is trimodal allowing the definition of three populations corresponding to approximately 0, 1 or 2 haplotype sharing. The means and standard deviations of the three populations were found. As expected, comparisons between family members sharing 2 haplotypes resulted in high HI scores with one exception. More interestingly, this approach distinguishes between the 1 and 0 haplotype groups, with some informative exceptions. This distinction was considered too difficult to attempt visually. The approach provides promise in the quantification of degrees of histo-compatibility.


http://www.sciencedirect.com/science/article/B6T3B-3RW785-62/e3d5059c3752afe4cd439d79161fb7d

ABSTRACT: Recent advances in the understanding and identification of chemokines and their receptors have provided evidence for their consideration as candidate loci with respect to genetic susceptibility/resistance to MS. Increased levels of the chemokine, macrophage inflammatory protein (MIP)-1[alpha], have been demonstrated in the cerebrospinal fluid of both patients with MS and mice with EAE, and anti-MIP-1[alpha] antibodies have been shown to prevent EAE. Recently, a common deletion mutation in the gene for the major receptor for MIP-1[alpha], chemokine receptor 5 (CCR5) has been described. Homozygotes for the mutation fail to express this receptor. Moreover, homozygotes are highly protected against HIV infection; this has potential implications for the cell entry of infectious agents in other multifactorial diseases where a viral component may be involved. In view of these aspects, a group of 120 unrelated Australian relapsing/remitting MS and 168 unrelated control subjects were screened for the CCR5[Delta]32 mutation. There was no significant difference in the allele frequency of CCR5[Delta]32 gene between the MS patients (0.1125) and the control population (0.0921). The presence of two CCR5[Delta]32 homozygotes in the MS patients indicates that the absence of CCR5 is not protective against MS. These data suggest that CCR5 is not an essential component in MS expression, though this may be due to redundancy in the chemokine system where different chemokine receptors may substitute for CCR5 when it is absent.


http://www.sciencedirect.com/science/article/B6T3B-45CTM7Y-B/2/t27fb4fe01b4261983153d4c987b8094

Interleukin-4 (IL-4) is a cytokine of the Th2 subtype. It is suggested that Th2 cytokines are involved in induction of tolerance towards the graft after organ transplantation. Therefore, we studied the association between the frequencies of IL-4 producing helper T lymphocytes (IL-4
HTL) and acute rejection in a panel of 31 cardiac transplant patients. It was also investigated whether these frequencies were influenced by: (1) a single nucleotide polymorphism (SNP) at position -590 in the promoter region of the IL-4 gene, which influences the production level of IL-4; and (2) the expression of an IL-4 splice variant (IL-4[delta]2), which inhibits the IL-4 receptor. Frequencies of IL-4 HTL were determined by limiting dilution analysis. Genotyping for the SNP was carried out by sequencing. The ratio of wild type versus IL-4[delta]2 mRNA was determined by quantitative RT-PCR of mRNA isolated from stimulated MNC of cardiac transplant patients. Frequencies of IL-4 HTL were significantly higher in patients who did not suffer from acute cardiac transplant rejection, than in patients that suffered from at least one rejection episode requiring treatment in the first year after heart transplantation. The genotype of the promoter SNP and the ratio between wild type/splice variant IL-4 mRNA did not influence the measured frequencies of IL-4 HTL or the presence of transplant rejection itself.


http://www.sciencedirect.com/science/article/B6T3B-47PPRNH-3/2/5ddf45d0659b09e12c82a84c2eb27d2

The location of the human TNF genes within the MHC complex has prompted much speculation about the role of TNF alleles in the etiology of MHC-associated autoimmune diseases. On sequencing the 5' regulatory region of the human TNFA gene a G (TNFA-308G) to A (TNFA-308A) transition polymorphism at position -308 was discovered. We have developed a simple PCR assay to facilitate the screening of the -308 polymorphism at the DNA level. In view of the possible linkage between the TNFA-308A allele and a certain MHC type, TNFA-308 genotypes in HLA-typed healthy individuals (n = 88) were determined. A statistically significant association between the TNFA-308A allele and HLA-DR3, DQB1*0201, DQA1*0501, A1, B8, and the Ncol 5.5-kb RFLP of the TNFB gene was observed. In addition, we determined the frequency of the TNFA-308A allele in patients with FS (n = 13), an HLA-DR4-associated disease. In this study, no association was found of Felty's syndrome with the TNFA-308A allele, indicating that this allele does not appear to be a susceptibility factor for FS. Human Immunology 41, 259-266 (1994).


http://www.sciencedirect.com/science/article/B6T3B-47J6V1H-K3/2/df62eb967c84a30f9c417e6ff607198c

Molecular genotyping of HLA class II genes using group-specific DNA amplification by the PCR followed by probing with (PCR-SSO) probes is too time consuming for the typing of cadaveric organ donors. Recently, amplification of DNA using PCR-SSP has proved a reliable and rapid method for typing HLA-DRB1 genes. PCR-SSP takes 2 hours to perform and is therefore suitable for the genotyping of cadaveric donors. We have designed a set of primers that in eight PCR reactions will positively identify the HLA-DQB1 alleles corresponding to the serologically defined series HLA-DQ2, DQ4, DQ5, DQ6, DQ7, DQ8, and DQ9. Presently, 30 homozygous cell lines and 138 individuals have been typed by the DQB1 PCR-SSP technique and compared with a combination of serology and RFLP with 100% concordance. No false-negative or false-positive amplifications were recorded. All combinations of DQB1 can be readily identified. DQB1 PCR-SSP can take as little as 130 minutes from start to finish, including DNA preparation.
Polymerase chain reaction (PCR)-based human leukocyte antigen (HLA) typing methods currently used in most histocompatibility laboratories, such as PCR-sequence-specific primers (PCR-SSP) and PCR-sequence-specific oligonucleotide probes (PCR-SSO), are time-consuming and are at risk of contamination during the post-PCR process. The aim of this study was to develop a real-time PCR (rtPCR)-based HLA-DRB1 and -DRB3/4/5 low-medium resolution typing method to avoid these problems. This new method combined the use of specific primers and probes for HLA-DRB alleles. One pair of DRB gene primers and two DRB-specific probes (FAM and VIC) were used per reaction in each of a set of 16 PCR reaction tubes. To provide an internal positive control, each tube also contained a pair of primers and a TET probe for glyceraldehyde phosphate dehydrogenase. This allowed a very significant reduction in the number of reactions and the processing time, whereas typing resolution increased. After being successfully tested on 100 samples, the technique was validated in 200 clinical samples that had previously been typed for HLA-DRB using a standard PCR-based method. Identical results were obtained with all samples. This new method also reduced ambiguous results and was faster and less cumbersome than currently used PCR-SSP or PCR-SSO techniques.
The tumor necrosis factor (TNF) and TNF receptor (TNF-TNFR) superfamily plays crucial roles in immune regulation and host immune responses. The superfamily has been also associated with many immune-mediated diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease, and diabetes. In order to investigate genetic variants of the TNF-TNFR superfamily, a total of 63 known single nucleotide polymorphisms (SNPs) in the coding region (cSNPs) of the TNF-TNFR superfamily genes were selected from the public SNP database. Among 63 cSNPs tested in this study, only 24 SNPs (38%) were validated to be polymorphic in the Korean population by primer extension-based SNP genotyping. By means of the new enhanced single strand conformational polymorphism (SSCP) method, we also identified a total of 78 SNPs, including 48 known SNPs and 30 novel SNPs, in the 44 human TNF-TNFR superfamily genes. The newly discovered SNPs in the TNF-TNFR superfamily genes revealed that the Korean population had very different patterns of allele frequency compared with African or white populations, whereas Korean allele frequencies were highly similar to those of Asian (correlation coefficient $r = 0.88$, $p = 0.90$, $p < 0.001$). The validated SNPs in the TNF-TNFR superfamily would be valuable for association studies with several immune-mediated human diseases.


http://www.sciencedirect.com/science/article/B6T3B-47HPGPW-J2/22f8c5f015dabf2e7fb4fdd3fe8b5253

We present our results in the identification of polymorphic sites within the second exon of the human leukocyte antigen A (HLA-A) region using the DNA microarray technology. Allele specific detection was performed by polymerase chain reaction followed by ligase detection reaction (LDR) in combination with a universal array, a powerful method for high throughput DNA sequence analysis. By this approach we confirmed 32 human samples previously characterized by direct DNA sequencing, thus demonstrating the interest of this approach.


http://www.sciencedirect.com/science/article/B6T3B-3W256Y0-62/863eca9a64af3ebf968c48b2d8c1f530

The third complementarity-determining region (CDR3) is the only nongermline-encoded hypervariable region of the T cell receptor [beta] (TCRB) chain, and it is the region that has been predicted to confer fine specificity of the TCR for peptide-MHC complexes. For this reason analysis of TCRB CDR3 heterogeneity may provide insight into immune mechanisms operative in infectious and autoimmune diseases. PBMC stimulated with either mitogen (PHA), superantigen (TSST-1), or nominal antigen (tetanus toxoid) have been compared with unstimulated PBMC using a two-dimensional approach. Analysis of the expressed TCRBV gene repertoire CDR3 length profile coupled with SSCP methodology enabled the discrimination of sequences with the same CDR3 length. For both freshly isolated and PHA- stimulated PBMC, a normally distributed spectrum of CDR3 lengths (five or more products) was observed. These products differed by 3 bp (1 amino acid) due to the strict requirement for in-frame rearrangements in the CDR3 region of TCR. By contrast, tetanus toxoid-stimulated PBMC had restricted profiles for most TCRBV families after as few as 7 days of incubation. The oligoclonal nature of samples showing CDR3 length restriction was revealed by SSCP analysis and confirmed by sequence determination. Superantigen stimulation resulted in unique patterns of diversity, which included polyclonal expansion of specific TCRBV families as well as oligoclonal expansion of most other TCRBV
families. These data reveal complex yet distinct patterns of TCR diversity in response to different T cell activation stimuli.


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Diversity in the peripheral T cell receptor repertoire of rhesus (Macaca mulatta) and pig-tailed macaques (Macaca nemestrina) has been studied by examining the profile of CDR3 lengths in TCR [beta] chains. Expressed CDR3 length distribution profiles for individual TCRBV families were obtained from total peripheral blood mononuclear cells (PBMC) and T cell subsets isolated from PBMC. These studies reveal that the T cell receptor repertoire of PBMC from healthy macaques often exhibits skewing in TCRBV family CDR3 profiles. The skewing of TCRBV family CDR3 profiles was evident as discrete expanded length(s) and was detected in up to 50% of the PBMC profiles. Analyses of separated T cell populations demonstrated that the CD8+ T cell subset was responsible for the majority of observed skewing in CDR3 length profiles. However, CD4+ T cells were also shown to contribute to the skewed peripheral PBMC repertoire in these animals. While certain TCRBV families frequently displayed skewed profiles, there was no concordance in the particular CDR3 lengths expanded among the different animals. Furthermore, an additional feature of the peripheral blood of the animals studied was the presence of an unusual population of extrathymic CD4+ and CD8+ (double-positive) T cells (up to 9.6% in the PBMC of rhesus macaques). The double-positive T cells could be differentiated from CD4 single-positive and CD8 single-positive T cells by their increased surface expression of LFA-1 and decreased CD62L expression. The percentage of the double-positive T cells was higher in rhesus than pig-tailed macaques and contributed substantially to the peripheral T cell repertoire.


http://www.sciencedirect.com/science/article/B6T3B-3S2BWT5-5/2/24c30a74cc3bf611c91aca9fb0fee75

ABSTRACT: Pemphigus vulgaris (PV) is an autoimmune disease of the skin and mucous membranes characterized by an autoantibody response against an epidermal cadherin. We performed high resolution HLA class II typing in 19 patients with PV from Rawalpindi, Pakistan and 19 non-Jewish European PV patients from Boston by sequence-specific oligonucleotide probe hybridization. The results were compared with two separate ethnically matched control populations. We found that PV patients from Pakistan had significantly increased frequencies of DRB1*1404 (p = 0.01), DQA1*0101 (p = 0.02), and DQB1*0503 (p = 0.01). Among the patients of non-Jewish European ancestry, DRB1*1401 (p < 0.01), DQA1*0101 (p = 0.05) and DQB1*0503 (p = 0.06), were increased in PV patients. Formal linkage analysis between the major histocompatibility complex and the PV antibody was performed in 67 relatives of the 19 Pakistani patients. The results showed strong evidence for linkage of HLA-DRB1*1404, DQA1*0101, DQB1*0503, with the presence of PV antibody in relatives' families with a significant logarithm of the odds score of 6.06. Based on the three dimensional structure of class II molecules, we propose that HLA-DQA1*0101 and DQB1*0503, encode a negatively charged P9 peptide binding pocket of the DQ molecule and are significantly associated with susceptibility to PV in non-Jewish populations.
Natural killer (NK) cell alloreactivity observed during stem cell transplantation (SCT) can be either beneficial (graft-versus-leukemia effect) or detrimental to the host (graft-versus-host disease). Killer immunoglobulin-like receptors (KIR), expressed on NK and CD8 memory T cells, are regulated at a posttranscriptional level and, because there are currently no KIR-specific antibodies available, the analysis of these receptors remains elusive. To better define the role of cells expressing KIR after SCT, we studied KIR transcript repertoires in 29 grafted patients who received myeloablative or nonmyeloablative regimens. We restricted our analysis to 3DL1, 3DL2, 2DL4, 2DS3, and 2DS4 KIR transcripts 6 months after SCT. Absolute counts of NK and CD8 T cells were determined by flow cytometry, and KIR transcripts were quantified by real-time reverse transcription polymerase chain reaction at days 14, 28, 60, 100, and 180 after transplantation. Three groups of patients were identified. Groups I and III were characterized by the absence or a delayed appearance of KIR transcripts, which correlated with the highest risk of acute graft-versus-host disease (aGvHD). In contrast, in group II, a significant transcript peak was observed early, and only one patient suffered from aGvHD (p = 0.025). Thus determining the kinetics of KIR transcription should make it possible to identify transplanted patients at a high risk of developing aGvHD.


In cryostat sections of 84 head and neck squamous cell carcinomas (HNSCC) HLA class I and [beta]2m expression was analysed using monomorphic and locus specific monoclonal antibodies. Loss of expression was heterogeneous and none of the tumours tested showed a total loss of HLA class I and/or [beta]2m when analysed with W6/32, which recognises HLA class I determinants and anti-[beta]2m MoAbs. Weak HLA class I and [beta]2m expression was found in 9 tumours (11%) and heterogeneous expression was found in 2 tumours (2%). When analysed with locus-specific antibodies (HCA2 and HC10, anti-HLA-A and anti-HLA-B/C, respectively) 37 tumours (44%) showed a loss, weak or heterogeneous expression of one or both loci. Tumours showing a down-regulated HLA class I expression were analysed for mutations in either allele of the [beta]2m gene by sequencing based mutation analysis (SBMA). Exon 1 and exons 2 and 3 were amplified separately by PCR using M13-tailed intron-specific primers. PCR products were sequenced in two directions. In none of the tumours mutations in the [beta]2m gene were detected. In 59% of the tumours with down-regulated HLA class I expression, lost or down-regulated TAP 1 expression was found when analysed with anti-TAP 1 antibodies. This indicates an important role for TAP in down-regulation of HLA class I expression in HNSCC.

It is well known that type 1 diabetes mellitus (T1DM) is a complex genetic disease resulting from the autoimmune destruction of pancreatic beta cells. Several genes have been associated with susceptibility and/or protection for T1DM, but the disease risk is mostly influenced by genes located in the class II region of the major histocompatibility complex. The attraction of leukocytes to tissues is essential for inflammation and the beginning of autoimmune reaction. The process is controlled by chemokines, which are chemotactic cytokines. Some studies have shown that CCR2-64I and CCR5-Δ32 might be important for protection of susceptibility to some immunologically-mediated disorders. In the present study, we demonstrate the lack of association between CCR2-64I and CCR5-Δ32 gene polymorphism and TIDM and we describe a new method for a simple and more precise genotyping of the CCR2 gene.


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Human papillomaviruses type 16 and 18 are the major cause of cervical cancer. However, genetic factors contribute to the propensity of persistent HPV infection and cervical carcinoma. Allelic variants of the human leukocyte genes have shown to be associated with cervical neoplasia. The strongest associations have been found with the genes in the HLA class II region. The aim of this study was to analyze the association of two non-HLA class II markers with invasive cervical cancer. Microsatellite polymorphism of the TNFA gene located in the class III region and a short tandem repeat polymorphism of the MICA gene located in the centromeric end of the HLA class I region were analyzed. Eighty-five patients and 120 matched control individuals from a population-based cohort from Northern Sweden participated in this nested case-control study. MICA was not associated with cervical carcinoma. TNFa-11 frequency was increased in the HPV18 DNA positive patients (OR = 2.84, p = 0.0481, CI = 1.04-7.78, pc = NS). TNFa-11 was not associated with susceptibility to HPV16 infection, but it increased the risk for cervical cancer with the HLA DQ6 (DQA 1*0102-DQB 1*0602) haplotype. Our findings indicate that the association of TNFA with cervical cancer is different with CIN. The extended HLA DQ6-TNFa-11 haplotype is increasing the risk for development of cervical cancer significantly (OR = 3.08, p = 0.0104, CI = 1.30-7.31).


http://www.sciencedirect.com/science/article/B6T3B-47J6V0T-JT/2/0f69e72b651c33a5bc0650e10bace7e7

An association of HLA-DQ3 with SCC of the cervix has been reported by researchers in Germany and Norway. This article documents a similar-sized study with patients and controls from northwest England. We report in detail on serologically determined HLA polymorphism in SCC patients with respect to HPV 16 infection, MHC class II expression within the tumor, serologic response to HPV, and other relevant clinical variables. We have also extended our studies to include DNA-based analysis using PCR and SSO probes for HLA-DQ. No significant association of any HLA-A, -B, -C, -DR, or -DQ antigen with SCC patients was found. While a possible explanation of the differences among studies could be a reflection of disease heterogeneity, the
several tumor and clinical factors examined do not account for the observed differences from previous reports. Further studies are needed for a greater understanding of the interaction of HPV and HLA type in the development of cervical neoplasia.


To evaluate the long-term reconstitution of the T cell immune repertoire in recipients of an allogeneic Bone Marrow Transplantation (allo-BMT), we have analyzed the T cell receptor (TCR) repertoire in the periphery and the T cell response against tetanus toxoid in two T-B+ Severe Combined Immunodeficiency Disease (SCID) patients more than 11 years after HLA haplo-identical allo-BMT. Our studies demonstrate that in the periphery of allo-BMT recipients, on the basis of TCR V-gene segment usage, the T cell immune repertoire long after allo-BMT is diverse, as is that of the donor. However, when donor and allo-BMT recipient were compared, differences were noted in the TCR Complementarity Determining Region 3 (CDR3) size distributions and in the T cell response against tetanus toxoid. In particular, the tetanus toxoid specific T cell clones differed in their use of HLA restriction elements, and expressed different T cell receptors. Moreover, we have uncovered donor-type tetanus toxoid specific T cell clones which were established from allo-BMT recipient derived peripheral blood lymphocytes and were found to be restricted by the non-shared recipient allele. This observation suggests a role for recipient-mediated T cell selection processes, in the thymus or at extra-thymic sites.


Susceptibility to autoimmune hepatitis type I (AIH-1) has been associated with HLA-DR3, DR52, and DR4 antigens in Caucasian and Oriental patients. However, in Brazil, disease susceptibility is primarily linked to DR13 and DR52. In this highly admixed population, we find different DR13-associated haplotypes, presenting a unique opportunity to discriminate relevant genes within a tightly linked genomic region. To identify the susceptibility locus, we sequenced DR13 alleles of 39 patients with AIH-1 and 22 controls. Patients were almost exclusively DRB1*1301, but half of controls typed DRB1*1302. HLA-DQ haplotypes were varied. Oligotyping of DRB3 locus of all patients and also within the HLA-DR13 positive group showed an allele distribution comparable to controls, confirming that the stronger association lies in the DRB1 locus. On the other hand, if DRB1*1301 is the major susceptibility factor in our sample, the only amino acid different from DRB1*1302 in position 86, corresponding to pocket 1 in the peptide-presenting groove, may be important. We propose that peptide presentation leading to pathogenesis of AIH-1 may be quite stringent, but will also be affected by other strong genetic or environmental susceptibility factors, which would explain the various HLA molecules associated to the disease in the different populations.

Cumulative evidence indicates that the human interleukin-4 receptor [alpha] chain gene (IL-4R[alpha], CD124) is highly polymorphic in contrast to other cytokine receptor genes. Our group recently identified the IL-4R[alpha] variant R551 as being strongly associated with decreased kidney allograft survival. Due to the key immunoregulatory role of IL-4 and controversial reports on the association of IL-4R[alpha] variants with atopy, we present here the development of polymerase chain reaction-primer sets for sequence-specific amplification of all seven hitherto described amino acid polymorphisms, and we investigated 158 blood donors prospectively. By using an Expectation-Maximization algorithm, we calculated the presence of 11 putative human IL-4R[alpha] haplotypes and identified 4 putative IL-4R[alpha] haplotypes with a cumulative frequency of >90%. None of the polymorphisms showed a significant association with the phenotype atopy. All mutant alleles showed a trend toward decreased total IgE levels. This association was only significant (p U-test) for the A375, R406, and P478 variants in non-atopic blood-donors (n = 90), presumably due to the high variance of IgE levels among the smaller group of atopic individuals. We postulate that IL-4R[alpha] mutations are associated to different extents with a decrease in function of the receptor but do not present a major atopy locus.


Genes may be silenced at the transcriptional level by 'genomic imprinting' in such a way that only one of the parental alleles is expressed. Imprinting may be tissue-specific and in some cases it seems also to be time-dependent during development. The phenomenon has been studied in pre- and post-implantation developmental processes. Animal studies of genomic imprinting of major histocompatibility complex (MHC) antigens in the placenta have shown discordant results. To address this issue in the human placenta, we examined the expression of the non-classical human leukocyte antigen (HLA) class I gene, HLA-G. Genomic imprinting of the HLA-G locus could have implications for the interaction in the feto-maternal relationship. Restriction Fragment Length Polymorphism (RFLP), allele-specific amplification and Single Strand Conformation Polymorphism (SSCP) analysis followed by DNA sequencing were performed on Reverse Transcription (RT) Polymerase Chain Reaction (PCR) products of HLA-G mRNA to examine the expression of maternal and paternal alleles. Our results demonstrate that HLA-G is co-dominantly expressed in first trimester trophoblast cells. A "new" non-synonymous base substitution in exon 4 was detected. We also investigated the different alternatively spliced forms of HLA-G mRNA in first trimester trophoblast and found the full-length transcript to be the far most abundant.

polymorphism in the clinical presentation of type 1 diabetes in French population has been reported. To test the putative involvement of SDF-1 gene polymorphism in predisposition to or clinical heterogeneity of type 1 diabetes in Japanese population, we conducted the case-control study. The SDF1-3'A variant (801 G to A in the 3'-untranslated region) was determined by the polymerase chain reaction-restriction fragment length polymorphism technique in 184 patients with abrupt-onset type 1 diabetes and 106 healthy control subjects. No significant difference in allele and genotype frequencies of SDF1-3'A variant was found between type 1 diabetic patients and healthy controls. However, the SDF1-3'A variant was strongly associated with early-onset diabetes in a recessive model (AA versus AG + GG, p = 0.017). The mean age-at-onset in patients carrying SDF1-3'AA genotype was significantly younger than that in patients with SDF1-3' AG or GG genotype (p = 0.028). The frequencies of SDF1-3' A variant were significantly increased in HLA-DR4/9 patients compared with non-DR4/9 patients (p = 0.008). These results suggest that the SDF-1 gene polymorphism is associated with the age-at-onset of type 1 diabetes in Japanese population.


This study investigated whether interleukin-10 (IL-10) gene promoter region polymorphisms are associated with susceptibility to or clinical presentation of type 1 diabetes. The frequency of -1082G/A, -819C/T, and -592C/A polymorphisms was analyzed in 128 Japanese patients with type 1 diabetes and in 107 healthy control subjects in a case-controlled study. The allelic and haplotypic frequencies of the IL-10 gene promoter region polymorphisms were similar in patients with type 1 diabetes and in control subjects. However, the -819T and -592A allele were associated with adult-onset (>18 years) of the disease (p = 0.037). Furthermore, the frequency of ATA haplotype was increased in adult-onset patients than that in early-onset patients (p = 0.037). Among the genotypes comprising ATA haplotype, the frequency of ATA/ATA was significantly higher in adult-onset patients than in early-onset patients (p = 0.004). These results suggest that the IL-10 gene promoter polymorphisms are associated with the age-at-onset in Japanese patients with type 1 diabetes.


The central class III region of the human major histocompatibility complex contains highly polymorphic genes that are associated with immune disorders and may serve as susceptibility factors for viral infections. Many HLA haplotype specific rearrangements, duplications, conversions and deletions, occur frequently in the C4 gene region. Genetic deficiencies of complement components are associated with recurrent occurrence of bacterial infections. We have studied the complement profile and the class III genes 5'-RP1-C4A-CYP21A-TNXA-RP2-C4B-CYP21B-TNXB-3' in a 4-year-old Caucasian patient. He has suffered from several pneumonias caused by respiratory viruses, eight acute otitis media, prolonged respiratory infections and urinary tract infection. Complement C4 was constantly low, but the other complement components, from C1 to C9, C1NH, factor B and properdin, were within normal limits. Immunological evaluation gave normal lymphocyte numbers and functions with the
exception of subnormal T cell response to pokeweed mitogen. Molecular studies of the C4 gene region in the patient revealed homozygous deletion of CYP21A-TNXA-RP2-C4B generating total deficiency of C4B and the flanking 5’ region up to C4A, and in the father a missing CYP21A gene. Further investigations are needed to elucidate the relationship between C4B deficiency and susceptibility to infections.


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Association frequencies of TCR J[beta] gene segments with six V[beta] families (V[beta] 3, 6.1-3, 8, 9, 12, and 18) were analyzed in T-cell populations obtained from healthy blood donors. The six selected V[beta] families are located at various chromosomal positions relative to other recombinatorial elements (D[beta], J[beta], C[beta]). We report here that in CD4+ as well as CD8+ T-cell subsets, all 13 J[beta] gene segments were used in combination with all the V[beta]s tested and that no correlation between the genomic position of the individual V[beta]s and J[beta] gene segment usage was observed. J[beta] gene segment usage was found to be nonrandom in general, with J[beta] 2.7 and J[beta] 2.4 exhibiting highest and lowest frequency of utilization, respectively. J[beta] family 2 was used more frequently than J[beta] family 1 by the two T-cell subsets. Some individual J[beta] gene segments were skewed toward either CD4+ or CD8+ T cells. Thus, J[beta] 1.3 and J[beta] 1.6 were consistently biased toward expression in CD4+ T cells. In contrast, when combined with V[beta]8 or V[beta]9, J[beta] 2.1 results were skewed dramatically toward expression in CD8+ T cells. We also found 70 cases of expanded individual V[beta]/J[beta] associations in a total of 1092 investigated combinations, 62 of which were confined to the CD8+ T-cell populations. CD8+ T-cell populations are thus much more likely to contain TCR V[beta]/J[beta]-restricted expansions than CD4+ T cells.


http://www.sciencedirect.com/science/article/B6T3B-44R1B3K-4/2/57bf409178bf5893cc24afe46991ba49

CD28-CD4+ T-cell subpopulation is expanded in kidney allograft patients with long graft survival. To seek for the roles of CD28-CD4- T cells in the long-term acceptance of kidney allografts, we characterized this population by analyzing cell surface molecules, TCR V[beta] repertoire, mixed lymphocyte reaction (MLR), and cytokine production. The number of CD28-CD4+ T cells increased correlatively with time after transplantation in this group of patients. The CD28-CD4+ T cells did not express detectable levels of CD25, CD69, V[alpha]24, or CTLA-4 but expressed heterogeneous amounts of CD45 RA on the surface. Freshly sorted CD28-CD4+ T cells revealed a restricted V[beta] repertoire, whereas the V[beta] usage of CD28+CD4+ T cells from the same patients was much diversified. Expression levels of TGF-[beta] and IFN[gamma] gene were significantly higher in the CD28-CD4+ T cells than in the CD28+CD4+ T cells from the kidney allograft patients. These findings suggest that an oligoclonal CD28-CD4+ T-cell population is continuously activated in patients with long allograft survival, which may be linked with the long-term acceptance.

Mhc-DRB and -DQA1 second-exon and -DRB 3'-untranslated-region nucleotide sequences of three lowland gorillas with no known family relationship with each other and of two HLA homozygous typing cell lines were determined and compared with published primate Mhc-DRB and -DQA1 sequences. Eleven distinct MhcGogo-DRB second-exon sequences were found, which represent the gorilla counterparts of the HLA-DRB1*03, -DRB1*10, -DRB3, -DRB5, -DRB6 allelic lineages. One Gogo-DRB second-exon sequence does not have an obvious human counterpart and is tentatively designated Gogo-DRBY*01. The gorilla equivalents of the HLA-DRB2 and -DRB8 loci were identified as judged on Mhc-DRB 3' - untranslated-region sequences. In addition, four different Gogo-DQA1 alleles belonging to three different allelic lineages were detected. The Mhc-DRB-DQA1 haplotypes of these gorillas were deduced based on the obtained Mhc-DRB and -DQA1 sequences and the two published Mhc-DRB haplotypes of the lowland gorilla Sylvia. All deduced Gogo-DRB-DQA1 haplotypes show gene constellations different from known HLA-DRB-DQA1 haplotypes, while some of the Gogo-DRB haplotypes presented here contain more DRB genes than the HLA-DRB haplotypes. Based on phylogenetic trees, bootstrap analyses, and the gorilla, chimpanzee, and human Mhc-DRB haplotypes described, we propose that at least two Mhc-DRB loci, here tentatively designated Mhc-DRBI and -DRBII, existed on an ancient primate Mhc-DRB haplotype. The Mhc-DRB1*01, DRB1*02 (DRB1*15 and DRB1*16), -DRB1*03 (-DRB1*03, -DRB1*08, -DRB1*11, -DRB1*12, -DRB1*13, and DRB1*14), and -DRB1*10 allelic lineages and -DRB3 and -DRB6 loci probably evolved from the hypothetical primate Mhc-DRBI locus, whereas the present primate Mhc-DRB2, -DRB4, and -DRB6 loci originate from the ancient Mhc-DRBII locus of this core primate Mhc-DRB haplotype. Human Immunology 36, 205-218 (1993)


Block matching is a valuable tool for selecting donors for bone marrow transplantation. Identical, electrophoretic profiles of unrelated bone marrow donor-recipient pairs have been shown to be associated with long-term survival and a reduction of graft versus host disease (GVHD). This study was undertaken to determine the sequences of the PCR products which are generated. PCR products obtained with beta-block primers following the amplification of DNA extracted from cell lines homozygous for 7.1 and 8.1 ancestral haplotypes were cloned and sequenced. The PCR products were characterised and the beta block profiles reconstructed. The data indicate that the profiles consist of homoduplexes and heteroduplexes which are formed by the products of probably 3 different sequence locations.


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The heterodimeric TAP (transporter associated with antigen processing) complex plays a key role in immune surveillance. By forming the portal between the cytoplasm and the endoplasmic reticulum, it enables cells to present antigenic peptides by human leukocyte antigen class I antigens to cytotoxic T cells, so infected and malignant cells can be eradicated. Because the nature of the peptide determines whether an immune response is evoked, peptide selective transport by TAP may influence immune surveillance. Currently, seven TAP1 and four TAP2 alleles are known. Each may have its own effect on peptide transport. In this study, we investigated whether TAP1 and TAP2 alleles are associated with the development of head and neck squamous-cell carcinoma (HNSCC). We developed a high-throughput SNaPshot™ assay to determine the frequencies of the TAP1 and TAP2 alleles in 79 Dutch control subjects and 94 patients with HNSCC. Strikingly, all control and HNSCC samples contained a TAP1*0101 allele, with the exception of one tumor patient. The most frequent alleles were TAP1*0101 (88.2%) and TAP2*0101 (81.2%). No significant difference was found between control subjects and patients with HNSCC. Here, we report the TAP1 and TAP2 allele frequencies in the Dutch population, the development of a high-throughput TAP typing technique, and a new TAP1*0501 allele.


http://www.sciencedirect.com/science/article/B6T3B-47J6T1Y-5T/2/92616852a86a3bb0570b653a0097141

Antibodies to the platelet HPA-1a antigen can elicit in the newborn a condition known as neonatal alloimmune thrombocytopenic purpura (NAITP). Previous studies based on RFLP analysis showed that 100% of HPA-1a-negative women who produced anti-HPA-1a antibodies (responders) were HLA-DRw52a (DRB3*0101). However, this specificity could also be found in some HPA-1a-negative women not producing anti-HPA-1a antibodies (nonresponders). We have analyzed in detail by PCR-SSOP the HLA-DR, -DQ, and -DP loci of 36 responders and 10 nonresponders. We found that while the allele DRB3*0101 was present in the vast majority of responders (91%), there were exceptions. Furthermore, the DQB1*0201 allele was found to be present in almost all responders (94%), but again was also found in nonresponders. The risk of alloimmunization to HPA-1a in an HPA-1b homozygous mother significantly increases with the presence of either allele, the odds ratio being 39.7 for DQB1*0201 and 24.9 for DRB3*0101. Sequencing of exon 2 of these two alleles from responders indicated no sequence difference when compared with the consensus sequences. This indicates that they do not represent variants when compared with the same alleles found in some nonresponders.


http://www.sciencedirect.com/science/article/B6T3B-47J6V0T-JR/2/d78ce7a96e857abc26bb65c7cd33f5e94

In this study we analyzed the impact of a MHC class-II-deficient environment on the differentiation of CD4+CD8- T lymphocytes into functional defined subsets of lymphokine-producing T-helper cells. To this end a CD4+CD8- T-cell line and CD4+CD8- T-cell clones, isolated from PBMCs of a type III BLS patient, were stimulated in vitro with anti-CD3 and PMA and assessed for lymphokine transcription patterns. The results of these analyses show that CD4+CD8- T cells that have matured in a MHC class-II-deficient environment display lymphokine transcription patterns that resemble those of MHC class-II-expressing family control-derived CD4+CD8- T cells.
Diversity in the HLA-B22 group was investigated in the Korean population using PCR-SSOP and DNA sequencing analyses. Allelic typing of the B22 gene was performed by gene amplification of the polymorphic exons 2 and 3 of the HLA-B genes from 91 B22 positive individuals followed by a hybridization assay using 63 digoxigenin-labelled probes. Five different SSOP patterns including an unexpected pattern were identified and correlated well with the observed serologic types and with data obtained from DNA sequencing analyses. Novel allele, B*5507, was identified from two unrelated individuals who exhibited standard B54 serologic reactivity but an unexpected SSOP pattern. The DNA sequence of B*5507 is identical to B*5502 in exons 2 and 3 except for a single nucleotide substitution at codon 45 (GAG -> GGG) altering glutamic acid to glycine. Among the already known B molecules, this substitution has been observed only in the B54 molecule encoded by B*5401 allele. This is the evidence that Gly-45 is a crucial site forming the B54 serologic epitope. Interestingly, both alleles (B*5401 and B*5507) exhibit strong association with Cw*0102. Along with previous data, B22 appears to be a very diverse group in the Korean population consisting of at least seven different alleles. B*5401, B*5502, and B*5601 are the most frequent alleles. B*5507, B*5501, B*5504, and B*5604 appear at lower frequencies. Data obtained from this study will be useful in hematopoietic stem cell donor searches as well as in determination of a typing strategy for the HLA-B22 types in this population.

The HLA-DPB1 alleles in 93 Australian aborigines, from two geographically separate areas within Australia, were studied by AFLP analysis. There was a restricted range of DPB1 alleles seen in the aboriginal population, and the distribution of alleles varied between the two aboriginal groups. DPB1*0501 was the most common allele in the aborigines from the Central Desert, whereas DPB1*0401 was the most frequent allele in the Northern Coast aborigines. A new AFLP pattern was observed, and was found to correspond to the allele DPB1*2201, recently identified by SSO analysis. The DPB1 allele frequency distribution for both of the aboriginal groups was different from that seen for the Australian Caucasoid population.

This study was designed to investigate how antiendothelial antibodies (EAbs) are involved in acute irreversible renal graft rejection. Eluates from 25 renal allografts, lost by irreversible rejection (n = 22) and by renal vein thrombosis (controls n = 3), were tested against a panel of cultured human umbilical vein endothelial cells (HUVEC). All patients were under
immunosuppression at the time of nephrectomy. EAbs binding and membrane expression of adhesion molecules ELAM-1 and VCAM-1 were analyzed by flow cytometry (FACS) and by semiquantitative RT-PCR for mRNAs coding for those molecules. The absence of anti-HLA antibodies against the donor was ascertained at transplant, and before and after nephrectomy by the negativity of specific crossmatches performed using the most sensitive techniques. EAbs eluted from eight rejected kidneys bound to HUVEC. They did not induce any cytotoxicity, but their incubation with HUVEC (4 h at 37°C; 2.5 mg/ml) led to upregulation of mRNAs coding for VCAM-1 (35- to 60-fold increases) and ICAM-1 (8- to 12-fold increases) as compared with control EAbs. Membrane expression of adhesion molecules was also strikingly increased, with 80% of the cells expressing VCAM-1 and 65% expressing ELAM-1 upon incubation. EAbs were detected in eight out of nine (88.8%) eluates from kidneys lost from acute vascular rejection, but in none of the 13 (0.0%) kidneys lost from other types of rejection (p < 0.0001). We conclude that EAbs, capable of activating human endothelial cells, can be recovered from acutely rejected kidneys and may play a direct role in the pathogenesis of acute rejection.


http://www.sciencedirect.com/science/article/B6T3B-3YS8MPG-H/2/07a3e477a40e0a1aa595e1ea0d1118d

A highly polymorphic (CA)n microsatellite marker (DQCAR), located between the DQA1 and the DQB1 genes, was characterized in four ethnic groups. Based on length polymorphism, 12 alleles could be defined. The marker is located 1- to 2-kb telomeric to the DQB1 gene and 10 kb centromeric to the DQA1 gene and was shown to be in tight linkage disequilibrium with HLA-DQ. Analysis of the linkage disequilibrium pattern revealed little additional diversity in DQ1-associated haplotypes. Almost all DQ1 subjects examined were DQCAR 103 or DQCAR 107 (13 and 15 CA repeats, respectively). In contrast, significant haplotypic diversity was observed for most DQ2-, DQ3-, and DQ4-associated haplotypes. These haplotypes often had longer allele sizes (DQCAR > 111, more than 17 CA repeats) and more DQCAR alleles per haplotype. These haplotypes also carried DQCAR alleles of different sizes, even though they bore the same DQA1 and DQB1 alleles, and sometimes the same DRB1 allele as well. These results indicate that DQCAR could be a useful marker to better define disease associations with HLA. Our results are also consistent with the hypothesis that CAR alleles with higher numbers of repeats have higher mutation rates and that recombination within the HLA-DR/DQ region is haplotype dependent.


http://www.sciencedirect.com/science/article/B6T3B-3Y0HP3C-C/2/27236ba7127435db368f99acb02e7894

The aim of the study was to assess the influence of constraints of V-D-J rearrangement on the nonrandom junctional diversity of productive T-cell receptor [beta]-chain genes in peripheral T-cells. Mature peripheral T lymphocytes are expected to display a biased repertoire of T cell receptors (TCRs), enriched for those that can recognize peptides presented by the major histocompatibility complex (MHC) molecules. Therefore, functional TCR rearrangements of peripheral T-cells are unsuitable to reveal the bias of the TCR repertoire, introduced by V-D-J rearrangement. To overcome this problem, we have studied nonfunctional TCR genes representing a repertoire of rearranged TCR gene sequences without any known post-rearrangement selection. Detailed molecular analysis of a database generated from more than
500 functional (TCRBV20S1) and nonfunctional (TCRBV10S1P and TCRBV19S1P) T-cell receptor genes from peripheral blood T-cells permitted a comparative analysis of recombination frequencies of each germline-encoded V, D, and J-segments, as well as exonucleolytic nibbling and addition of nucleotides in functional and nonfunctional transcripts. Our data demonstrate that V-D-J recombination generates a more diverse CDR3 length distribution than found among productive TCRBV genes, suggesting that selection constrains the CDR3 to an optimal junctional region length. Furthermore, the well established biased patterns of D- and J-usage in the rearranged TCRBV genes in human peripheral blood lymphocytes were also present in nonfunctional transcripts. Therefore, V-D-J diversity is biased mainly by constraints of the rearrangement process rather than intrathymic T-cell selection and peripheral expansion of particular T-cell clones.


Clonally expanded T cells might be involved in the pathogenesis of Crohn's disease (CD). To test the impact of CD on the regional distribution of expanded T cells, this study analyzed the T cell receptor [beta] (TCRB) repertoire within colonic biopsy specimens from 12 CD patients and 6 noninflammatory controls by TCR spectratyping. Migration characteristics of dominant CDR3 bands from different sites of the normal mucosa suggested focal, segmental, or ubiquitous spreading of individual expanded clones. Similar patterns were observed when inflamed and noninflamed areas of the colon of CD patients were compared, suggesting that regional expansion of T cells was more closely related to anatomic proximity than to local inflammatory activity. CDR3-sequence analysis of TCRBV12+ T cells, which were selectively expanded in the inflamed colon of 3 CD patients, failed to reveal a public CDR3 motif. Our data indicate the existence of distinct patterns of regional T cell expansions in the normal gut mucosa, which are not significantly disrupted by chronic intestinal inflammation. This does not exclude a pathogenic role of expanded T cells in CD through more subtle changes, but emphasizes the need to distinguish them from a discontinuous distribution of clonally expanded T cells in normal colon.

Naumov, Y. N., E. N. Naumova, et al. (1996). "CD4+ and CD8+ circulating [alpha]/[beta] T-cell repertoires are equally complex and are characterized by different levels of steady-state TCR expression." Human Immunology 48(1-2): 52.

http://www.sciencedirect.com/science/article/B6T3B-3W256Y0-7/2/ebba3c9bc45f5744c30627c59b039a80

The repertoire complexity of CD4+ and CD8 + T cells was measured in three healthy blood donors for a number of TCR BV gene families by TCR spectra-typing. This method subdivides V family-specific PCR products based on CDR3 length. Genomic DNA was analyzed to determine the distribution of the cells bearing particular V-J rearrangements. cDNA was analyzed to measure the levels of transcripts arising from those same cells. The complexity and distribution of T cells in each lineage were equal for most BV families. Certain families showed frequent skewing in CD8 cells. Analysis of the intensity profiles of RNA versus DNA spectratypes indicated that in general, there is a constant ratio of transcript per cell for all rearranged sizes within a particular family. This ratio appeared higher in CD4 cells. Thus, steadystate levels of TCR mRNA were measured and found to be higher in CD4+ than in CD8+ cells.

http://www.sciencedirect.com/science/article/B6T3B-42397WG-7/2/e650d4ad13a2a70a01db1b61ecdc8bc1

We studied tumor necrosis factor (TNF), lymphotoxin-[alpha] (LT-[alpha]), and TNF receptors type 1 (TNFR-1) and type 2 (TNFR-2) gene polymorphisms as well as HLA class II DRB1 alleles in Japanese patients with human T-cell lymphotropic virus type I (HTLV-I) associated myelopathy (HAM) (n = 51), patients with adult T-cell leukemia/lymphoma (ATL) (n = 48), asymptomatic HTLV-I carriers (n = 50), and HTLV-I seronegative, normal controls (n = 112). There were significant differences between HAM patients and normal controls in the distributions of TNF promoter region polymorphism at position -857, the LT-[alpha] gene NcoI polymorphism, and the T-G substitution in exon 6 of the TNFR-2 gene. The distribution of the NcoI polymorphism of the LT-[alpha] gene was also significantly different between HAM patients and asymptomatic HTLV-I carriers. In contrast, we failed to detect any difference in the frequency of DRB1, TNF promoter at position -1031, -863, or the TNFR-1 promoter -383 polymorphism. The results suggest that the TNF/LT-[alpha] gene region within the HLA class III of chromosome 6 and the TNFR-2 gene region located on chromosome 1p36 might contribute to susceptibility to HAM, and that aberrant expression or function of these cytokines and the receptor could be involved in the development of HAM.


http://www.sciencedirect.com/science/article/B6T3B-47K22SY-2/2/56ee5cf74809b7c75edaf8d6d1c7b95

The human leukocyte antigen (HLA) class III region, located on chromosome 6p21, has been regarded as one of the susceptible loci for type 1 diabetes. Because it contains many genes related to inflammatory and immune responses, including tumor necrosis factor (TNF), lymphotoxin-[alpha] (LT-[alpha]), and allograft inflammatory factor 1 (AIF-1) genes, it is unclear which gene within the class III region is responsible for the susceptibility to the disease. We sequenced the AIF-1 gene region and detected three novel polymorphisms, all of which were diallelic and localized at introns. Then, we investigated AIF-1, TNF, and LT-[alpha] gene polymorphisms in 165 patients with type 1 diabetes, consisting of 90 patients with young-onset type 1 diabetes, 75 patients with adult-onset type 1 diabetes, and 200 control patients. We also analyzed TNF receptors type 1 (TNFR1) and type 2 (TNFR2) gene polymorphisms, located on chromosome 12p13 and 1p36, respectively. Although there were significant differences between type 1 diabetes patients and controls in the distributions of TNF promoter polymorphisms at position -1031 and -857, and LT-[alpha] gene NcoI polymorphism, none of them was independently associated with the disease after two-locus analysis with HLA class II alleles. We detected the significantly increased frequency of the -383C allele, located in the TNFR-1 promoter region, in both young-onset and adult-onset diabetes patients compared with controls. In addition, the -383C allele was found to be associated with higher expression of the TNFR1 gene than that of -383A allele in in vitro expression. These results suggest that the TNFR1 gene region might be a susceptible locus to type 1 diabetes in Japanese.

http://www.sciencedirect.com/science/article/B6T3B-47J6SN6-G/2/f5d24409809431835816dd045d88a917

We designed a primer for the PCR directed against a highly conserved sequence of the TCR V[beta] gene. The V[beta]-universal primer, in combination with a constant region-specific primer, enabled us to amplify TCR[beta] cDNA of allo-HLA class-II-reactive T-cell clones by PCR without prior knowledge of their V[beta] sequences. The amplified TCR cDNA was purified by agarose gel electrophoresis and subjected to direct sequencing. In nine of ten T-cell clones analyzed, direct TCR sequencing gave readable sequence ladders, including two-thirds of V[beta], junctional, and J[beta] regions. One T-cell clone gave an unreadable mixed-profile sequence ladder, indicating that this clone expressed more than one major TCR[beta] transcript. Even in this case, however, it was possible to determine two different TCR[beta] sequences separately using sequence primers specific to one of the 13 J[beta] segments deduced from the mixed ladder. Thus, direct sequencing utilizing the single V[beta]-universal primer enabled a simple, rapid, and reliable sequence determination of TCR[beta] cDNA of all T-cell clones analyzed.


http://www.sciencedirect.com/science/article/B6T3B-3XRY8CD-8/2/ed15b5c69f3d7737368f8a10b6a66abd

The TNF-[alpha] gene is located in the HLA region and has been implicated in the pathogenesis of Type I (insulin-dependent) diabetes mellitus (IDDM). We investigated the frequency of TNFa microsatellite alleles in 76 young-onset IDDM patients, 65 adult-onset IDDM patients, and 90 control subjects. We also examined the association of these TNFa alleles with HLA-DRB1 alleles, HLA-class I alleles, and TNF-[alpha] production. The frequency of the TNFa2 and TNFa9 alleles was increased in the young-onset IDDM patients compared to control subjects, but the increased frequency of TNFa2 was not significant after the correction for the number of comparisons was made. We did not find any association of TNFa2 or TNFa9 with any of the HLA-DRB1 alleles. In contrast, the frequency of the TNFa13 allele was decreased in both the young-onset and adult-onset IDDM patients compared to the control subjects, but the difference lost significance after the correction was made in the adult-onset IDDM. The TNFa13 allele was strongly associated with DRB1*1502. Patients with TNFa2 or TNFa9 had greater TNF-[alpha] production, while those positive for TNFa13 had lower TNF-[alpha] production than patients with non-TNFa2, a9, and a13 alleles. These results suggest that TNFa polymorphisms are associated with age-at-onset of IDDM and influence the inflammatory process of pancreatic [beta] cell destruction in the development of IDDM.


http://www.sciencedirect.com/science/article/B6T3B-456FC2M-3/2/8f464cabee33a3b3449adb6442f6e40d

Human leukocyte antigen (HLA) incompatibilities are the most important immunological barriers to bone marrow transplant success when using unrelated donors. Until recently, standards for
donor selection included serological methods for HLA class I antigens and DNA-based typing for HLA class II alleles. In our center cytotoxic T-lymphocyte precursor (CTLp) assays have been an integrated part of the search selection procedure as well. More recently, DNA-based typing for HLA class I became available. This allowed us to determine the correlation of CTLp frequencies directed against incompatibilities at the HLA-A, -B, and -C locus in 211 donor-recipient pairs. HLA class I incompatibilities are significantly (p < 0.001). However, this is mainly due to Cw*0303-0304 mismatches. In conclusion, although there is a highly significant correlation between the outcome of the CTLp frequency test and HLA allele class I typing, exceptions occur. It is unclear whether they are all clinically relevant but they certainly provide additional insight in allograft recognition.


Evolutionary relatedness among the highly polymorphic DR[beta] genes has been established based on shared nucleotide sequences and structural organization of DR[beta] loci. The evolution of promoter regions of the B1*0701, B1*0101, B1*1501, B5*0101 genes was analyzed by cloning and sequencing. This shows that the polymorphism and isomorphism of HLA DR[beta] genes extend into the 5’ flanking promoter region of the genes and that evolutionary relatedness also exists among the DR[beta] gene promoters. This suggests that DR[beta] gene promoters and coding regions coevolved. The effect of the naturally occurring nucleotide substitutions in the polymorphic and isomorphic DR[beta] promoters on transcriptional activity has been determined in a transient expression system. The transcriptional activity of two polymorphic DR[beta] promoters, B1*1501 and B1*0701, and two isomorphic DR2 promoters, B1*1501 and B5*0101, is the same for these promoters. Together these data suggest that naturally occurring substitutions do not significantly affect the transcriptional activity of these promoters. Human Immunology 41, 112-120 (1994)


The HLA-DRB1, -DRB3, -DRB4, and -DRB5 alleles of the Guarani and Kaingang Amerindians were characterized. Our previous serologic analyses detected three class II haplotypes among the Kaingang: DR2-DQ3, DR4-DQ3, and DR8-DQ4. In addition to these, the Guarani presented haplotype DR6-DQ3. Individuals typed serologically (67 Kaingang and 34 Guarani) were selected for molecular analyses. Using a set of 23 SSOs for hybridization of PCR products from generic DRB amplification six different haplotypes were identified, of which only three are shared by the two tribes. The oligonucleotide hybridization patterns are compatible, with haplotypes DRB1*1602-DRB5*02, DRB1*0404-DRB4*0101, DRB1*0802, and DRB1*0901-DRB4*0101 in the Kaingang tribe, and haplotypes DRB1*1602-DRB5*02, DRB1*0411-DRB4*0101, DRB1*1413-DRB3*0101, DRB1*0802, and DRB1*0901-DRB4*0101 among the Guarani. DRB1*1413 is a new allele, most closely related to DRB1*1402, which is common among South and North American Indians. At the segments analyzed, they differ solely at position 57, which is GAT (aspartic acid) in DRB1*1402 and AGC (serine) in DRB1*1413. This allele probably originated in South American Indians, resulting from a single segmental exchange event between alleles DRB1*1402 (the acceptor) and DRB1*0411.
Sequencing-based typing (SBT) is the most comprehensive method for characterizing human leukocyte antigen gene polymorphisms. Development of a SBT method for DQA1 is hampered because of a deletion of codon 56 in nearly half of the known DQA1 alleles. Sequence electropherograms of heterozygous samples comprising a deletion allele and a non-deletion allele display misalignment after codon 56 because of a three base-pair shift in the deletion allele. To overcome this problem, we have designed three group-specific primer sets to selectively amplify the deletion alleles from the nondeletion alleles. DNA samples are initially polymerase chain reaction (PCR)-typed using these primer sets along with an internal positive control primer set specific to growth hormone gene 1 (hGH1). The positive group-specific PCR reactions were selectively repeated without hGH1 control primers, and the amplicons were used as template in sequencing reactions. The sequence data were analyzed to obtain DQA1 types using ABI MatchTools software as well as the newly available Conexio Genomics Assign SBT Genotyping Software. The method was validated using a panel of reference DNA from the University of California, Los Angeles, International DNA Exchange Program. We conclude that the present SBT method is a technically simple and robust procedure to characterize the sequence polymorphisms in exon 2 of DQA1 gene.

The role of the DPB1 gene in genetic susceptibility to type I diabetes has yet to be elucidated. Studies of DPB1 alleles are conflicting. Analysis at the amino acid level, rather than consideration of allelic polymorphism, has been informative in determining disease susceptibility encoded by the DRB1 and DQ genes. In this study, therefore, amino acid variation at polymorphic sites of the DP[\beta] peptide chain encoded by the second exon of the DPB1 gene was analyzed in diabetic and control subjects from white Caucasian, North Indian Asian, and Jamaican populations. Human leukocyte antigen genotypes and haplotypes were analyzed using a logistic-regression approach and the data were conditioned for the effects on disease risk of the DRB1, DQA1, and DQB1 genes. Eight DP[\beta] amino acid residues were significantly associated with type I diabetes independent of DR and DQ (DP[\beta] 9, 33, 35, 36, 55, 56, 57, and 69). None of these residues, however, correlated consistently with disease risk in all three racial groups. This contrasts with findings for the DR[\beta], DQ[\alpha] and DQ[\beta] peptide chains, where the identity of the amino acid at particular sites has been found to correlate with predisposition to type I diabetes.

Certain T-cell receptor (TCT) [beta]-chain variable (V), joining (J), and constant (C) gene segments, as well as TCR[alpha]-chain V gene segments, are disproportionally represented in TCR [alpha] and [beta] cDNA libraries derived from PHA-stimulated peripheral blood lymphocytes. Sequences of 138 TCR[alpha] clones and 96 TCR[beta] clones were determined and of these 128 TCR[alpha] clones and 88 TCR[beta] clones were found to contain unique combinations of V, J, and C gene segments or to display diversity in N region nucleotides. The frequency of the V, J, and C genes used in the assembly of unique transcripts was ascertained. Of the 24 reported V[beta] genes, families, 21 were observed among the 88 TCR[beta] clones including four V[beta] families [V[beta]1, V[beta]2, V[beta]3, and V[beta]4] that were represented in the sample times more frequently than would be expected on the basis of copy number within the gene complex. Seventy-eight percent of the clones were positive for C[beta]2 and more than half of the clones (53%) used one of two J[beta]2 genes: J[beta]2.1 was present in 27 clones and J[beta]2.7 in 20 clones. TCR V[alpha] families were also disproportionately represented in this sample. Twenty-five of 30 V[alpha] families were observed in the sample of 128 clones including six recently reported V[alpha] families. Three V[alpha] families, V[alpha]2, V[alpha]8, and V[alpha]23, accounted for [ap]40% of the TCR[alpha] clones and were represented at 18%, 9.4%, and 13.3%, respectively. Both V[alpha]2 and V[alpha]8 gene families contain more than one gene; thus the number of clones observed in these families may, in part, be related to gene number. However, V[alpha]23, which appears to be a single-copy gene family, is significantly overrepresented in this sample. Although disproportional usage of V[beta] genes may be accounted for by superantigen exposure, reasons for disproportional usage of J[beta], C[beta], and V[alpha] genes are presently unknown.


http://www.sciencedirect.com/science/article/B6T3B-44SHDT2-B/2/ca2e0d5b565a5ff00c71514a97ce7743

Cytotoxic T-lymphocyte antigen 4 (CTLA4) gene polymorphism located in the 3’ untranslated region (UTR) was investigated in 141 Spanish patients (38 men and 103 women) with rheumatoid arthritis (RA) and in 194 ethnically-matched healthy controls. Twenty alleles having different numbers of (AT) repeats (from 7 to 32) were found in this population. (AT)7 and (AT)16 were the most frequent alleles, and accounted for almost two-thirds of the allelic frequency in the control population. Consequently, alleles were assigned as L (large: 16 or more AT repeats) or S (short: less than 16 AT repeats). When the L/S distribution in patients and controls were compared, an increase of L alleles was observed among patients (49.9% vs. 39.7%; p = 0.02; pc = 0.04; odds ratio [OR] = 1.46; 95% confidence interval [CI], 1.06-2.01). Hence, the frequency of S alleles was decreased among patients (51.1% vs. 60.3%; p = 0.02; pc = 0.04; OR = 0.69; 95%CI, 0.50-0.95). Moreover, a statistically significant decrease in the frequency of S/S individuals was observed among RA patients (27.7% versus 40.7%; p = 0.01; pc = 0.03; OR = 0.56; 95%CI, 0.34-0.91). These differences were irrespective of the HLA "shared epitope" (SE) status, and were observed similarly among SE+ as well as among SE- patients. After combining these data with other reported previously by us, from studies of CTLA4 49 (A/G) and -318 (C/T) polymorphisms, we conclude that the strongest association between CTLA4 gene polymorphisms and RA susceptibility occurs with the 3’ UTR polymorphism.


http://www.sciencedirect.com/science/article/B6T3B-47J6T30-
In an attempt to define the role of HLA class II genes in predisposition to primary Sjögren's syndrome, patients of two different ethnic groups (Israeli Jews and Greeks of non-Jewish origin) suffering from this disorder were studied. Oligonucleotide genotyping revealed the majority in both groups to carry either DRB1*1101 or DRB1*1104, alleles that are in linkage disequilibrium with DQB1*0301 and DQA1*0501. The high frequency of the two alleles in these SS patients is in contrast with the accepted association of primary SS with HLA-DR3 in Italian and American individuals. Molecular analysis of DQB1 and DQA1 alleles found in American Caucasian and American black SS (or SLE) patients demonstrated high frequencies of DQB1*0201 and DQA1*0501. The fact that the majority of SS patients, across racial and ethnic boundaries, carry a common allele, DQA1*0501, implies its involvement in the predisposition to primary SS. Based on sequence analysis and the computer imaging of the HLA class II molecule structure, a hypothetical model for the role of the DQ molecule in promoting primary SS was proposed.


http://www.sciencedirect.com/science/article/B6T3B-3S2BWT5-6/2/b097bb84ac09825b5677727efa2c75b98

ABSTRACT: Sequencing Based Typing (SBT) is a generic approach for the identification of HLA-A polymorphism. This approach includes the high resolution typing of the HLA-A broad reacting groups, HLA-A subtypes and will identify new alleles directly. The SBT approach described here uses a locus specific amplification of DNA from exon 1 to exon 5. The resulting 2,022 bp PCR product serves as a template for the subsequent sequencing reactions. Amplification is followed by direct sequencing of exons 2, 3 and 4 in both orientations with fluorescently labeled primers to define all polymorphic positions leading to a high resolution typing result. In this study the sequence of exons 2 and 3 of a panel of 49 cell lines was determined. In addition, the exon 4 region of 35 cell lines was also sequenced to evaluate the exon 4 polymorphism. The HLA-A type of most of the cells could be identified by sequencing only exons 2 and 3. However, the sequence of exon 4 was required to discriminate A*0201 from A*0209 and A*0215N. In this panel, an identical new "HLA-A*0103" was identified in two Caucasian samples.


http://www.sciencedirect.com/science/article/B6T3B-3Y51FFC-F/2/414ba15e5d4c67ec03f0ba9c4062f9cc

The function of the TAP gene products appears to be the transport of antigenic peptides into the lumen of the endoplasmic reticulum where peptides are loaded onto HLA molecules. The polymorphisms within the TAP genes and potential disease associations are the subject of intense current study. While several methods have been described for TAP1 genotyping, most of these methods are unable to definitively assign TAP1 genotypes to individuals heterozygous at more than one polymorphic position. A combination named TAP1U was observed in approximately 25% of study subjects. We developed a restriction enzyme based method that allows definitive TAP1 genotypes assignment to 100% of subjects. We also further developed and optimized TAP genotyping by PCR amplification of specific alleles (PASA) that resulted in significant time and cost savings. Hence, we report a novel method for assigning TAP genotypes for TAP1U subjects and the modified PASA reactions. These improvements facilitate the rapid...
and efficient assignment of TAP genotypes useful for large human disease-gene association studies.


http://www.sciencedirect.com/science/article/B6T3B-49C52S8-3/2/2c2ea97212b4218d4b9edad200d75f07

Cytokine genetic polymorphisms are the subject of disease-association studies that require large-scale human genotyping. Polymerase chain reaction based custom microarrays and microfluidics systems were used to develop genotyping assays for following cytokine polymorphisms: tumor necrosis factor-[alpha] G-308A, interleukin-4 (IL-4) C-589T, interferon-[gamma] (CA)n repeats, IL-1RN 86-bp variable number of tandem repeats (VNTR), and CCR5 32-bp indel. For G-308A, 70.9% of DNA samples assayed were homozygous for wild type, 25.5% were heterozygous, and none were homozygous for variant allele. For C-589T, 35.5% of DNA samples were homozygous for wild type, 38% were heterozygous, and 22% were homozygous for variant. For IL-1RN VNTR, 71% of DNA samples were homozygous and the remainder were heterozygous. For CCR5, 96.4% of amplicons were homozygous for wild type, and 3.6% were heterozygous containing deletion. For IFN-[gamma] (CA)n repeats, 35.6% had 2,2 alleles, 42.2% had 2,3 alleles, and 11% had 3,3 alleles with alleles 1 through 5 corresponding to 11 through 15 repeats, respectively. There was good concordance between the results we obtained and current "gold-standard" methodologies for analyzing single nucleotide polymorphisms and size polymorphisms. Electronic DNA concentration with high stringency predisposes microarray technology to hybridization fidelity and accuracy, and microfluidics systems outperform conventional methodologies for size polymorphisms. Comprehensive genotyping can be achieved for clinical epidemiologic studies on cytokine gene polymorphisms using this approach.


More than 590 human leukocyte antigen (HLA)-B alleles have been identified by sequence analysis. Although the polymorphic exon 2 and 3 sequences of all HLA-B alleles are described, the sequences of the other exons of a number of infrequent B-alleles are unknown. In this study, the exon 1, 4, or 5 sequences of 39 different HLA-B alleles were elucidated by allele-specific sequencing. Overall, these exon sequences showed identity with the majority of the known sequences from the corresponding allele groups, except for four alleles B*4010, B*4415, B*4416, and B*5606. The exon 1 sequence of B*4010 had nucleotide differences with all B*40 alleles, but was identical to the B*54, *55, *56, and *59 allele groups. B*4416 differed from B*440201 at position 988, which was previously considered a conserved position. B*4415 showed exon 1, 4, and 5 sequences deviating from the other B*44 alleles, but identical to B*4501. The exon 1 and 4 sequences of B*5606 differed from other B*56 alleles, but were in complete agreement with B*7801. The deviating exon sequences of B*4415 and B*5606 confirmed the evolutionary origin of these alleles suggested by the sequences of exons 2 and 3. The polymorphism observed in exons 1, 4, and 5 merely reflects the lineage-specificity of HLA-B.

Swelsen, W. T. N., C. E. M. Voorter, et al. (2005). "No HLA-A gene detectable on one of the haplotypes in
An unusual haplotype was detected in a family of a caucasian transplant patient. Human leukocyte antigen (HLA) analysis of the family demonstrated the absence of HLA-A on one of the haplotypes present in two family members. One was serologically typed A24, the other A2. Because they had one haplotype in common, the HLA-A allele of the shared haplotype was supposed to be a null allele. Different molecular typing methods identified only one allele in both individuals. The results suggest a deletion of the complete HLA-A gene or a major part of it. For confirmation, microsatellite analysis of the HLA-A region was performed with six microsatellite markers. Both family members were heterozygous for all markers, and a deletion of HLA-A could not be proven. Fluorescent in situ hybridization (FISH) was performed with cosmid and PAC probes encompassing the HLA-A gene. Both probes demonstrated an identical normal distribution pattern for diploid results. The absence of any serologic and molecular reaction with the results of the microsatellite and FISH analysis make a deletion of a narrow region, encompassing the HLA-A gene, the most plausible explanation.


The strategy for sequencing human leukocyte antigen (HLA)-A was based on separate amplification of exons 2 and 3, followed by forward and reverse heterozygous sequencing of the alleles. Validation of the method was obtained by sequencing 11 individuals carrying alleles from all different HLA-A allele groups, except *43. All alleles could be correctly identified except A*3401. Unexpected polymorphic positions were identified in exon 3, even in individuals homozygous for A*3401. The pseudogene HLA-COQ or HLA-DEL linked to A*3401 was coamplified and sequenced in addition. The problem was solved by using different amplification primers for exon 3 with mismatches for the two pseudogenes. A total of 252 unrelated individuals with at least one allele belonging to the A10 or A19 group were typed for HLA-A by this strategy. Ten different alleles were identified in the A10 group and 14 in the A19 group. As second allele a further 30 different subtypes from all different groups were sequenced. In 21 individuals, sequencing exon 1 was necessary to distinguish A*7401 from A*7402. The sequencing strategy, with separate amplification of the exons, has proven to be a robust method, resulting in reliable and efficient high-resolution HLA-A typing.


http://www.sciencedirect.com/science/article/B6T3B-42HNKXW-7/2/bd26b00d4418ae6236cc9d09d3c181f

Genetic variations in the locus encoding the transporter associated with antigen processing, subunit 1 (TAP1), were systematically studied using samples from Caucasians, Africans, Brazilians, and compared with data from chimpanzees. PCR-amplified genomic sequences corresponding to the 11 exons were analyzed by single-strand conformation polymorphism (SSCP) and sequencing. Six nonsynonymous and 2 synonymous single nucleotide polymorphisms (SNPs) were found to be common in one ethnic group or another, and they
involved codons 254 (Gly-GGC/Gly-GGT) in exon 3, 333 (Ile-ATC/Val-GTC) in exon 4, 370 (Ala-
GCT/Val-GTT) in exon 5, 458 (Val-GTG/Leu-TTG) in exon 6, 518 (Val-GTC/Ile-ATC) in exon 7,
637 (Asp-GAC/Gly-GGC), 648 (Arg-CGA/Gln-CAA) and 661 (Pro-CCG/Pro-CCA) in exon 10. At
each SNP site the sequence listed first was predominant in all ethnic groups. Several SNPs
segregated on the same chromosome regardless of populations and species. Together, the
SNPs produced 5 major human TAP1 alleles, 4 of which matched the officially recognized alleles
*0101, *02011, *0301, and *0401; the 5th allele differed from each of those by at least 4 SNPs.
Overall, TAP1*0101 was the predominant allele in all ethnic groups, with frequencies ranging
from 0.667 in Zambians to 0.808 in US Caucasians. The TAP1*0401 frequency showed the
greatest difference between Africans (0.221-0.254) and Caucasians (0.033), with Brazilians
(0.058) fitting in the middle. Consistent with earlier work based on Caucasians and gorillas, *0101
appeared to be the newest human TAP1 allele, suggesting a dramatic spread of *0101 into all
human populations examined. Characterization of TAP1 polymorphisms allowed the design of a
PCR-based genotyping scheme that targeted 7 SNP sites and required 2 separate genotyping
techniques.

sclerosis in Australian patients." Human Immunology 60(8): 715.

http://www.sciencedirect.com/science/article/B6T3B-3WY9RK0-9/2/9b34278b1f5a7555f29ce962bc3a6fb2

Genetic susceptibility to multiple sclerosis (MS) has so far been strongly localized to the MHC
class II region encoding the alleles of the haplotype HLA-DRB1*1501, -DQA1*0102, -
DQB1*0602. However, this haplotype is not carried by approximately 40% of MS patients; a
potential explanation could be that they carry other MHC class II alleles with similar function due
to the sharing of nucleotide sequences encoding critical amino acid residues. The DRB1 gene is
polymorphic at residue 86, encoding valine or glycine. In view of the increasing evidence for a
functional role for DRB1 aa86 in the binding and presentation of autoantigenic peptides such as
myelin basic protein, this study investigated associations with the residue 86 polymorphism in an
Australian MS population. A significant increase in the Val86/Val86 genotype was observed in the
MS patients, which was still present in the absence of the DRB1*1501 allele (p = 0.032). This
suggests that DRB1 aa86 may have an independent role in contributing to MS susceptibility. The
Val86/Val86 genotype was correlated with genotyping for other putative MS susceptibility genes,
including T cell receptor beta chain germline polymorphisms, HLA-DMB alleles, and -DQA1 and
-DQB1 alleles encoding critical amino acid residues, with a significant interaction only observed
with DQB1 Leu26 (p = 0.014). Additional studies of the HLA-DRB1 aa86 polymorphism in MS,
and its function, are needed to more fully understand this association.

Ueno, H., K. Katamura, et al. (1999). "Further characterization of memory T cells existing in a case of
CD8 deficiency." Human Immunology 60(11): 1049.

http://www.sciencedirect.com/science/article/B6T3B-3Y0HP3C-5/2/0f9e4d784c6a34638f4678f5b63dab54

CD8 deficiency is a rare primary immunodeficiency caused by a defect of ZAP-70, which plays a
pivotal role in T cell activation. We previously reported the existence of memory phenotype-CD4+
T cells in a case of CD8 deficiency, which demonstrates that activation signals through ZAP-70
are not essential to the phenotypic conversion of T cells from "naive" to "memory." In this study,
we further characterized CD45RO+ T cells in a CD8 deficient patient. We showed that the
patient's CD45RO+ T cell population had a wide variety of T cell receptor V[beta]-chain gene
usage, and contained few clonally expanded T cells, while many clonally expanded T cells were
present in the memory T cell population of age-matched healthy children. These results suggest that various kinds of antigens were involved in the differentiation of the patient's T cells, and that the differentiation into memory T cells was not accompanied by profound T cell proliferation. Moreover, our findings confirmed that the patient's CD45RO+CD4+ T cells had acquired effector-cytokine producing ability, indicating that there exists an alternative activation pathway which is independent of ZAP-70 for the acquisition of effector-cytokine producing ability.

van den Elsen, P. J., N. van der Stoep, et al. (2000). "Lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells and is caused by methylation of the IFN-[gamma] inducible promoter (PIV) of CIITA." Human Immunology 61(9): 850.

http://www.sciencedirect.com/science/article/B6T3B-41GWN1G-2/2/4719aacb7f9283b2771e5c3c1822f810

Lack of MHC-mediated antigen presenting functions of fetal trophoblast cells is an important mechanism to evade maternal immune recognition. In this study we demonstrated that the deficiency in MHC expression and antigen presentation in the trophoblast cell lines JEG-3 and JAR is caused by lack of class II transactivator (CIITA) expression due to hypermethylation of its interferon-[gamma] (IFN-[gamma])-responsive promoter (PIV). Circumvention of this lack of CIITA expression by introduction of exogenous CIITA induced cell surface expression of HLA-DR, -DP, and -DQ, leading to an acquired capacity to present antigen to antigen-specific T cells. Transfection of CIITA in JEG-3 cells also upregulated functional HLA-B and HLA-C expression. Note-worthy, this lack of IFN-[gamma]-mediated induction of CIITA was also found to exist in normal trophoblast cells expanded from chorionic villus biopsies. Together, these observations demonstrate that lack of CIITA expression is central to the absence of antigen presentation functions of trophoblast cells.


The polymerase chain reaction (PCR) in combination with the sequence-specific oligonucleotide probe (SSOP) was applied to analyze the polymorphism in the axon 2 of the HLA-B gene. In this study, genomic DNAs from 85 B-lymphoblastoid cell lines homozygous for HLA and peripheral blood granulocytes of 156 Japanese individuals were investigated. Two HLA-B-specific 5'-sided primers (CG4 and CG5) and two 3'-sided primers (CG2 and CG3) were designed for specific amplification of the axon. HLA-B alleles were classified into two groups (groups I and II) according to specific amplification with two types of the 3'-sided primers. The amplified DNAs were hybridized with 23 nonradioactively labeled SSOPs. Based on the hybridization patterns with the SSOPs, 34 HLA-B specificities were divided into 26 epitope combination (EC) groups. Fifteen HLA-B specificities were classified into four EC groups and these HLA-B specificities could not be distinguished from one another in the same EC group. Another 16 HLA-B specificities corresponded one by one to 16 distinct EC groups, and two subtypes of HLA-Bw 75, B27, and Bw48 were also identified enabling the accurate typing of 22 HLA-B alleles at the DNA level. Single-strand conformation polymorphism (SSCP) of the PCR products from group I HLA-B alleles was also investigated. The HLA-B alleles showed distinct electrophoretic patterns in nondenaturing polyacrylamide gels, depending on the nucleotide sequences of the exon 2, indicating that the SSCP analysis may be an alternative, useful and practical HLA-matching system of HLA-B specificity in tissue transplantation.
MHC class II alleles and haplotypes were determined from unrelated individuals and families of the Arhuaco (n = 107), Kogi (n = 42), Arsario (n = 18), and Wayu (n = 88) tribes located in the northern part of Colombia. Class II DRB, DQA1, and DQB1 alleles were determined by PCR-SSO and PCR-RFLP based methods. Four haplotypes, [DRB1*0407, DRB4*0101, DQA1*03, DQB1*0302]; [DRB1*0403, DRB4*0101, DQA1*03, DQB1*0302]; [DRB1*1402/1406, DRB3*0101, DQA1*0501, DQB1*0301]; and [DRB1*0802, DQA1*0401, DQB1*0402], were observed among these four tribes. In addition to these haplotypes, the Wayu Indians showed a frequency of 21.3% for the [DRB1*1602, DRB5*02, DQA1*0501, DQB1*0301] haplotype, 13.1% for the [DRB1*0411, DRB4*0101, DQA1*03, DQB1*0302] haplotype, and 8.1% for the [DRB*0411, DRB4*0101, DQA1*03, DQB1*0402] haplotype. Red cell antigen typing was used to calculate genetic admixture. The Kogi and Arsario showed no genetic admixture while the Arhuaco tribe showed admixture with genes of African origin and the Wayu showed admixture with Caucasians as well as genes of African origin. These findings were confirmed by the MHC class II allele and haplotype data obtained, as alleles and haplotypes of Caucasian and African origin were detected in the Wayu and Arhuaco and not in the Kogi or Arsario. These studies will be important in disease association and transplantation studies for Amerindian and Colombian populations and for correlating genetic traits with the anthropologic and linguistic data available in order to better understand the Amerindian populations.


Type 1 diabetes (T1D) is a complex autoimmune disease. Several genetic loci have been implicated in the susceptibility to this illness. Evaluated was the role of the CTLA4 exon 1 A49G polymorphism and its role as a risk factor for T1D in our population. DNA from 190 patients with T1D and their families and 96 control individuals were genotyped for CTLA4 exon 1 polymorphism and human leukocyte antigen (HLA)-DQB1*0201 and *0302 haplotypes by polymerase chain reaction (PCR) amplification-restriction enzyme analysis and PCR amplification that used sequence-specific primers, respectively. Patients were nonobese and 2 analysis and family-based association studies were performed and suggested the association of CTLA4 exon 1 G polymorphism with T1D (p = 0.0229). Furthermore, in HLA-DQB1*0201-positive patients with T1D, the GG and AA genotypes were higher and lower, respectively, than those found in control individuals. This study suggests that CTLA4 is a candidate susceptibility gene for T1D.
The clinical applicability of genomic HLA class II typing techniques has increased after the introduction of PCR-based typing strategies. In typing by PCR amplification using sequence-specific primers (PCR-SSP), amplification of specific alleles or groups of alleles is achieved, provided that the mismatch(es) of the SSP is located in the 3’ end of the primer. Thus, the specificity of the typing system becomes part of the amplification step, which reduces the total typing time to a minimum by simplifying the postamplification processing of samples. The set of primers presented here identifies all of the alleles of the DR4 group, DRB1*0401-DRB1*0411, as well as the DRB1*07 and DRB1*0901 alleles. In the present study of DR4 alleles, PCR-SSP was compared with hybridization with sequence-specific oligonucleotide probes following group-specific PCR amplification (PCR-SSO). The two typing strategies gave completely concordant results in the 90 DR4-positive and the 32 DR4-negative individuals and cell lines studied. DR7, DQ9/DR9,DQ9 discrimination using PCR-SSP, was compared with MspI DQA RFLP typing, also with concordant results in the 33 DR7- and/or DR9-positive and 36 DR7- and DR9-negative individuals and cell lines tested. No false-negative or false-positive typing results were obtained. Genomic typing by PCR-SSP was performed in the overall time of 2 hours, including rapid DNA preparation, PCR amplification, postamplification processing, documentation, and interpretation of results. This makes the PCR-SSP strategy for HLA class II typing attractive not only in population- and disease-association studies, but also in routine clinical practice, including donor-recipient matching prior to cadaveric transplantation.

Zipp, F., C. Windemuth, et al. (2000). "Multiple sclerosis associated amino acids of polymorphic regions relevant for the HLA antigen binding are confined to HLA-DR2." Human Immunology 61(10): 1021.

http://www.sciencedirect.com/science/article/B6T3B-41NCFTH-8/2/7c931e6c016ad71c7d071061ed1fbf17b

Among the candidate genes for multiple sclerosis (MS), the strongest influence is conferred by human leucocyte antigen (HLA) class II genes, in particular the DR2, DQ6, Dw2 haplotype (DRB1*1501, DQA1*0102, DQB1*0602). Similar to other autoimmune diseases, it is not clear yet how the presence of a specific HLA-DR or -DQ molecule translates into an increased disease susceptibility. Previous observations by us and others imply a HLA-DR2 dependent propensity of antigen-specific T-cell lines to produce increased amounts of TNF-[alpha]/[beta] as one mechanism how DR2 could contribute to susceptibility. In this article, we investigated the distribution of polymorphic stretches of the DRB1, DQA1, and DQB1 chains known to be relevant for antigen binding, in 66 unrelated patients with relapsing remitting MS and 210 unrelated controls. We found a significant association with disease for the appearance of proline at position 11, arginine at position 13, and alanine at position 71 of HLA-DR[beta]1. Surprisingly, we identified only residues preferentially expressed in the MS group that were related to HLA-DR2. Thus, the contribution of HLA class II to the pathogenesis of MS is not mediated by allele-overlapping antigen binding sites, but is confined to the disease associated HLA allele.


IL-2 receptor is expressed at low levels on adult blood lymphocytes, and at lower levels on cord blood cells. IL-2 receptor [alpha] and [beta] chain expression increases gradually from 0-18 months of age. The level of soluble CD25 (IL-2 receptor [alpha] chain) has been reported to be elevated in cord blood. Quantitative RT-PCR showed that adult cells express 10 times as much
CD25 mRNA as cord cells. Cord plasma showed only a marginal ability to strip CD25 from the membrane. To assess the functional consequences of low IL-2 receptor expression, cord and adult cells were activated in vitro. The response was stimulus-dependent, but cord cells upregulated CD25 readily. Cord and adult cells proliferated in an IL-2-dependent assay to a similar extent. Infants suffering acute infection showed marginally higher levels of membrane CD25 expression than infants without overt infection. Thus neonatal and infant lymphocytes express lower levels of IL-2 receptors than adult cells, reflecting lower mRNA concentrations at least for CD25; they are able to up-regulate receptors in response to in vitro stimulation and are able to respond in vitro to IL-2-dependent stimulation; however in vivo there may be a dampening down of the IL-2 system in infancy.

Human Pathology  (33)


http://www.sciencedirect.com/science/article/B6WGD-49BXWP3-M/2/e04d919045323a5828e14d21f8531668

Balanced translocations are rare in myelodysplasia (MDS) and acute myeloid leukemia (AML) with multilineage dysplasia; however, the t(3;5)(q25;q35) and insertion variant occur in a subset of patients. To evaluate the possible genes involved in this translocation, we studied 6 cases with a t(3;5) by fluorescence in situ hybridization with probes directed against the nucleophosmin (NPM), EVI1, and Ribophorin genes, as well as a newly developed myeloid leukemia factor 1 (MLF1) BAC clone. The histologic spectrum of the cases was variable, ranging from refractory cytopenia with multilineage dysplasia to AML with multilineage dysplasia in the World Health Organization classification. An NPM/MLF1 fusion was identified in 5 of 6 cases, whereas the EVI1 and Ribophorin genes were not involved in any of the cases. The NPM/MLF1-positive cases were predominantly young adult males (median age, 33 years) who responded well to hematopoietic stem cell transplantation. These findings suggest that an NPM/MLF1 fusion is the primary molecular abnormality in t(3;5) MDS and AML with multilineage dysplasia, and also that cases with NPM/MLF1 may be clinically distinct from other MDS-associated disease.


http://www.sciencedirect.com/science/article/B6WGD-49KGPM6-K/2/511061193133b299967b41fb4fdd8db2

The histological transformation from a follicular lymphoma (FL) to a diffuse large B-cell lymphoma (DLBL) occurs in 22% to 30% of all cases of FL. The aim of this study was to identify specific chromosomal gains/losses associated with transformation of FL to DLBL, in addition to the well-known mechanisms like p53 mutation and protein expression and c-myc translocation and up-regulation. This is the first study to meet 2 important conditions for such a comparison. First, we demonstrate that the FL and the DLBL were clonally related, based on identical immunoglobulin gene rearrangements in 5 of the 6 cases. Second, we used laser microdissection microscopy to
isolate only the neoplastic cells from the initial FL samples. The results indicate that no single chromosomal abnormality seems to be responsible for the transformation of FL to DLBL. P53 protein overexpression was found in 4 and c-myc translocation in 3 of the 6 transformed DLBLs, but not in the initial FL samples. Additional chromosomal abnormalities were detected by comparative genomic hybridization in all 6 cases when the DLBL was compared with the FL. In the 5 cases with transformation of grade 1 or 2 FL to DLBL, gains at chromosomes 7 (5 of 5 cases), 10p1 (3 of 5 cases), 12 (3 of 5 cases), and 20p13 (2 of 5 cases) and loss at 9q (4 of 5 cases) were the most frequently found abnormalities. A gain on chromosome 7p, in combination with a loss on 9q, was found in 4 of the 5 DLBL that transformed from FL grade 1 or 2.


http://www.sciencedirect.com/science/article/B6WGD-4CBD3N7-5/2/cf3508cfecdb475c51b299617b87cd5

Microsatellite instability (MSI) is commonly seen in tumors associated with the hereditary nonpolyposis colorectal cancer syndrome and is caused by defects in the DNA mismatch repair genes. MSI has also been observed in various sporadic cancers, including colorectal, gastric, and endometrial. The role and incidence of MSI in ovarian clear cell carcinoma remain unknown. This study was conducted to evaluate the frequency of MSI in ovarian clear cell carcinomas and to evaluate the sensitivity and specificity of immunohistochemistry in predicting mismatch-repair gene deficiency. A total of 42 ovarian clear cell carcinomas were analyzed for MSI using a panel of 5 microsatellite markers (BAT25, BAT26, D5S346, D2S123, and D17S250). Alterations in the expression of hMLH1 and hMSH2 proteins in these tumors were examined. Of the 42 ovarian clear cell tumors analyzed, 6 demonstrated a high level of MSI (MSI-H), 3 demonstrated a low level of MSI (MSI-L), and the remaining 33 exhibited microsatellite stability (MSS). No correlation was found between MSI level and patient age or tumor stage or size (P >0.05). Loss of expression of either hMLH1 or hMSH2 was observed in 4 of the 6 (67.7%) MSI-H tumors, whereas 34 of the 36 (94.4%) MSI-L or MSS tumors expressed both the hMLH1 and hMSH2 gene products. Our results indicate that MSI-H is involved in the development of a subset of ovarian clear cell carcinomas. A strong correlation exists between alterations in the expression of hMLH1 and hMSH2 and the presence of MSI-H in these tumors. However, immunohistochemical testing alone may miss a small fraction of cases with MSI-H.


http://www.sciencedirect.com/science/article/B6WGD-4C4D7KX-P/2/1ad096a2216b859bd7676e8e10902ddc

Two cases of placental transmogrification of the lung are reported. The lesions presented in the left lung, in one case as a giant bulla of the upper lobe and in the other as a cystic nodule of the lower lobe. A segmentectomy was performed in both cases, and the patients were alive and well 5 years and 2 months after surgery, respectively. In our opinion, pulmonary placental transmogrification is not a variant of emphysema, as generally considered, but rather probably represents a benign proliferation of immature interstitial clear cells with secondary cystic change. This report presents a histological, immunohistochemical, ultrastructural and molecular study of these peculiar cells, together with a review of the literature.

http://www.sciencedirect.com/science/article/B6WGD-4CBD3N7-B/2/e1df38cd47c364b64d8d395865d84686

The monitoring of gastric lymphomas is often hampered by the inherently limited sampling provided by small endoscopic biopsy specimens. To investigate the feasibility of using gastric washing fluid for monitoring patients with known gastric lymphoma and for diagnosing gastric involvement in patients with extranodal nongastric lymphoma, we collected 49 gastric washings from 39 patients (29 patients with gastric lymphoma and 10 patients with nongastric extranodal lymphoma). Collection was done at the time of follow-up biopsy and when no endoscopic abnormalities were found. DNA was extracted from the washing fluid and analyzed for clonal IgH gene rearrangement by Southern blotting (J6 probe) and/or polymerase chain reaction (PCR) (using VH-FR3 and JH primers). Forty-one of 49 samples (84%) yielded sufficient DNA for molecular analysis. Sixteen of 41 analyzable gastric washing samples (39%) failed Southern blot analysis due to degraded or insufficient DNA. Concordance between the results of Southern blot analysis of the washing and histology of the simultaneous biopsy specimen was found in 20 (80%) of the remaining 25 samples. The IgH PCR result was concordant with biopsy histology in 33 out of 41 washing samples (80%). The overall concordance between molecular clonality studies of washings (Southern blotting and/or PCR) and biopsy histology was 83% (34 of 41). Of the 7 (18%) discrepant specimens, 2 were diagnosed histologically as lymphoma, but the simultaneous washings were negative by molecular studies. Five biopsy specimens were histologically benign, but the corresponding washings demonstrated clonal IgH gene rearrangement (3 cases by PCR and 2 cases by Southern blotting). This study demonstrates the diagnostic utility of molecular clonality analysis of gastric washings.


http://www.sciencedirect.com/science/article/B6WGD-4CTDD8S-14/2/eb2c1ebd05b442b357549ce9e6c1f12bb

High-grade astrocytomas are tumors that are uncommon in children. Relatively few studies have been performed on their molecular properties and so it is not certain whether they follow different genetic pathways from those described in adult diffuse astrocytomas. In this study, we evaluated 24 pediatric high-grade astrocytomas (11 anaplastic astrocytomas and 13 glioblastomas) all of which were sporadic and primary. We studied mutations of p53, phosphatase and tensin homolog (PTEN), loss of heterozygosity (LOH) of chromosomes 17p13, 9p21 and 10q23-25, amplification of epidermal growth factor receptor (EGFR), and overexpression of EGFR and p53 protein. In addition, we searched for microsatellite instability (MSI) by using MSI sensitive and specific microsatellite markers. p53 mutations were found in 38% (9/24) of the high-grade astrocytomas and all brain stem tumors except 2 (71%, 5/7) had p53 mutations. PTEN mutations were found in 8% (2/24) of high-grade astrocytomas. However, no EGFR amplification was found in any of them. LOH was found at 17p13.1 in 50% (3/6 informative tumors), 9p21 in 83% (5/6 informative tumors), and 10q23-25 in 78% (7/9 informative tumors). Four tumors showed MSI, and 2 of them that showed widespread MSI were regarded as tumors with replication error (RER+) phenotype. All 4 tumors with MSI showed concurrent LOH of 9p21 and 10q23-25. Combining gene alterations, LOH, MSI, and gene mutations, inactivation of both alleles of PTEN and p53 was found in 72% (12/17 informative tumors) and 50% (3/6 informative tumors) of the cases respectively. We conclude that development of pediatric high-grade astrocytomas may follow pathways different from the primary or secondary paradigm of adult glioblastomas. In a subset of
these tumors, genomic instability was also implicated.


Chronic hepatitis may progress to cirrhosis and hepatocellular carcinoma (HCC). Progressive accumulation of mutations and genomic instability in chronic viral hepatitis might flag an increased risk of HCC development. Genomic instability at dinucleotide microsatellite loci in chromosomes 2, 13, and 17 and at 2 mononucleotide repeat loci was examined in liver tissues from 41 patients, including 30 without HCC (18 patients with chronic hepatitis and 12 with cirrhosis) and 11 with HCC. Genomic instability was detected in 51% of the 41 cases. Allelic imbalance at informative dinucleotide loci occurred in 37% of the cases. In 14 cases (34%), allelic imbalance was detected in chronic hepatitis or cirrhosis without HCC. Allelic imbalance at the chromosome 13 locus was detected in 50% of the cases of chronic hepatitis C. Allelic imbalance at the TP53 chromosome locus and/or at the chromosome 13 locus was significantly more frequent than alterations at the chromosome 2 locus (P = .026). Low-level microsatellite instability was found in 20% of all cases examined and high-level microsatellite instability in 3 patients (7.5%), including 2 cases of chronic hepatitis and 1 case of cirrhosis. Our results show that allelic imbalance occurs frequently in hepatitis-related HCC as well as in chronic hepatitis in patients without HCC. Allelic imbalance at the D13S170 chromosome 13 locus (13q31.2) occurs frequently in chronic hepatitis, suggesting that genomic alterations affecting the long arm of chromosome 13 might be used to monitor the natural progression of chronic hepatitis-associated liver carcinogenesis.


Disseminated disease is very important in the clinical assessment of pediatric sarcomas. Several reports suggest that reverse transcriptase polymerase chain reaction (RT-PCR) holds great promise in the early staging of cancer patients in general. However, the complexities of these protocols hamper adequate standardization, and their application as routine diagnostic tools has been difficult. The aim of this study is to assess the actual minimal number of tumor cells that may be detected by RT-PCR in a blood sample. Specific tumor cell dilutions from a Ewing's sarcoma cell line reconstituted in peripheral blood from healthy individuals were "ficolled" and submitted to RNA extraction for cDNA preparation and PCR amplification of the t(11-22) (q24;q12) fusion transcript. After PCR amplification, we were able to detect the EWS/FLI-1 chimeric gene product at a dilution of 10 tumor cells per 1 or 2 mL of blood. Our simple method supports a role for routine clinical use of RT-PCR in the detection of circulating Ewing's sarcoma cells.

Iwata, K., N. Takamura, et al. (2004). "Loss of heterozygosity on chromosome 9q22.3 in microdissected basal cell carcinomas around the Semipalatinsk Nuclear Testing Site, Kazakhstan." Human
A high incidence of skin cancers has been noted around the Semipalatinsk Nuclear Testing Site (SNTS) in Kazakhstan. Recently, basal cell carcinoma (BCC) susceptibility genes, human homolog of the Drosophila pathed gene (PTCH), and the xeroderma pigmentosa group A-complementing gene (XPA), have been cloned and localized on chromosome 9q22.3. To clarify the effect of low-dose irradiation on the occurrence of BCC, we used microdissection and polymerase chain reaction to identify loss of heterozygosity (LOH) at 9q22.3 using BCC samples obtained from this region. Ten Japanese samples were analyzed as controls. LOH with at least 1 marker was identified in 5 of 14 cases from around SNTS, whereas only 1 case with 1 marker was identified among the 10 Nagasaki cases. The total number of LOH alleles from SNTS (8 of 45) was significantly higher than the number from Nagasaki (1 of 26) (P = 0.03). The higher incidence of LOH on 9q22.3 in BCC from around SNTS suggests involvement of chronic low-dose irradiation by fallout from the test site as a factor in the cancers.

Summary

This report describes a vasculitis and subsequently developing angiodestructive lymphoma in an 11-year-old Japanese-Filipino girl exhibiting mosquito allergy with the background of chronic active Epstein-Barr virus (EBV) infection. She developed necrotic skin ulcer at the site of mosquito bite, and histopathological examination revealed EBV-positive mononuclear cell infiltration throughout the wall of small-sized muscular artery. These EBV-positive lymphoid cells were oligoclonal in Southern blot analysis for EBV terminal repeats. Effectiveness of steroid therapy also supports the nonneoplastic nature. Approximately 1 year later, she developed progressive large skin ulcer without mosquito bites. Microscopically, the angiocentric or angiodestructive pattern of EBV-positive atypical cells supported the diagnosis of extranodal natural killer/T-cell lymphoma. Southern blot analysis revealed the monoclonal neoplastic nature of EBV-positive cells. In contrast to the primary mosquito bite lesion, natural killer/T-cell lymphoma cells exhibited the higher expression of EBV latent membrane protein 1 mRNA and the apparent protein expression detected by immunohistochemistry.

Gliomatosis peritonei, a rare condition that occurs almost exclusively in the setting of ovarian immature teratoma, is characterized by the occurrence of nodules of mature glial tissues in the peritoneum. It is controversial whether glial tissues are derived from maturation of the associated teratomatous tissue that has implanted in the peritoneum, or glial differentiation of subperitoneal stem cells. In this study, we employed the unique genetic characteristics of ovarian teratomas.
(often with a duplicated set of maternal chromosomes and thus homozygous at many polymorphic microsatellite loci) versus normal tissues (heterozygous pattern due to presence of maternal and paternal genetic materials) to investigate the origin of gliomatosis peritonei. DNA samples were extracted from microdissected paraffin-embedded tissues, including the glial implants, the associated ovarian teratomas, and normal tissues, to determine their patterns of microsatellite loci in a multiplex polymerase chain reaction system. Two cases were not informative because the ovarian teratoma showed a heterozygous microsatellite pattern. In the 5 informative cases, the normal tissues showed a heterozygous pattern in the microsatellite loci, the associated teratomas showed a homozygous pattern, and the glial tissues showed a heterozygous pattern. Thus, gliomatosis peritonei is genetically unrelated to the associated teratoma but is probably derived from nonteratomatous cells, such as through metaplasia of submesothelial cells.

http://www.sciencedirect.com/science/article/B6WGD-4B0NG2V-R/2/2a43659b628fc03c34cca266bc388b7

Liposclerosing myxofibrous tumor (LSMFT) is a benign fibro-osseous lesion that is characterized by mixture of histologic elements including lipoma, fibroxanthoma, myxoma, ischemic ossification, and fibrous dysplasia (FD)-like features. These tissue components are seen in the original reports of FD; however, the relationship between LSMFT and FD is not clear. Point mutation of the [alpha] subunit of G protein (Gs [alpha]), which increases cyclic adenosine monophosphate formation, has been recognized as the cause of McCune-Albright syndrome as well as polyostotic and monostotic FD of bone. Gs [alpha] mutation was demonstrated at the Arg201 codon in 2 patients of LSMFT was demonstrated in the present study. Although direct sequencing analysis using the fresh-frozen materials could not detect the mutation, the polymerase chain reaction fragmentation length polymorphism (PCR-RFLP) disclosed the missense point mutation Gs [alpha] at the Arg201 codon in 2 cases involving LSMFT. This result strongly suggests that a subset of LSMFT is a variant form of FD.

http://www.sciencedirect.com/science/article/B6WGD-4D04T5C-26/2/965082c4b4fdec46b9fd84888ec6def

Bartonella (formerly Rochalimaea) henselae (Bh) plays a central role in cat scratch disease. A polymerase chain reaction (PCR)-based assay that can detect Bh DNA in formalin-fixed, paraffin-embedded (FF-PE) samples would have utility in the evaluation of processed lymph nodes suggestive of this disorder. Fresh or FF-PE cultures of Bh and related species were analyzed. Thirteen lymph nodes (12 FF-PE and one fresh cell suspension) with necrotizing suppurative granulomatous inflammation and seven FF-PE negative control lymph nodes were also evaluated. PCR was performed using a novel, heminested protocol. Amplified products were analyzed by gel electrophoresis. The fresh and FF-PE Bh cultures showed a specific PCR product with an analytical sensitivity of 0.5 pg bacterial DNA. Seven (54%) of 13 clinical lymph node samples with morphological features suggestive of cat scratch disease also had detectable Bh DNA, whereas none of the seven negative control lymph nodes yielded positive results. We have designed a rapid and sensitive PCR test that can reliably detect Bh DNA in fresh and FF-PE samples. Our findings indicate that this assay has clinical utility in the diagnosis of cat scratch disease.
Combined small cell and non--small cell carcinoma is relatively rare in the lung. Examination of the clonal relationship of different components in this type of tumor may give a clue to the rarity. We retrieved 6 such tumors; all 6 had small cell carcinoma and adenocarcinoma components, and 3 had an additional squamous cell carcinoma component. We examined the point mutations in the p53 gene and allelic loss (ie, the loss of heterozygosity [LOH] pattern) of chromosome 3p in each component. p53 mutations were detected in the small cell carcinoma component of 5 tumors and in the non--small cell carcinoma components of 2 tumors. In 1 case, the squamous cell carcinoma component had a p53 mutation locus identical to that in the small cell carcinoma component, but in the other case, the adenocarcinoma component had a different mutation than that in the small cell carcinoma component. Chromosome 3p LOH loci in the squamous cell carcinoma component were present in the small cell carcinoma component in all 3 cases, but some LOH loci were not identical in the small cell carcinoma and adenocarcinoma components in 3 cases. These results suggest that the small cell and squamous cell carcinoma components of combined small cell lung carcinomas have an intimate clonal relationship. On the other hand, the adenocarcinoma component often may be derived from a separate clone or, more likely, undergo a progressive process separate from the squamous cell--small cell carcinoma beginning in a very early stage, that is, before the appearance of p53 and chromosome 3p abnormalities. This tumorigenesis process may explain the relative rarity of combined small cell and non--small cell carcinoma, which occurs primarily in the peripheral lung, an infrequent site of squamous cell carcinoma.


A subset of patients with non-Hodgkin's lymphoma (NHL), present with or subsequently develop lymphocytic effusions. Differential diagnosis between reactive lymphocytosis and recurrent low-grade NHL is difficult by cytology alone. We studied the use of polymerase chain reaction (PCR)-based techniques to detect concurrent/recurrent NHL. Both primary tumors and atypical lymphocytic effusions of 12 low-grade B-NHL patients and 4 T-NHL patients were studied. Six pleural effusions (reactive/carcinomatous), in patients with no history of NHL, were included. Samples were amplified by PCR, using Fr3, Fr2, LJH, and VLJH primers specific for the immunoglobulin heavy chain (IgL) gene and V[gamma]-8, V[gamma]9, V[gamma]10, V[gamma]11 and J[gamma]1/J[gamma]2 consensus primers specific for the T-cell receptor gamma (TCR-[gamma]) gene. IgL gene PCR products were analyzed by polyacrylamide gel electrophoresis (PAGE). TCR-[gamma] gene PCR products were analyzed using a novel nonradioactive single-stranded conformational polymorphism (SSCP) procedure. IgL gene rearrangement analysis demonstrated monoclonality in 11/12 primary low-grade B-NHLs. Identical monoclonal bands were found in both primary tumor and effusion in 9 patients. TCR-[gamma] gene rearrangement analysis demonstrated monoclonality in 4 of 4 primary T-NHLs. Identical monoclonal banded patterns were found in both primary tumor and effusion in 3 patients. Our results strongly support the diagnosis of concurrent/recurrent NHL in 13 of 16 (81%) cases of
atypical lymphocytic effusions. IgH/PAGE and TCR-[gamma]/SSCP analyses are useful tools in
the diagnoses of lymphocytic effusions in patients with NHL.

chronic villitis by polymerase chain reaction." Human Pathology 25(8): 815.

http://www.sciencedirect.com/science/article/B6WGD-4C2RX5R-P1/2/0db58deb0fa373c95cd6fa98ee8dd673

Placental chronic villitis was observed in 44 cases (2.12%) of 2,073 histologically examined
placentas. Infiltrating lymphocytes in chronic villitis were determined by immunohistochemistry to
be predominantly helper/inducer T cells. Detection of the cytomegalovirus (CMV) gene was
performed on paraffin-embedded sections by polymerase chain reaction (PCR) using two
different primers (CMV immediate early gene and CMV late antigen gp 64). Both CMV immediate
eye gene and late antigen gp 64 gene were detected in one case. Cytomegalovirus late antigen
gp 64 gene was observed in only three cases. Among these four cases, the cytomegalic inclusion
body was observed only in a single case with light microscopic examination. In two cases
generalized CMV infection was manifested during the early infantile period and the patient died of
the disease. The other two cases were asymptomatic. Our data suggest that approximately 9% of
the cases of chronic villitis are caused by CMV infection, and most of them are difficult to detect
morphologically. Detection of CMV gene by PCR using primers, especially late antigen gp 64
gene, is very useful for assessing the cause of placental chronic villitis.

with lymphomatous potential." Human Pathology 34(6): 617.

http://www.sciencedirect.com/science/article/B6WGD-48VN45R-V/2/46313b84a92efed4e230d68b7986b7f5

Cutaneous lymphoid hyperplasia (CLH) has been proposed to be the benign end of a continuum
of lymphoproliferative disorders with cutaneous lymphoma at its malignant extreme. An
intermediate condition, known as "clonal CLH," was first recognized by us and shown to be a
transitional state capable of eventuating in overt lymphoma. To better determine the prevalence
of dominant clonality and risk of lymphoma among CLH cases, we studied the immunohistology
and clonality of fresh-frozen samples from 44 CLH patients referred to a multidisciplinary
cutaneous lymphoproliferative disorders program. Using a large panel of lymphoid markers, the
cases were divided into 38 typical mixed B-cell/T-cell type CLH and 6 T-cell-rich type (T-CLH),
the latter containing > 90% T cells. Of the 44 patients, 38 had solitary or localized lesions (4
cases of T-CLH), and 6 had regional/generalized lesions (2 cases of T-CLH). Forty cases were of
idiopathic etiology. Suspected etiologies among 4 other cases included mercuric tattoo pigment,
doxepin, clozapine, and bacterial infection. Immunoglobulin heavy chain (IgH) and T-cell receptor
(TCR)-gamma gene rearrangements (GR) were studied using polymerase chain reaction assays,
which are approximately 80% sensitive. Overall, 27 cases (61%) showed clonal CLH: 12 IgH+
(27%; 3 cases of T-CLH); 13 TCR+ (30%; 1 case of T-CLH); and 2 IgH+/TCR+ (4%; neither case
was T-CLH). Two cases (4%; 1 case of T-CLH) progressed to cutaneous B-cell lymphoma. Both
of these patients presented with regional lesions. Our findings indicate that clonal overgrowth is
common in CLH, links CLH to lymphoma, and probably involves both B- and T-cell lineages
(although TCR GR by B cells and vice versa could not be ruled out). The high prevalence
of dominant clonality in our series may have resulted from the sensitivity of our PCR assays as well
as patient selection.
Strong epidemiological evidence links human papilloma viruses (HPV) with the development of cervical intraepithelial neoplasia (CIN) and invasive cancers of the uterine cervix. The localization of HPV DNA sequences high up in the female genital tract (in benign and malignant lesions) is not that uncommon, but its precise significance is uncertain. In particular, the detection of HPV DNA sequences by polymerase chain reaction (PCR) needs careful interpretation, because the source of the amplicon may emanate from tumor cells, direct contamination from the cervix, or possibly from extratumoral sites in the endometrium. We have previously reported the identification of koilocyte-like changes in the squamous epithelium of some endometrial adenoacanthomas. Adenoacanthomas (adenocarcinoma with squamous metaplasia) are mixed epithelial tumors arising in the endometrium composed of malignant glandular areas admixed with benign metaplastic squamous epithelium. The rarer adenosquamous carcinoma containing both malignant glandular and squamous areas is also described. The origin of benign/malignant squamous epithelial islands in endometrial tumors has been the subject of speculation, with some investigators considering an origin from metaplastic glandular endometrial cells. In this study, we examined 10 normal endometrial samples, 20 adenocarcinomas, 41 adenocarcinomas with squamous metaplasia, and two adenosquamous carcinomas, (including control cervical material where possible) for the presence of HPV DNA sequences using nonisotopic in situ hybridization (NISH), type-specific HPV PCR, general primer PCR (to detect sequenced and unsequenced HPVs), and PCR in situ hybridization (PCR-ISH). We did not identify HPV DNA sequences in normal endometrial tissue. In adenocarcinomas (endometrioid type), HPV was only identified in 2 of 20 cases by PCR, both of which were HPV 11 positive. We were unsuccessful in identifying HPV in endometrial carcinomas by NISH or by PCR-ISH, raising the possibility of contamination from the cervix in the two positive cases. In adenoacanthomas, a low-risk HPV type (HPV 6) was found in 19 of 41 cases. NISH signals were intranuclear in location in squamous regions of adenoacanthomas. Additional positive nuclei were uncovered using PCR-ISH, which increases the sensitivity of standard NISH detection. HPV DNA sequences were located in some malignant endometrial glandular epithelial cells, but this accounted for a minority of samples. HPV DNA sequences were not detected in extratubular sites. Mixed infection by two different HPV types was identified in two cases. Most cases showed similar HPV types in cervical and endometrial lesions, although discordant cases were uncovered. In adenosquamous carcinomas, one case showed mixed infection with HPV 6 and 33 by PCR. The apparent segregation of low-risk HPV type (HPV 6) with benign squamous metaplastic epithelium in adenocarcinoma with squamous metaplasia, and high-risk type (HPV 33) with malignant squamous epithelium in adenosquamous carcinoma, raises important questions in relation to the role of HPVs in mixed epithelial tumors of the endometrium and their interplay in the pathogenesis of squamous metaplasia at extracervical sites.
changes underlying the progression from APA to adenocarcinoma are unknown. DNA from paraffin-embedded tissue of 6 APAs was evaluated for microsatellite instability (MI), MLH-1 promoter hypermethylation, and CTNNB-1 mutations. Tissue sections were also subjected to MLH-1, MSH-2, and [beta]-catenin immunostaining. MI was not detected in any case. Two tumors exhibited MLH-1 promoter hypermethylation and showed focal negative MHL-1 immunostaining; 1 of these showed marked architectural complexity and cellular pleomorphism. Five cases presented [beta]-catenin nuclear immunoreactivity, but none of them had CTNNB-1 mutations. The results of this study suggest that APA and complex endometrial hyperplasia may share some molecular alterations. Some APAs exhibit MLH-1 promoter hypermethylation with focal lack of MLH-1 immunostaining, a molecular abnormality involved in the transition from complex atypical hyperplasia to endometrioid adenocarcinoma.


http://www.sciencedirect.com/science/article/B6WGD-4F4H9PP-1/2/57d5fec64763145ee6f678397b4ef2c1

SummarySupratentorial primitive neuroectodermal tumors (SPNETs) and medulloblastomas (MBs) are histologically similar intracranial tumors found in different anatomic locations of the brain. Our group has previously demonstrated that loss of chromosome 8p is a frequent event in MBs. The aim of this study was to evaluate whether DLC-1, a newly identified tumor-suppressor gene on chromosome 8p22, is involved in the tumorigenesis of MBs and the histologically similar SPNETs. We first assessed for alterations of gene expression in microdissected tumors and detected lack of DLC-1 transcript in 1 of 9 MBs (case M44) and 1 of 3 SPNETs (case M1). Neither somatic base substitutions nor homozygous deletion were found in tumors without DLC-1 transcript. We then explored the possibility of hypermethylation of the CpG island in DLC-1 as the mechanism of suppressed expression. Methylation-specific polymerase chain reaction revealed promoter hypermethylation of DLC-1 in M1 but not in M44. Bisulfite sequencing further verified a densely methylated pattern of 35 CpG sites studied in M1 that were not found in normal brain, indicating that inactivation of DLC-1 by hypermethylation is involved in SPNET. Based on this finding, we examined an additional 20 MBs, 8 SPNETs, and 4 MB and 2 SPNET cell lines for hypermethylation of the CpG island of DLC-1, finding that none of these samples exhibited DLC-1 methylation. In conclusion, our results demonstrate that transcriptional silencing of DLC-1 through promoter hypermethylation may contribute to tumorigenesis in a subset of SPNETs, and that loss of DLC-1 expression in MBs may be related to mechanisms other than promoter hypermethylation, genomic deletion, and mutation.


http://www.sciencedirect.com/science/article/B6WGD-4BHSXSD-H/2/1a8e53a77a91483359df6f2ed1401261

Previous studies have established that expression of plakoglobin is down-regulated during malignant transformation. The aim of this study was to evaluate for the first time the expression of plakoglobin at the mRNA and protein levels in primary oropharyngeal squamous cell carcinomas (SCCs) and determine the extent to which the patterns of expression correlated with clinical parameters. Plakoglobin expression was evaluated in 37 new tumor cases and normal oral epithelium using immunofluorescence, reverse transcriptase-polymerase chain reaction (RT-PCR), and Northern and Western blotting analysis. The results indicated that the steady-state
levels of plakoglobin protein were down-regulated in all tumors compared with normal epithelium. Furthermore, in 87.1% of the tumors, plakoglobin immunoreactivity displayed an abnormal cytoplasmic localization that was inversely correlated with tumor size and directly correlated with a poor clinical outcome for the patient. Northern blotting analysis revealed that down-regulation of mRNA expression occurred in only 65.6% of the tumors, with plakoglobin mRNA levels similar to normal epithelium in the remaining cases. In the tumors expressing mRNA levels similar to those of normal tissue, a 3.7-kb transcript was detected in addition to the expected 3.4-kb transcript observed in normal epithelium. RT-PCR analysis of the 3’ untranslated region of the 3.7-kb plakoglobin mRNA transcript identified a 297-base insertion from +2369 to +2666 that had been previously reported only in transformed cell lines (GenBank M23410). Interestingly, the prognosis was poor for patients with tumors expressing both RNA transcripts. These results are consistent with the concept that complex regulation of plakoglobin expression and intracellular routing may contribute to malignant transformation. The study also shows evidence that the level of expression and intracellular localization of plakoglobin may be useful in predicting the course of disease in patients with oropharyngeal SCC.


Renal angiomyolipomas are benign tumors composed of variable amounts of mature fat, smooth muscle, and thick-walled blood vessels. They occur either sporadically or in association with tuberous sclerosis. Such tumors are considered as hamartomas, but few data are available concerning their pathogenesis. Indeed, it is not known whether angiomyolipoma is a congenital malformation or a neoplastic process. To answer this question, we assessed the clonality of sporadic angiomyolipomas using molecular analysis. Seven women (mean age, 59 years) with renal angiomyolipomas were included. DNA of the tumor and the normal adjacent kidney was extracted from archival paraffin-embedded tissue. DNA methylation pattern at a polymorphic site on the HUMARA gene was studied by polymerase chain reaction (PCR) amplification after methylation-sensitive enzyme digestion. This procedure enables the differentiation between polyclonal and monoclonal lesions according to their X-chromosome inactivation pattern. Five of the seven women included were informative for the HUMARA gene. The mean size of the angiomyolipomas was 53 mm (range, 18 to 110). In one case, a tumor thrombus was observed in the inferior vena cava. Clonal analysis showed that all of the angiomyolipomas and the tumor thrombus studied were monoclonal. This study shows that sporadic angiomyolipomas are monoclonal lesions consistent with neoplastic disorders. This result strongly supports the hypothesis that angiomyolipomas arise from the donal proliferation of an uncommited cell, which will further evolve toward different cell types.


Epithelial tumors of the ovary are the most common ovarian tumors of adult women. They exist in several different histological patterns and exhibit varying degrees of aggressiveness. Molecular genetic studies in epithelial ovarian cancer have shown that loss of heterozygosity (LOH) for regions of chromosome 17 is a common event, probably reflecting the inactivation of one or more tumor suppressor genes present on this chromosome. We examined 87 sporadic epithelial
ovarian tumors of different grade and histological type at 16 loci on this chromosome and found that 35% of them showed LOH for chromosome 17. Of these, 84% showed LOH for all informative markers, suggesting that loss of the entire chromosome 17 homologue may have occurred. Interestingly, chromosome 17 loss was observed frequently in serous tumors (49%), was less common in endometrioid tumors (15%), and was rare in mucinous tumors (4%) (P = .01 and P = .0002, respectively). Our findings support the concept that the histological subtypes of epithelial ovarian cancer may be the result of different molecular genetic events.


http://www.sciencedirect.com/science/article/B6WGD-4D04T5C-22/2/441b6f1f2683d7881bff7d21f5776975

In 11 of 35 clinically proven cases of sarcoidosis, we detected DNA sequences coding for the mycobacterial 65-kDa antigen. In four cases, the sequences were homologous to Mycobacterium avium; seven sequences were related to other nontuberculous Mycobacteria. The insertion sequence 1110, characteristic for Mycobacterium avium, was present in three cases. The insertion sequence 6110 of the Mycobacterium tuberculosis complex (M. tuberculosis, africanum, bovis, BCG) was not detectable in any of the 11 cases, ruling out the presence of members of the Mycobacterium tuberculosis complex. Therefore, it seems reasonable to speculate about a mycobacterial cause in some cases of sarcoidosis.


http://www.sciencedirect.com/science/article/B6WGD-45SJ0P-82/2/f9960598494692cc20ce9db47b8c4212

A monoclonal proliferation of germinal center cells within a lymph node follicle was incidentally discovered during the staging surgical procedures in a patient with Clark III-level cutaneous melanoma. In one of the 19 axillary lymph nodes examined, we identified a single morphologically atypical lymphoid follicle, predominantly composed of medium-sized cells and immunoreactive for B-cell antigens and for the markers of germinal center origin CD10 and bcl-6. A monoclonal rearrangement of the immunoglobulins heavy chains (IgH) was documented by polymerase chain reaction after laser capture microdissection. The cells of the aberrant follicle expressed the bcl-2 protein at higher levels than the surrounding T lymphocytes in the absence of bcl-2 gene rearrangement. We propose for this lesion the designation of incipient follicular lymphoma. The present findings also confirm the previously reported association between melanoma and lymphoproliferative disorders. HP32:1410-1413. Copyright (c) 2001 by W.B. Saunders Company


http://www.sciencedirect.com/science/article/B6WGD-4D04T0F-H/2/d68131ace8cf34ea33bad9f9ae22b9018

Serous surface carcinoma (SSC) is a neoplasm histologically indistinguishable from typical
serous carcinomas that arise from the ovary but has a distinct clinical presentation. It is characterized by widespread peritoneal dissemination at presentation, but the ovaries are grossly normal in size and shape. If the carcinoma involves the ovaries microscopically, the tumor is confined to the surface or is minimally invasive. The recognition of this entity is important, because in some studies it appears to have a poorer prognosis than stage-matched serous cancers of the ovary. Loss of heterozygosity (LOH) of the p53 (17p) and BRCA1 (17q) tumor suppressor genes has been frequently identified in sporadic ovarian carcinomas. Although 17p LOH is correlated with common p53 gene mutations, inactivating mutations of the BRCA1 gene are uncommon in sporadic ovarian cases. In contrast, germline BRCA1 mutations are responsible for some hereditary forms of ovarian cancer, where it has been suggested that germline BRCA1 mutations confer a more favorable prognosis. In this study, 12 sporadic SSC were assessed for the presence of allelic deletions on the p53 and BRCA1 gene loci. DNA from both tumor and normal cells was obtained for LOH studies using tissue microdissection. Polymerase chain reaction (PCR) amplification was performed with the polymorphic DNA markers TP53 (17p13.1/p53 gene) and D17S579 (17q/BRCA1 gene). LOH in the p53 and BRCA1 loci was detected in 62.5% and 66.6% of the cases, respectively. In 50% of tumors informative for both markers, it is possible that an entire chromosome may be lost. In conclusion, we have shown that LOH of the p53 and BRCA1 loci is a frequent event in sporadic SSC, similar to what has been described in the usual form of serous ovarian carcinoma. Mutational analysis will be necessary to determine the exact role of these genes in this group of tumors.


Dysplasia in Barrett's esophagus (BE) is a precursor to adenocarcinoma and most commonly occurs as a flat, grossly undetectable lesion. Rarely, dysplasia in BE may grow as a polypoid lesion. Most BE-associated polypoid dysplastic lesions have been referred to as "adenomas" because of their histological similarity to a colonic adenoma. BE-associated polypoid dysplastic lesions have been less well characterized than the flat type. Therefore, our aim was to characterize the clinicopathologic and molecular features of five cases of BE-associated polypoid dysplasia and to review the literature on this entity. The cases were evaluated clinically, histologically, immunostained for MIB-1 and p53, and genotyped for loss of heterozygosity (LOH) at the adenomatous polyposis coli (APC) locus. Mucosal biopsy specimens of five BE patients without dysplasia, and five BE cases with high-grade flat dysplasia, were used as controls. The study patients were all male (average age, 71 years) who presented with symptoms of gastroesophageal reflux disease. Endoscopically, all five cases had a well-defined sessile or pedunculated polypoid lesion ranging from 0.4 to 1.5 cm in size in the mid (n = 1) or distal (n = 4) esophagus and were associated with specialized-type BE (four long segment, one short segment). Histologically, the polyps consisted of intestinalized epithelium with low- and high-grade dysplasia. All five cases contained adenocarcinoma (four within the polyp, one in adjacent BE). All polyps showed increased cell proliferation in the form of surface MIB-1 staining and showed positive p53 staining. Three of three (100%) informative cases showed LOH at the APC locus in the dysplastic epithelium and in areas of adenocarcinoma. All five flat dysplasia controls also showed surface MIB-1 staining and p53 positivity, and three of three informative controls showed LOH for APC. None of the nondysplastic BE controls showed any of these findings. Three patients were treated with esophagectomy and two with polypectomy. All were alive, without metastasis, from 2 months to 6 years later. A literature review of esophageal "adenomas" uncovered 12 cases. Four of these had no clinical or pathological information, two were, in fact, gastric heterotopic lesions, one was composed entirely of intestinal-type epithelium, and five were polypoid dysplastic lesions similar to the cases described here (three male, two female; mean age, 59 years). Four of these five cases were associated with adenocarcinoma in the polyp (two
intramucosal, two submucosal). In summary, BE-associated polypoid dysplasia share similar clinical, pathological, and molecular features as flat dysplasia and are often associated with adenocarcinoma. Thus, we agree with other authors who recommend that the term adenoma, which usually carries a benign connotation, be abandoned in favor of a descriptive diagnostic term, such as "BE-associated polypoid dysplasia." BE patients with this lesion should be considered strong candidates for esophageal resection similar to lesions of this kind that occur in inflammatory bowel disease.


http://www.sciencedirect.com/science/article/B6WGD-4D04PBD-5B/2/89c2fac9ff23a8939c534e7ee0eda20

Endometrial carcinoma is the most common invasive malignancy of the female genital tract, and it exists as two different clinicopathologic forms: an estrogen-dependent, "usual" type and an estrogen-independent "special variant" type. Despite the frequency of endometrial cancer, little is known about the molecular genetic events that contribute to its pathogenesis. The accumulation of genetic alterations identified through the study of loss of heterozygosity (LOH), gene mutation, and gene activation in tumor DNA has been associated with the establishment and progression of a variety of human malignancies. A relatively low incidence of LOH has been reported in usual type endometrial cancers; however, special variant tumors have rarely been included in the reported studies. To understand the molecular events that contribute to both forms of endometrial cancer, 31 tumors have been surveyed for events of LOH on all chromosomes. The study groups included 18 tumors of the usual type and 13 special variant tumors. Polymorphic loci were studied by Southern blot analysis and polymerase chain reaction (PCR) of microsatellite loci. Normal tissue in each case served as a control. Both frequency and patterns of LOH differed greatly between the two tumor types. Although LOH was frequently detected in the special variant tumors, it was rare in the usual type tumors. LOH was detected in only 8 of the 18 usual tumors, with chromosomes 17, 13, and 2 being the most frequently affected (22%, 20%, and 19%, respectively). In contrast, LOH was detected in all cases of special variant tumors, with chromosomes 17p, 14, and 12 showing the highest LOH (83%, 77%, and 40%, respectively). Two cases of microsatellite instability (MI) were detected among the usual type tumors. These findings suggest that the clinicopathologic phenotypes observed in these tumor types are likely caused by different tumorigenic pathways that reflect alterations of different cancer-controlling genes.


http://www.sciencedirect.com/science/article/B6WGD-4CWS14K-1V/2/10df7fd9e1dd1f18f8970c6a9f36f59d

Abnormalities in the p16INK4A, CDK4, and Rb genes, which regulate transition through G1 phase of the cell cycle, have been implicated in the progression of diverse types of cancer. To evaluate the involvement of p16INK4A, CDK4, and Rb in the tumorigenesis of meningiomas, the status of these genes or gene products were examined. The genetic alteration of the p16INK4A gene was examined by homozygous deletions and by mutation analysis. The methylation status of the p16INK4A was determined by Southern blotting. Neither homozygous deletions nor point mutations of the p16INK4A gene were observed in any of the 23 meningiomas. Partial rather than complete methylation of the p16INK4A gene at SacII or SmaI sites was shown in five (23.8%) meningiomas. The methylation status of the p16INK4A gene was not consistently associated with
the expression of pl6INK4A in meningiomas. These results suggest that the true relationship between methylation and expression of pl6INK4A may be obscured in a complex manner by various mechanisms that regulate pl6INK4A expression. Aberrant expressions of pRb and CDK4 were not observed in any of the meningiomas we examined, indicating that abnormalities of the pRb and CDK4 appear to be rare in meningiomas.

Tzen, C.-Y., Y.-W. Huang, et al. (2003). "Is atypical follicular adenoma of the thyroid a preinvasive malignancy?" Human Pathology 34(7): 666.

Among the follicular neoplasms of the thyroid, the definition and nature of atypical adenoma have been confusing. Despite the original speculation about the biologic behavior of preinvasive malignancies, this term is currently used as an expression of uncertainty. To examine the molecular features of a typical adenoma, we analyzed the p53 genes in 2 atypical adenomas and 12 control lesions (6 typical follicular adenomas and 6 follicular carcinomas). Mutations of p53 were detected in the bizarre cells of the atypical adenomas, but not in the bland-looking follicular cells or in the control specimens. Both atypical adenomas showed an identical point mutation in codon 273 (CGT->CAT), a common mutation in various human cancers, including anaplastic carcinoma of the thyroid. This finding supports the view that atypical follicular adenoma is a precursor of thyroid anaplastic carcinoma and suggests that "atypical adenoma" should not be used to express diagnostic uncertainty about the nature of a lesion.


The vast majority of in situ breast cancers represent focal lesions all derived from a single clone and requiring local treatment alone. We focused our attention on rare cases of multicentric in situ carcinomas affecting different quadrants, which required mastectomy. Defining the origin from single- or multiple-cell clones of separate independent neoplastic foci in the breast may be of pathogenetic interest and of importance in deciding the type of therapy to be administered. We employed a molecular assay based on loss of heterozygosity (LOH) and human androgen receptor assay (HUMARA) analysis of microdissected samples from 19 mastectomies. Two or more tissue samples were obtained from 7 patients with multicentric lobular in situ carcinoma (LCIS), either classical or large-cell variety; and 12 patients with multicentric ductal in situ carcinomas (DCIS), either low-grade (7 cases) or high-grade (5 cases) variety. Separate foci of high-grade (comedonic) DCIS were found to be monoclonal in nature. On the contrary, definite evidence favoring the origin from different cell clones of separate carcinomatous foci within the same breast was obtained in 2 cases of low-grade DCIS and in 6 cases of LCIS. A genetic imbalance might be the factor favoring the development of multifocal heterogeneous foci of in situ breast cancer. Such a small subgroup of in situ cancers affecting diffusely the entire breast and originating from independent foci presents both clinical and pathogenetic interest.

Until now, no definitive molecular evidence proving or disproving a true progression from superficial to invasive bladder tumors has been reported. A total of 36 lesions from 6 patients affected by invasive bladder cancer after multiple superficial recurrences were analyzed for loss of heterozygosity on 8 loci of chromosome 9 and 2 loci of chromosome 17. In addition, the clonal composition of the tumors from two female patients was examined using the human androgen receptor assay. Our data suggest that papillary bladder lesions can and sometimes do make a true progression into invasive life-threatening tumors; however, this progression is not an invariable sequence because it was definitely proven in 2 but not confirmed in 3 of the cases we examined.


Polymerase chain reaction (PCR) is being increasingly used in clinical laboratories for the diagnosis of human papillomavirus. From the L1 region, there are two commonly used consensus primer systems designated CP5+/G6+ and MY09/MY11. Both detect a wide variety of human papillomaviruses (HPVs). In this investigation, the authors compared the sensitivity of these approaches with the modification of hot-start PCR on 148 neutral-buffered formaldehyde-fixed cervical biopsies classified as cervical intraepithelial neoplasia (CIN) I to III. The authors chose hot-start PCR because in a previous study it proved more sensitive than cold-start PCR. Furthermore, the authors combined GP5+/GP6+ and MY09/MY11 in a two-step amplification (nested PCR) to analyze further those cases that proved negative with either GP5+/GP6+ or MY09/MY11. The authors found that the two consensus primer systems were equally sensitive with a correlation of 98%. By using GP5+/GP6+, the authors achieved an HPV positivity rate of 95% and with MY09/MY11 94%. Nested PCR did not improve HPV positivity in the CINs included in this study.

initial 310-bp polymerase chain reaction product that included the angiotensin type-2 (-1332 G/A) locus. The mean LV mass index for the male patients was 94.3{+/-}19.6 g/m2 (n=125) and for the female patients was 71.2{+/-}12.0 g/m2 (n=72). Seventy-three (37.1%) of all patients had an elevated LV mass index, defined as the mean LV mass index for normal volunteers plus 2 SD (males 77.8{+/-}9.1 g/m2, n=30; females 61.5{+/-}7.5g/m2, n=30). Comparison of LV mass index of the A_/AA genotype (mean LV mass index=82.4{+/-}21.1 g/m2; n=123) against that of the G_/GG genotype (mean LV mass index=88.1{+/-}19.0 g/m2; n=89) as a continuous variable was significant by ANOVA (P=0.044). \( \chi^2 \) Comparison between subjects with and subjects without left ventricular hypertrophy revealed an excess of the G_/GG genotype among the group with LV hypertrophy (P=0.031). We observed an association between the angiotensin type-2 receptor (-1332 G) allele and the presence of left ventricular hypertrophy in hypertensive subjects.


http://hyper.ahajournals.org/cgi/content/abstract/41/3/414

Obesity is associated with volumetric arterial hypertension and with early increase in heart rate and decreased heart rate variability. The consequences of obesity-related hypertension on heart gene regulation are poorly known and were investigated in a model of obesity-related hypertension induced by high fat diet in dogs. When compared with control animals (n=6), a 9-week high fat diet (n=6) provoked significant weight gain and increased blood pressure load and heart rate but failed to significantly change left ventricular mass assessed by echocardiography. Subtractive hybridization of dog heart cDNA libraries were used to generate sublibraries containing differentially expressed cDNAs that were in turn spotted onto membranes to create custom microarrays. Hybridizations of these microarrays with complex probes representing mRNAs expressed in right atria and left ventricles from obese hypertensive and control dogs were performed. Thirty-eight differentially expressed genes were identified; altered expression was confirmed by Northern blot analysis in 15. In addition, real-time quantitative polymerase chain reaction confirmed differential expression for 80% of the randomly chosen tested genes. Once identified, transcripts were categorized into groups involved in metabolism, cell signaling, ionic regulation, cell proliferation, protein synthesis, and tissue remodeling. In addition, we found a set of 11 cDNAs encoding proteins with unknown functions. This study clearly shows that obesity-related hypertension, lasting for only 9 weeks, causes marked changes in gene expression in right atrium as well as the left ventricle that may contribute to early functional changes in heart function and to long-term structural changes such as left ventricular hypertrophy and remodeling.

Immunity (22)


http://www.sciencedirect.com/science/article/B6WSP-44JHVW4-B/2/8f00d1e2e67e3f3545b130c7494120a7

The major murine systemic lupus erythematosus (SLE) susceptibility locus, Sle1, corresponds to
three loci independently affecting loss of tolerance to chromatin in the NZM2410 mouse. The congenic interval corresponding to Sle1c contains Cr2, which encodes complement receptors 1 and 2 (CR1/CR2, CD35/CD21). NZM2410/NZW Cr2 exhibits a single nucleotide polymorphism that introduces a novel glycosylation site, resulting in higher molecular weight proteins. This polymorphism, located in the C3d binding domain, reduces ligand binding and receptor-mediated cell signaling. Molecular modeling based on the recently solved CR2 structure in complex with C3d reveals that this glycosylation interferes with receptor dimerization. These data demonstrate a functionally significant phenotype for the NZM2410 Cr2 allele and strongly support its role as a lupus susceptibility gene.


Contact sensitivity responses require both effective immune sensitization following cutaneous exposure to chemical haptens and antigen-specific elicitation of inflammation upon subsequent hapten challenge. We report that antigen-independent effects of IgE antibodies can promote immune sensitization to haptens in the skin. Contact sensitivity was markedly impaired in IgE-/- mice but was restored by either transfer of sensitized cells from wild-type mice or administration of hapten-irrelevant IgE before sensitization. Moreover, IgE-/- mice exhibited impairment in the reduction of dendritic cell numbers in the epidermis after hapten exposure. Monomeric IgE has been reported to influence mast cell function. We observed diminished contact sensitivity in mice lacking Fc[epsilon]RI or mast cells, and mRNA for several mast cell-associated genes was reduced in IgE-/- versus wild-type skin after hapten exposure. We speculate that levels of IgE normally present in mice favor immune sensitization via antigen-independent but Fc[epsilon]RI-dependent effects on mast cells.


Here we describe a family of GPI-anchored cell surface proteins that function as ligands for the mouse activating NKG2D receptor. These molecules are encoded by the retinoic acid early inducible (RAE-1) and H60 minor histocompatibility antigen genes on mouse chromosome 10 and show weak homology with MHC class I. Expression of the NKG2D ligands is low or absent on normal, adult tissues; however, they are constitutively expressed on some tumors and upregulated by retinoic acid. Ectopic expression of RAE-1 and H60 confers target susceptibility to NK cell attack. These studies identify a family of ligands for the activating NKG2D receptor on NK and T cells, which may play an important role in innate and adaptive immunity.

Cyclophilin A (CypA/Ppia) is a peptidyl-prolyl isomerase (PPIase) that binds the immunosuppressive drug cyclosporine. The resulting complex blocks T cell function by inhibiting the calcium-dependent phosphatase calcineurin. To identify the native function of CypA, long suspected of regulating signal transduction, we generated mice lacking the Ppia gene. These animals develop allergic disease, with elevated IgE and tissue infiltration by mast cells and eosinophils, that is driven by CD4+ T helper type II (Th2) cytokines. Ppia-/- Th2 cells were hypersensitive to TCR stimulation, a phenotype consistent with increased activity of Itk, a Tec family tyrosine kinase crucial for Th2 responses. CypA bound Itk via the PPIase active site. Mutation of a conformationally heterogeneous proline in the SH2 domain of Itk disrupted interaction with CypA and specifically increased Th2 cytokine production from wild-type CD4+ T cells. Thus, CypA inhibits CD4+ T cell signal transduction in the absence of cyclosporine via a regulatory proline residue in Itk.


http://www.sciencedirect.com/science/article/B6WSP-41BD8FH-7/2/fedeaac147760eed5a9662650fc28ad0

The 2C transgenic TCR is positively selected on Kb and is alloreactive for and negatively selected on Ld. To test an avidity model for positive selection, mice were bred to express different levels of surface Ld by varying the number of gene copies encoding [beta]2- microglobulin ([beta]2m) or Ld heavy chain. Whereas mice expressing 35% Ld ([beta]2m+/+ Ld+/+) negatively selected the 2C TCR, mice expressing 2% Ld ([beta]2m-/- Ld+/-) positively selected the 2C TCR. Furthermore, 2C cytotoxic T lymphocytes selected on 2% Ld showed peptide-specific cytolytic activity against Ld/p2Ca targets. These findings provide clear in vivo evidence that positive selection can occur on very low levels of the same class I antigen capable of negative selection when expressed at higher levels.


http://www.sciencedirect.com/science/article/B6WSP-433W6T5-7/2/93e74a015904d7bb60f1fc9a03e415b3

Homing behavior and function of autoimmune CD4+ T cells in vivo was analyzed before and during EAE, using MBP-specific T cells retrovirally engineered to express the gene of green fluorescent protein. The cells migrate from parathyrmic lymph nodes to blood and to the spleen. Preceding disease onset, large numbers of effector cells invade the CNS, with only negligible numbers left in the periphery. In early EAE, most (>90%) infiltrating CD4+ cells were effector cells. Migratory effector cells downregulate activation markers (CD25, OX-40) but upregulate several chemokine receptors and adsorb MHC class II on their membranes. Within the CNS, the effector cells are reactivated, with upregulated proinflammatory cytokines and downmodulated T cell receptor-associated structures, presumably reflecting autoantigen recognition in situ.

We have characterized a cytokine produced by Th2 cells, designated as IL-25. Infusion of mice with IL-25 induced IL-4, IL-5, and IL-13 gene expression. The induction of these cytokines resulted in Th2-like responses marked by increased serum IgE, IgG1, and IgA levels, blood eosinophilia, and pathological changes in the lungs and digestive tract that included eosinophilic infiltrates, increased mucus production, and epithelial cell hyperplasia/hyper trophy. In addition, our studies show that IL-25 induces Th2-type cytokine production by accessory cells that are MHC class II high, CD11c dull, and lineage−. These results suggest that IL-25, derived from Th2 T cells, is capable of amplifying allergic type inflammatory responses by its actions on other cell types.


http://www.sciencedirect.com/science/article/B6WSP-4194TJC-C/2/76cef6421af9e05b3408ff67a870f007

The constitutive and cytokine-induced levels of major histocompatibility (MHC) class I expression are tightly controlled at the transcriptional level. In this study, it is shown that the cis-acting regulatory element site [alpha] of the MHC class I promoter is essential for the IFN[gamma]-induced transactivation of MHC class I gene expression through the ISRE. Moreover, it was discovered that the class II transactivator (CIITA), which is itself under the control of the IFN[gamma] induction pathway, strongly transactivates MHC class I gene expression and exerts its activity through site [alpha]. Therefore, site [alpha] is a crucial regulatory element, mediating the classic route of IFN[gamma] induction via the ISRE as well as a novel route of MHC class I transactivation involving CIITA.


http://www.sciencedirect.com/science/article/B6WSP-41CR732-6/2/c4c746f671b9afe40fc505135a7a041c

We generated mice harboring germline mutations in which the enhancer element located 9 kb 3' of the immunoglobulin [kappa] light chain gene (3'E[kappa]) was replaced either by a single loxP site (3'E[kappa][Delta]) or by a neomycin resistance gene (3'E[kappa]N). Mice homozygous for the 3'E[kappa][Delta] mutation had substantially reduced numbers of [kappa]-expressing B cells and increased numbers of [lambda]-expressing B cells accompanied by decreased [kappa] versus [lambda] gene rearrangement. In these mutant mice, [kappa] expression was reduced in resting B cells, but was normal in activated B cells. The homozygous 3'E[kappa]N mutation resulted in a similar but more pronounced phenotype. Both mutations acted in cis. These studies show that the 3'E[kappa] is critical for establishing the normal [kappa]/[lambda] ratio, but is not absolutely essential for [kappa] gene rearrangement or, surprisingly, for normal [kappa] expression in activated B cells. These studies also imply the existence of additional regulatory elements that have overlapping function with the 3'E[kappa] element.

http://www.sciencedirect.com/science/article/B6WSP-41BD45H-5/2/24d7236144c8dca27e0cc878aadb682

Families of clonally expressed major histocompatibility complex (MHC) class I-specific receptors provide specificity to and regulate the function of natural killer (NK) cells. One of these receptors, mouse Ly49A, is expressed by 20% of NK cells and inhibits the killing of H-2Dd but not Db-expressing target cells. Here, we show that the trans-acting factor TCF-1 binds to two sites in the Ly49A promoter and regulates its activity. Moreover, we find that TCF-1 determines the size of the Ly49A NK cell subset in vivo in a dosage-dependent manner. We propose that clonal Ly49A acquisition during NK cell development is regulated by TCF-1.


http://www.sciencedirect.com/science/article/B6WSP-41CR78M-9/2/b83b46db39dfd29aaf12ce8003d82d8

Melanoma lines MEL.A and MEL.B were derived from metastases removed from patient LB33 in 1988 and 1993, respectively. The MEL.A cells express several antigens recognized by autologous cytolytic T lymphocytes (CTL) on HLA class I molecules. The MEL.B cells have lost expression of all class I molecules except for HLA-A24. By stimulating autologous lymphocytes with MEL.B, we obtained an HLA-A24-restricted CTL clone that lysed these cells. A novel gene, PRAME, encodes the antigen. It is expressed in a large proportion of tumors and also in some normal tissues, albeit at a lower level. Surprisingly, the CTL failed to lyse MEL.A, even though these cells expressed the gene PRAME. The CTL expresses an NK inhibitory receptor that inhibits its lytic activity upon interaction with HLA-Cw7 molecules, which are present on MEL.A cells and not on MEL.B. Such CTL, active against tumor cells showing partial HLA loss, may constitute an intermediate line of anti-tumor defense between the CTL, which recognize highly specific tumor antigens, and the NK cells, which recognize HLA loss variants.


http://www.sciencedirect.com/science/article/B6WSP-41BD3W4-9/2/e2ba8a4019d9093fa2a636a2e9fa101f

Successful in-frame rearrangement of immunoglobulin heavy chain genes or T cell antigen receptor (TCR) [beta] chain genes in lymphocyte progenitors results in formation of pre-BCR and pre-TCR complexes. These complexes signal progenitor cells to mature, expand in cell number, and suppress further rearrangements at the immunoglobulin heavy chain or TCR[beta] chain loci, thereby ensuring allelic exclusion. We used transgenic expression of a constitutively active form of c-Raf-1 (Raf-CAAX) to demonstrate that activation of the Map kinase pathway can stimulate both maturation and expansion of B and T lymphocytes, even in the absence of pre-TCR or pre-BCR formation. However, the same Raf signal did not mediate allelic exclusion. We conclude that maturation of lymphocyte progenitors and allelic exclusion require distinct signals.

http://www.sciencedirect.com/science/article/B6WSP-4598W92-7/2/2f1abcfb730bed06a24ee2b59c554425

Notch1 signaling drives T cell development at the expense of B cell development from a common precursor, an effect that is dependent on a C-terminal Notch1 transcriptional activation domain. The function of Deltex1, initially identified as a positive modulator of Notch function in a genetic screen in Drosophila, is poorly understood. We now demonstrate that, in contrast to Notch1, enforced expression of Deltex1 in hematopoietic progenitors results in B cell development at the expense of T cell development in fetal thymic organ culture and in vivo. Consistent with these effects, Deltex1 antagonizes Notch1 signaling in transcriptional reporter assays by inhibiting coactivator recruitment. These data suggest that a balance of inductive Notch1 signals and inhibitory signals mediated through Deltex1 and other modulators regulate T-B lineage commitment.


http://www.sciencedirect.com/science/article/B6WSP-41BD623-4/2/1abe42d3df9e14ee5f8d891ded5315a6

The generation of an adaptive immune response against intracellular pathogens requires the recruitment of effector T cells to sites of infection. Here we show that the chemokine IP-10, a specific chemoattractant for activated T cells, controls this process in mice naturally infected with Toxoplasma gondii. Neutralization of IP-10 in infected mice inhibited the massive influx of T cells into tissues and impaired antigen-specific T cell effector functions. This resulted in >1000-fold increase in tissue parasite burden and a marked increase in mortality compared to control antibody-treated mice. These observations suggest that IP-10 may play a broader role in the localization and function of effector T cells at sites of Th1 inflammation.


http://www.sciencedirect.com/science/article/B6WSP-48M81Y9-5/2/160ca12b137662d01959cf7a243b5fdc

This study challenges the concept that herpes simplex virus type 1 (HSV-1) latency represents a silent infection that is ignored by the host immune system, and suggests antigen-directed retention of memory CD8+ T cells. CD8+ T cells specific for the immunodominant gB498-505 HSV-1 epitope are selectively retained in the ophthalmic branch of the latently infected trigeminal ganglion, where they acquire and maintain an activation phenotype and the capacity to produce IFN-[gamma]. Some CD8+ T cells showed TCR polarization to junctions with neurons. A gB498-505 peptide-specific CD8+ T cell clone can block HSV-1 reactivation from latency in ex vivo trigeminal ganglion cultures. We conclude that CD8+ T cells provide active surveillance of HSV-1 gene expression in latently infected sensory neurons.

The degree of heavy chain (H) editing, the types of V[kappa] editors, and the pattern of J[kappa] usage are correlated with a range of the affinity of anti-DNA. This range was determined by the number and location of arginine (R) residues in the VH. We, here, changed a key arginine residue in the VH of anti-DNA transgene to glycine, which sharply reduces the affinity for dsDNA. However, complete reversion of this anti-DNA to germline enhances the affinity for phosphatidylinerse (PS). The B cells of this low-affinity anti-DNA and anti-PS transgenic mouse are tightly regulated by receptor editing. Thus, anti-PS B cells are another example of a constitutive self-antigen regulated in the bone marrow.


Receptor editing is a means by which immature bone marrow B cells can become self-tolerant. Rearrangements of heavy (H) and/or light (L) chain genes are induced by encounter with autoantigens to change the specificity from self to nonself. We have developed site-directed transgenic mice (sd-tg) whose transgenes code for the H chain of antibodies that bind DNA. B cells that express the transgenic H chain associate mainly with four of the 93 functional V[kappa] genes of the mouse. Numerous aspartate residues that might inhibit DNA binding by the VH domain distinguish these L chain V[kappa] sequences, but engaging these V[kappa] editors often requires multiple rearrangements. Among the edited B cells is a subset of multispecific cells that express multiple receptors. One consequence of multispecificity is partial autoreactivity; these multispecific B cells may contribute to autoimmunity.


We have used gene-targeted mutation to assess the role of the T cell receptor [delta] (TCR[delta]) enhancer (E[delta]) in [alpha][beta] and [gamma][delta] T cell development. Mice lacking E[delta] exhibited no defects in [alpha][beta] T cell development but had a severe reduction in thymic and peripheral [gamma][delta] T cells and decreased VDJ[delta] rearrangements. Simultaneous deletion of both E[delta] and the TCR[alpha] enhancer (E[alpha]) demonstrated that residual TCR[delta] rearrangements were not driven by E[alpha], implicating additional elements in TCR[delta] locus accessibility. Surprisingly, while deletion of E[delta] severely impaired germline TCR[delta] expression in double-negative thymocytes, absence of E[delta] did not affect expression of mature [delta] transcripts in [gamma][delta] T cells. We conclude that E[delta] has an important role in TCR[delta] locus regulation at early, but not late, stages of [gamma][delta] T cell development.

We have used gene targeted mutational approaches to assess the role of the T cell receptor \(\alpha\) (TCR\(\alpha\)) enhancer \(E\alpha\) in the control of TCR\(\alpha\) and TCR\(\delta\) gene rearrangement and expression. We show that \(E\alpha\) functions in cis to promote V\(\alpha\) to J\(\alpha\) rearrangement across the entire J\(\alpha\) locus, a distance of greater than 70 kb. We also show that \(E\alpha\) is required for normal \(\alpha\beta\) T cell development; in this lineage, \(E\alpha\) is required for germline J\(\alpha\) expression, for normal expression levels of rearranged V\(\alpha\)J\(\alpha\) genes, and for expression of a diverse V\(\alpha\) repertoire. In \(\gamma\delta\) T cells, \(E\alpha\) is not required for V\(\delta\)DJ\(\delta\) rearrangement, but, surprisingly, is required for normal expression levels of mature V\(\delta\)DJ\(\delta\) transcripts and for expression of germline J\(\alpha\) transcripts. Our findings imply that \(E\alpha\) function is not limited to the TCR\(\alpha\) components of the TCR\(\alpha/\delta\) locus or to the \(\alpha\beta\) lineage; rather, \(E\alpha\) function is important in both \(\alpha\beta\) and \(\gamma\delta\) lineage T cells.


The presence and expression of killer inhibitory receptor (KIR) and CD94:NKG2 genes from 68 donors were analyzed using molecular typing techniques. The genes encoding CD94:NKG2 receptors were present in each person, but KIR gene possession varied. Most individuals expressed inhibitory KIR for the three well-defined HLA-B and -C ligands, but noninhibitory KIR genes were more variable. Twenty different KIR phenotypes were defined. Two groups of KIR haplotypes were distinguished and occurred at relatively even frequency. Group A KIR haplotypes consist of six genes: the main inhibitory KIR, one noninhibitory KIR, and a structurally divergent KIR. Allelic polymorphism within five KIR genes was detected. Group B comprises more noninhibitory KIR genes and contains at least one additional gene not represented in group A. The KIR locus therefore appears to be polygenic and polymorphic within the human population.


Somatic hypermutation (SHM) requires selective targeting of the mutational machinery to the variable region of the immunoglobulin heavy chain gene. The induction of SHM in the BL2 cell line upon costimulation is associated with hyperacetylation of the chromatin at the variable region but not at the constant region. The V region-restricted histone hyperacetylation resulting from costimulation occurs independent of AID expression and mutation. Interestingly, costimulation in the presence of Trichostatin A causes hyperacetylation of histones associated with the constant region and extends mutations to the constant region. Under this condition, promoter proximal mutations are observed in the variable region as well. The overexpression of AID results in a similar deregulation of mutational targeting. Our results indicate that the stimulation of SHM in BL2 cells activates two independent pathways resulting in histone modifications that permit induced levels of AID to selectively target the variable region for mutation.

http://www.sciencedirect.com/science/article/B6WSP-4C6TBJIH-S/2/78637d7e9b3ea787be71321ada95c9c3

We previously identified possible intermediates in V(D)J recombination at the TCR[delta] locus and characterized molecules with signal ends and with covalently sealed (hairpin) coding ends in thymocytes of scid mice by Southern blotting. Here, we use a sensitive ligation-mediated PCR assay to demonstrate that all coding ends detected in acid thymocytes are covalently sealed. Neither coding nor signal ends exhibit loss or addition of nucleotides. These data imply that hairpin formation is coupled to the initial cleavage at the signal/coding border, and that the cleavage step in V(D)J recombination is conservative. In scid/+ or wild-type thymocytes, hairpin coding ends are at least 1000-fold less abundant than signal ends. These results provide insight into the mechanism of V(D)J recombination.

Immunobiology (1)


http://www.sciencedirect.com/science/article/B7GW1-4DN9WTG-1/2/4bd44c28f7651ae6213cae4c0047a877

In bitches, the onset of pyometra, an infection of the uterus, characteristically occurs in the first half of the diestrous stage in the estrous cycle, in which the blood concentration of progesterone peaks and that of estradiol-17[beta] is lowest. To investigate the immunological mechanisms governing stage-specific onset of pyometra, peripheral blood mononuclear cells (PBMNCs) were collected from beagle bitches during different stages of the estrous cycle and examined using various immunological assays. When we examined the proliferative response of PBMNCs to PYO-252, that is a clone of Escherichia coli isolated from the uterus of a dog afflicted with pyometra, the response of PBMNCs significantly decreased in the first half (day 10) of diestrus, but increased in proestrus/estrus. No significant differences were observed in the responses to concanavaline A between stages of the cycle. Throughout the estrous cycle, canine PBMNCs did not respond to lipopolysaccharide derived from E. coli. The response of PBMNCs collected in anestrus to PYO-252 was significantly enhanced upon the addition of estradiol-17[beta] to the culture. In contrast, these responses were significantly suppressed in the presence of progesterone. Progesterone progenitor or metabolite molecules, which have a low affinity for the progesterone receptor, did not affect proliferative responses. Expression of gamma interferon (IFN[gamma]) in response to PYO-252 was also significantly enhanced by estradiol-17[beta], but suppressed by progesterone. This evidence suggests that in the first half of the diestrous stage, suppressed activity of cellular immunity results from increasing progesterone concentration and minimal estrogen release. This marked decrease of immune resistance allows the expansion of E. coli, which enter the uterine cavity through the loosened cervical canal during estrus, leading to pyometra onset.


Immune response against self antigens is normally prevented by an elaborate immunotolerance mechanism. A potential problem for recipients of gene therapy is, therefore, an immune response against the newly introduced gene product. To examine this issue we tested the immune response to the native proteins in knockout (KO) mice in which the genes for [alpha]A- or [alpha]B-crystallin were disrupted by partial or complete gene deletion, respectively. [alpha]A- and [alpha]B-crystallins are two immunologically distinct polypeptides which form the large (~800 kDa) complex in the lens referred to as [alpha]-crystallin. When immunized with murine [alpha]-crystallin, [alpha]B-crystallin KO mice, in which the corresponding gene was completely deleted, responded well to the absent self antigen. In contrast, [alpha]A-crystallin KO mice, with the partial gene deletion, resembled wild type (WT) mice in being immunotolerant toward the native crystallin. Although no functional [alpha]A-crystallin could be detected in the lens of [alpha]A-crystallin KO mice, mRNA transcript coding for a truncated [alpha]A-crystallin gene was found in thymi of these mice, suggesting that thymic expression of a residual fragment of the protein is responsible for the tolerance induction. These data suggest that nonfunctional proteins may induce immunotolerance and protect recipients of gene therapy from immunity against the native proteins.


http://www.sciencedirect.com/science/article/B6T75-44W458H-1/2/9e6905d20ad9a06aa63c140fba57c24f

We have shown that two of the matrix metalloproteinases (MMPs), matrilysin and stromelysin-1, are capable of cleaving all of the human IgG subclasses. The cleavage occurs at a conserved site in the CH2 domain of the heavy chain of IgG, releasing a single chain Fc-like fragment. We have not been able to demonstrate cleavage of IgA, IgD, IgM or IgE classes, which lack the cleavage site, nor could we show cleavage of IgG by collagenase, gelatinase, macrophage metalloelastase or membrane-type (MT)-MMP. This cleavage of IgG, by separating the antigen-binding (Fab')2 from the Fc portion, will remove much of the immunoglobulins' functionality, e.g. complement fixation, Fc receptor binding. In the context of a tumour producing matrilysin or stromelysin, this may represent a way in which the tumour protects itself from ADCC. In inflammed or damaged tissues where plasma protein leakage occurs, degradation by MMPs may be a mechanism for clearance of IgG.


http://www.sciencedirect.com/science/article/B6T75-48GVKTX-
Although [gamma][delta] T-cells form only a small portion of circulating T-cells in mice and humans, they are more frequent in many other types of mammals and this has led to speculation regarding their roles and the evolutionary significance of their relative abundance. Moreover, whilst clear homologues of four types of T-cell receptor (TCR) chains ([alpha], [beta], [delta] and [gamma]) have been identified in vertebrates as distantly related as eutherian mammals and cartilaginous fish, there are still many gaps in our knowledge of these TCR components from various taxa. Such knowledge would further illuminate the evolution and function of these receptors and of [gamma][delta] T-cells. Here, we report the molecular cloning of a TCR-[delta] chain cDNA from the tammar wallaby (Macropus eugenii) which represents the first component of the [gamma][delta] TCR to be characterised from a marsupial. A PCR-based survey of variable (V) segment usage in tammar wallaby mammary-associated lymph node indicated that, although [gamma][delta] T-cells may be sparse in this type of tissue, this species has at least three subfamilies of V genes that have been broadly conserved across vertebrate evolution. Two V subfamilies found in the tammar wallaby were relatively similar and may have diverged more recently, an event that probably occurred at some point in the marsupial lineage.

Horvath, B. V., A. Falus, et al. (2002). "Inverse regulation of interleukin-6 (IL-6) and IL-6 receptor in histamine deficient histidine decarboxylase-knock-out mice." Immunology Letters 80(3): 151.

http://www.sciencedirect.com/science/article/B6T75-44XDTFY-2/2/baf5e6cac2c6f46c38c7c1d5c82473ef

Interleukin-6, a multifunctional cytokine upon binding to its receptor on hepatocytes regulates production of acute phase proteins involved in local and systemic inflammation. Gene expression and biosynthesis of IL-6 and its receptor (IL-6 R/gp130) is under complex regulation. Histamine, in addition to its principal role in immediate type hypersensitivity has been described to modulate IL-6 production and expression of IL-6 receptor. In this study, the IL-6 and IL-6 receptor expression was examined in histamine deficient histidine decarboxylase (HDC) knock-out mouse model. Our data suggest that in histamine deficient mice the inducibility of IL-6 is significantly reduced, whilst more IL-6 receptor/gp130 mRNA expresses in the liver than in wild type (HDC+/+) mice. These in vivo findings confirm earlier in vitro results and emphasize the efficacy of antihistamines in local IL-6 related processes.


http://www.sciencedirect.com/science/article/B6T75-3XMPN51-5/2/d219fccc3894818785d4cbea3beb152

The effect of histamine and histamine antagonists was examined on gene expression and biosynthesis of bacterial endotoxin (LPS) induced interferon [gamma] (IFN[gamma]) both in human peripheral mononuclear cells (PMBC) and in T-cell enriched fractions. We found, that histamine inhibited the LPS induced transcription of IFN[gamma] gene and biosynthesis of IFN[gamma] protein in PMBC and also in CD19-depleted cell populations. The inhibitory effect of histamine could be reversed by the H2 histamine receptor (HR2) antagonists cimetidine and ranitidine both in PMBC and in CD19-depleted cells, but not with triprolidine, an H1 receptor antagonist, suggesting that the inhibition of IFN[gamma] production is mediated through H2 receptors in these cell populations. In contrast to the inhibitory effect of histamine, cimetidine...
alone (in the absence of exogenous histamine) strongly stimulated both the IFN[\gamma] mRNA and protein production, whereas this effect was hardly seen by and other H2 receptor blocker, ranitidine. This superinduction of IFN[\gamma] gene by cimetidine disappeared if the CD19+ cells are removed from PMBC. These results suggest, that inhibition of IFN[\gamma] gene expression by histamine is a direct effect of histamine on H2 receptor of T lymphocytes; however, the superinduction of IFN[\gamma] by cimetidine requires the presence of other (probably primarily B) cell subsets.


http://www.sciencedirect.com/science/article/B6T75-437XR49-9/2/e20b3b2089846b7de70104066dca2297

Although a high level of IgE is produced after primary infection with Nippostrongylus brasiliensis (Nb), most of the IgE antibodies (Abs) are not specific to the worm. Analyses with Western blotting and enzyme-linked immunosorbent assay (ELISA) revealed that the IgE Abs from Nb-infected BALB/c mice did not show reactivity with Nb-derived excretory-secretory proteins (NES) and antigens present in the cell-free extracts of the worm. Monoclonal IgE Abs obtained from the Nb-infected mice were not reactive with these Nb antigen either. To characterize Nb-induced IgE response, we used (QM x C57BL/6)F1 (QBF1) mice that bear the knock-in 17.2.25 VHDJH segment (VHT) encoding a VH region specific to 4-hydroxy-3-nitrophenylacetyl hapten, and express VHT-encoded antigen receptors on 80-85% of their B cells. Consistent with the frequency of VHT-positive B cells, more than 80% of IgE Abs induced in QBF1 B cells that were cultured with LPS plus IL-4 were found to bear VHT-encoded H chains. In contrast, when QBF1 mice were infected with Nb, less than 10% of Nb-induced IgE Abs were found to use VHT. The QBF1-derived IgE did not react with Nb antigens either. Taken together, data suggest that Nb-induced IgE response in mice is not merely the result of polyclonal activation of B cells, but may involve a mechanism that revises Ig genes secondarily.


http://www.sciencedirect.com/science/article/B6T75-4BG3SG7-3/2/3cf7ee9df076ff67fb4167d0d47e2245

Dendritic cells (DCs) are powerful antigen-presenting cells (APCs) that have attracted attention in recent years from the viewpoint of DC vaccine therapy against cancer. However, the existence of a strongly immunosuppressed state in cancer-bearing individuals inhibits DC maturation, which is one of the problems facing anti-cancer DC vaccine therapy. Protein-bound polysaccharide K (PSK), which is extracted from the cultured mycelium of Coriolus versicolor (Fr.) Quel, is used as an anti-cancer agent in Japan. PSK is reported to improve the immunosuppressed state and might be associated with DC maturation directly. We examined the effect of PSK on the maturation of DC derived from CD14-positive cells obtained from human peripheral blood monocytes using a negative selection method. CD14-positive cells cultured in the presence of PSK significantly increased the expression of HLA class II antigen and CD40; significantly increased the number and expression of CD80-, CD86- and CD83-positive cells; decreased Fluorescein isothiocyanate (FITC)-dextran uptake, augmented IL-12 production; augmented the allogeneic mixed lymphocyte reaction; and induced antigen-specific cytotoxicity. These results indicate that PSK promotes both the phenotypic and functional maturation of DC derived from human CD14-positive mononuclear cells. The clinical significance of the combined use of PSK in DC vaccine therapy remains for study.
In normal human subjects a small proportion of peripheral blood T-cells simultaneously express both CD4 and CD8 differentiation antigens. In this study we characterized a subset of CD4+ clones, from a healthy donor, that is specific for the thyrotropin receptor (TSHR) and that showed cells co-expressing the CD8 receptor. To address whether the expression of the CD8 receptor on the cell membrane was associated to differences in the physiology of the T-cells, we isolated, from the same clone, CD4 single positive (SP) cells from those co-expressing CD4/CD8 receptors (DP cells) and stimulated them in vitro with antigen presenting cells (APC) carrying TSHR. The results demonstrated that CD8 co-expression has a profound effect on the physiology of T helper (Th) cells. In comparison to cells expressing the CD4 receptor alone, DP T-cells showed: (1) increased proliferation; (2) higher and more sustained release of free Ca2+ in the cytosol, under stimulus; (3) lower levels of IL-2 and IL-4 released in the supernatants; (4) increased amounts of IFN-\[gamma\] released.

A population of peripheral B cells have been shown to express recombination activating gene products, RAG-1 and RAG-2, which are considered to be involved in revising the B cell antigen receptor (BCR) in the periphery. BCR engagement has been reported to turn off RAG expression in peripheral B cells, whereas the same treatment has an opposite effect on immature B cells in the bone marrow. In contrast to receptor editing that is involved in the removal of autoreactivity in immature B cells, it has been shown that secondary V(D)J rearrangement in peripheral B cells, termed receptor revision, contributes to affinity maturation of antibodies. Here, we show that RAG-2 expression in murine splenic B cells was abrogated by the coligation of BCR with complement receptors (CD21/CD35) much more efficiently than by the engagement of BCR alone. On the other hand, the same coligation augmented proliferation of anti-CD40-stimulated B cells. These findings suggest a crucial role for CD21/CD35 in directing the conservation or the revision of BCRs in peripheral B cells.

Okada, H., H. Momota, et al. (1993). "Elimination of HIV-infected cells by lymphocytes armed with a
The T-cell receptor (TCR) can acquire a new antigen binding site by treatment with a bifunctional antibody (BFA) prepared with mAb against a specified antigen and an epitope of the TCR. Lymphocytes armed with BFA directed to CD3 and an HIV antigen were able to eliminate all HIV antigen-positive cells during incubation with a mixture of HIV-infected and uninfected cells. HIV antigen-positive cells even from persistently infected cells were undetectable with immunofluorescence staining although HIV genes were detectable by polymerase chain reaction (PCR) amplification indicating that only dormantly infected or low producer cells, if any, survived. This suggests that HIV antigen-positive cells could be eliminated by administration of BFA-armed lymphocytes leaving HIV patients with only dormantly infected or low producer cells.


Estrogens have been shown to modulate immune responses. Several studies have demonstrated the capacity of T cells, B cells, and monocytes to respond to estrogens and estrogen receptor (ER) expression in these cell types has been reported. However, little is known regarding the relative expression in these cells of ER[alpha] and the more recently identified ER[beta]. In the present study, results of quantitative TaqMan\textsuperscript{TM} RT-PCR analyses indicate that ERs are differentially expressed in PBMC subsets. CD4+ T cells express relatively high levels of ER[alpha] mRNA compared with ER[beta], whereas B cells express high levels of ER[beta] mRNA but low levels of ER[alpha]. Peripheral blood CD8+ T cells and monocytes express low but comparable levels of both ERs. This quantitative analysis of ER expression in distinct PBMC subsets may provide a basis for dissecting the mechanisms of immune modulation by estrogens and identifying therapeutic targets for the treatment of inflammatory and immunologic disorders.


Fc[gamma]RIIa is one of a family of specific cell surface receptors for immunoglobulin. Fc[gamma]RIIa, which binds immune complexes of certain IgG isotypes, plays important roles in immune homeostasis. However, the precise characteristics of IgG binding and three-dimensional structure of Fc[gamma]RIIa have not been reported. This study describes the affinity of the Fc[gamma]RIIa:IgG interaction as well as biochemical characterisation of recombinant Fc[gamma]RIIa that has been used to generate high quality crystals. Equilibrium binding analysis of the Fc[gamma]RIIa:IgG interaction found, IgG3 binds with an affinity of KD=0.6 \text{[mu]}M, as expected. Unlike other Fc[gamma]R, IgG4 also bound to Fc[gamma]RIIa, KD=3 \text{[mu]}M, clearly establishing Fc[gamma]RIIa as an IgG4 receptor. Biochemical analysis of mammalian and insect cell derived Fc[gamma]RIIa established the genuine N-terminus with Q being the first amino acid in the sequence Q, A, A, A, P. extending the N-terminus further than previously thought. Furthermore, both potential N-linked glycosylation sites are occupied. Electrospray ionisation
mass spectrometry (ESMS) indicate that the N-glycans of baculovirus derived Fc[gamma]RIIa are core mannose oligosaccharide side chains. Finally, we describe the first crystallisation of diffraction quality crystals of soluble Fc[gamma]RIIa. Orthorhombic crystals diffract X-rays beyond 2.1 Å resolution in the space group P21212 with cell dimensions a=78.8 Å, b=100.5 Å, c=27.8 Å. This marks a significant advance towards understanding the three-dimensional structure of Fc[gamma]RIIa and related FcR proteins that share high amino acid identity with Fc[gamma]RIIa.


http://www.sciencedirect.com/science/article/B6T75-46MT7DR-1/2/060310fc7b16a60508e10538761fa9c7

Chondroitin sulfate (CS) is a glycosaminoglycan that is widely present in animals organisms, and it has anti-inflammatory and chondroprotective properties. To examine the effects of CS on the immune system, splenocytes obtained from ovalbumin (OVA)-sensitized BALB/c mice were challenged with OVA in the presence of CS, and cytokine levels in the medium of the cultured cells were measured. CS induced secretion of Th1-type cytokines (IFN-[gamma], IL-2, and IL-12) by OVA-sensitized splenocytes but suppressed secretion of Th2-type cytokines (IL-5 and IL-10). Flow cytometric assay showed a significantly higher percentage of helper T cells (CD4+CD8- cells) among the splenocytes cultured with OVA and CS than with OVA alone. Analysis of the IFN-[gamma] mRNA level of the splenocytes by the real-time quantitative RT-PCR technique revealed higher levels in the splenocytes cultured with OVA and CS than in the splenocytes cultured with OVA alone. This is the first demonstration that CS inhibits antigen-induced IgE production through induction of cytokine secretion by Th1 cells, and this finding suggests a potential use of CS in preventing IgE-mediated allergy.


http://www.sciencedirect.com/science/article/B6T75-48JSXFG-2/2/5084f51bc4fccc85c130fbb92577b5f56

Neurotrophins, including nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 (NT-3) are essential factors for the development of the nervous system. In this report, we demonstrate gene expression of neurotrophins and their receptors in T helper 1 (Th1) and T helper 2 (Th2) cells induced from naive CD4+CD45RB+ T cells of ovalbumin-specific DO11.10 T cell receptor transgenic mice. Interestingly, the TrkC gene, which encodes a high affinity receptor for NT-3, was expressed in Th2 cells, but not in Th1 and naive CD4+ T cells. Expression of the TrkC gene was markedly augmented by addition of anti-IFN-[gamma] monoclonal antibody (mAb) into the culture, whereas it was blocked by anti-IL-4 mAb. Moreover, NT-3 synergistically enhanced anti-CD3 mAb-induced IL-4 production by Th2 cells, but did not affect IFN-[gamma] production by Th1 cells. These data suggest that NT-3, through its receptor TrkC, plays a critical role in regulating the Th1/Th2 balance.

We have analysed the relative T cell receptor (TCR) BV gene usage in T cells from hearts and spleens of CBA/HJ mice chronically infected with the Tulahuen strain of Trypanosoma cruzi. During chronic infection, CBA/HJ mice recruit T cells at the major site of inflammation (i.e. the heart), with over-representation of certain TCRBV gene subfamilies (TCRBV8S2 and TCRBV8S3). In contrast, no signal or a very weak message from a limited number of T cells was recorded from one heart of the control group. No alteration of TCRBV distribution was recorded in spleens of chronically infected CBA/HJ. Our findings indicate that there is a preferential TCRBV gene usage in the T cell response in the hearts of chronically infected mice. Furthermore, the pattern of CDR3 lengths in inflammatory T cells was altered.


Depletion of lymphocyte subsets in vivo using monoclonal antibodies against cell surface markers has helped to define the roles for these subsets in many immune processes. However, in some cases the mechanisms through which these lymphocytes act remain partially elucidated or completely unknown. A new approach to these biological problems is the use of transcriptional analyses to find mRNAs whose abundance in tissues is altered by depletion of lymphocyte subsets. We have verified the use mRNA differential display (DD) for this purpose and applied it in a study of CD8+ lymphocyte mediated clearance of herpes simplex virus (HSV) from the nervous systems of experimentally infected mice. The results of the differential displays and characterisation of a large mRNA identified using this strategy are presented.


Eosinophils play a pivotal role in the mechanism of allergic diseases including asthma. Interleukin-5 (IL-5) and eotaxin are critical cytokines/chemokines for eosinophil activation. Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]) is a nuclear receptor that regulates lipid metabolism. Recent evidence has suggested that PPAR[gamma] serves as a negative regulator in the immune system. In the present study, we investigated the expression of PPAR[gamma] and effect of PPAR[gamma] agonist on human eosinophils. We demonstrated that purified eosinophils and Eol-1 cells express PPAR[gamma] at the mRNA and protein levels. The PPAR[gamma] agonist troglitazone reduced the IL-5-stimulated, but not spontaneous, eosinophil survival in a concentration-dependent manner. Moreover, the eotaxin-directed eosinophil chemotaxis was dose-dependently inhibited by troglitazone. Our results suggest that the administration of the PPAR[gamma] agonists thiazolidinediones could be a new therapeutic modality for the treatment of allergic diseases such as asthma.
The neutrophil antigen (NA)1 and 2 is coded by two recognized allelic forms of Fc gamma receptor IIIb (Fc[gamma]RIIIB). Fc[gamma]RIIIB is a low affinity receptor and preferentially removes immune complexes from the circulation. Systemic lupus erythematosus (SLE) is an autoimmune and polygenic disorder characterized by accumulation of autoimmune complexes. The majority of SLE patients in our medical center are of Chinese ethnicity, followed by Malay and Indian. Recently, studies have focussed on the Fc receptors in different ethnic groups and their relation to SLE. We chose to study the gene distribution of this receptor in the Chinese and Malays population in Malaysia. We designed a polymerase chain reaction-allele specific primers (PCR-ASP) method to distinguish the two allelic forms. Genomic DNA was isolated from the peripheral blood of 183 Chinese and 55 Malays SLE patients as well as 100 Chinese and 50 Malays healthy controls. Genotyping of Chinese SLE patients revealed that the gene frequencies for Fc[gamma]RIIIB-NA1 and Fc[gamma]RIIIB-NA2 were 0.648 and 0.347, while in the ethnically matched healthy controls they were 0.68 and 0.32, respectively. One out of the 183 Chinese SLE patients was identified as a NA-null due to the absence of PCR product for both alleles. The Fc[gamma]RIIIB-NA1 and Fc[gamma]RIIIB-NA2 allele frequencies for both the Malays SLE and healthy controls were 0.62 and 0.38.

Recently evidence has been provided for a genetic control of T-cell dependent cytokine production by HLA-class II. Candidate genes in multiple sclerosis, a T-cell mediated autoimmune disease, are the disease-associated DR2, DQ6, Dw2 haplotype. Previous observations by us and others imply a HLA-DR2 dependent propensity of antigen-specific T-cell lines to produce increased amounts of TNF-[alpha]/[beta]. Here, we tested a possible association between HLA or disease status with cytokine production employing the simple and widely used method of bulk cultures. Peripheral blood cells of 48 patients and 68 healthy individuals were analyzed. We observed no significant differences of the cytokine production in relation to disease status or any HLA polymorphism. Our data indicate that, in contrast to monoclonal T-cell cultures, bulk cultures are not suitable to detect immunogenetic control of T-cell function.

The immunopharmacological characteristics of angelan, a polysaccharide purified from Angelica gigas Nakai, were investigated in relation to the specificity to immune cells. The treatment of angelan increased the expression of IL-2, IL-4, IL-6, and IFN-[gamma]. The expression of IL-6 and IFN-[gamma] was rapidly augmented but that of IL-2 responded later. In the case of IL-4, angelan stimulated at early time after exposure but down-regulated thereafter. These results suggested that macrophages and natural killer cells involved in nonspecific immunity were primarily activated and helper T cells were secondarily affected by angelan. Angelan also had lympho-proliferative potential to B cells, specifically. The specificity of angelan was also elucidated in a cell fractionation experiment. The activated B cells by angelan also increased antibody production. The direct activation of B cells, macrophages, and accessory cells and the indirect activation of helper T cells coordinately increased immune functions such as in vitro and in vivo T-dependent immunization and antibody production. The experiment of host resistance to syngeneic tumors also showed that angelan potentiated the immune functions. In conclusion, angelan, a purified polysaccharide from an oriental herbal drug, showed characteristic immunostimulation, which was different from clinically used polysaccharides such as lentinan and PSK.


http://www.sciencedirect.com/science/article/B6T27-3RWW799-6/2/98abc0c394c50a65ce6ae92101cea3d6

Immunocyctokines, such as interleukin-1 (IL-1), have been shown to be involved in the activation and/or induction of a variety of transcription factors which may modulate the expression of genes possessing DNA binding sites on which these transcription factors act. The promoter DNA sequence of the [mu] opioid receptor gene contains IL-1 response elements such as NF-IL6, and, therefore, the receptor gene may be responsive to IL-1. To investigate the effect of IL-1 on the opioid receptor gene, the in vitro expression of [mu] opioid receptor mRNA in neural microvascular endothelial cells (NMVEC) was determined before and after IL-1 treatment. PCR analysis revealed that there was virtually no [mu] opioid receptor expression at basal levels and no increase after either IL-1[alpha] or IL-1[beta] treatment. However, simultaneous treatment with both IL-1[alpha] and IL-1[beta] increased [mu] opioid receptor expression. This upregulation of [mu] opioid receptor expression provides direct evidence of a relationship between opioid and cytokine actions, and suggests that opioid-dependent pathways may be modulated in the disease state.

Infect. Immun. 65


http://iai.asm.org/cgi/content/abstract/70/6/2763

The NRAMP1 gene (Slc11a1) encodes an ion transporter protein involved in the control of
intraphagosomal replication of parasites and in macrophage activation. It has been described in mice as the determinant of natural resistance or susceptibility to infection with antigenically unrelated pathogens, including Leishmania. Our aims were to sequence and map the canine Slc11a1 gene and to identify mutations that may be associated with resistance or susceptibility to Leishmania infection. The canine Slc11a1 gene has been mapped to dog chromosome CFA37 and covers 9 kb, including a 700-bp promoter region, 15 exons, and a polymorphic microsatellite in intron 1. It encodes a 547-amino-acid protein that has over 87% identity with the Slc11a1 proteins of different mammalian species. A case-control study with 33 resistant and 84 susceptible dogs showed an association between allele 145 of the microsatellite and susceptible dogs. Sequence variant analysis was performed by direct sequencing of the cDNA and the promoter region of four unrelated beagles experimentally infected with Leishmania infantum to search for possible functional mutations. Two of the dogs were classified as susceptible and the other two were classified as resistant based on their immune responses. Two important mutations were found in susceptible dogs: a G-rich region in the promoter that was common to both animals and a complete deletion of exon 11, which encodes the consensus transport motif of the protein, in the unique susceptible dog that needed an additional and prolonged treatment to avoid continuous relapses. A study with a larger dog population would be required to prove the association of these sequence variants with disease susceptibility.


http://iai.asm.org/cgi/content/abstract/71/8/4463

Listeria monocytogenes is a gram-positive facultative intracellular food-borne pathogen that can cause severe infections in humans and animals. We have recently adapted signature-tagged transposon mutagenesis (STM) to identify genes involved in the virulence of L. monocytogenes. A new round of STM allowed us to identify a new locus encoding a protein homologous to AgrA, the well-studied response regulator of Staphylococcus aureus and part of a two-component system involved in bacterial virulence. The production of several secreted proteins was modified in the agrA mutant of L. monocytogenes grown in broth, indicating that the agr locus influenced protein secretion. Inactivation of agrA did not affect the ability of the pathogen to invade and multiply in cells in vitro. However, the virulence of the agrA mutant was attenuated in the mouse (a 10-fold increase in the 50% lethal dose by the intravenous route), demonstrating for the first time a role for the agr locus in the virulence of L. monocytogenes.


http://iai.asm.org/cgi/content/abstract/70/6/2995

Lipopolysaccharide (LPS) has recently been shown to facilitate macrophage foam cell formation and has been suggested to be a proatherogenic factor. The mechanism of LPS induced cholesterol accumulation, however, is unclear. In this report, using the macrophage-like RAW 264.7 cell line, we provide experimental evidence that LPS's proatherogenic effects may at least in part reflect altered cholesterol metabolism. Data presented demonstrate that in a dose-dependent manner, LPS is able to down regulate the mRNA expression of the two primary high-density lipoprotein (HDL) receptors, scavenger receptor B1 (SR-B1) and ATP binding cassette A1 (ABCA1), with a 50% inhibitory concentration of less than 0.2 ng/ml, as well as to decrease SR-B1 protein expression by 80%. We also found that LPS treatment resulted in a significant decrease (to 20% of the control level) of the specific 125I-HDL binding as well as in 50%
inhibition of the HDL-mediated cholesterol efflux compared to untreated cells. In addition, we compared the potencies of various modified LPS preparations and demonstrated that the phosphorylated lipid A portion of LPS, which is highly conserved among gram-negative microorganisms, including Chlamydia, is primarily responsible for the effects of LPS on SR-B1 and ABCA1 expression. Inhibitors of NF-κB activation were observed to efficiently block the suppressive effect of LPS on SR-B1 and ABCA1, suggesting a mechanism involving NF-κB. These data indicate that the LPS effects on cholesterol metabolism may contribute to the proatherogenic properties of LPS.


http://iai.asm.org/cgi/content/abstract/70/6/2915

Most strains of Shigella flexneri 2a and enteroaggregative Escherichia coli carry a highly conserved chromosomal locus which encodes a 109-kDa secreted mucinase (called Pic) and, on the opposite strand in overlapping fashion, an oligomeric enterotoxin called ShET1, encoded by the setA and setB genes. Here, we characterize the genetic regulation of these overlapping genes. Our data suggest that pic and the setBA loci are transcribed as complementary 4-kb mRNA species. The major pic promoter is maximally activated at 37°C in exponential growth phase. Our data suggest that the setB gene is transcribed from a promoter which lies more than 1.5 kb upstream of the setB structural gene; setA may be transcribed via readthrough of the setB transcript and possibly by its own promoter. The long leader of the setB gene provides a strong silencing effect on setB transcription. The signals which provide relief from setB silencing are not clear, but significant induction is observed in a continuous anaerobic culture of human fecal bacteria, suggesting that some complex characteristics of the human intestine act to lift repression of setB expression. Our studies provide the first insights into the mechanisms affecting expression of this unusual virulence locus.


http://iai.asm.org/cgi/content/abstract/73/4/2424

Neisseria lactamica, a harmless human commensal found predominantly in the upper respiratory tracts of infants, is closely related to Neisseria meningitidis, a pathogen of global significance. Colonization with N. lactamica may be responsible for the increase in immunity to meningococcal disease that occurs during childhood, when rates of meningococcal carriage are low. This observation has led to the suggestion that N. lactamica whole cells or components are potential constituents of novel meningococcal vaccines. However, the dynamics of carriage and population diversity of N. lactamica in children are poorly understood, presenting difficulties for the choice of representative isolates for use in vaccine development. This problem was addressed by the multilocus sequence typing of N. lactamica isolates from two longitudinal studies of bacterial carriage in infants. The studies comprised 100 and 216 subjects, with N. lactamica carriage monitored from age 4 weeks until age 96 weeks and from age 2 weeks until age 24 weeks, respectively. The maximum observed carriage rate was 44% at 56 weeks of age, with isolates obtained on multiple visits for the majority (54 of 75, 72%) of carriers. The N. lactamica isolates were genetically diverse, with 69 distinct genotypes recovered from the 75 infants. Carriage was generally long-lived, with an average rate of loss of under 1% per week during the 28 weeks following acquisition. Only 11 of the 75 infants carried more than one genotypically unique isolate during the course of the study. Some participants shared identical isolates with siblings, but none shared identical isolates with their parents. These findings have implications for the design of

http://iai.asm.org/cgi/content/abstract/72/7/3914

Enterotoxigenic Escherichia coli (ETEC) strains that produce multiple enterotoxins are important causes of severe dehydrating diarrhea in human beings and animals, but the relative importance of these enterotoxins in the pathogenesis is poorly understood. Gnotobiotic piglets were used to study the importance of heat-labile enterotoxin (LT) in infection with an ETEC strain that produces multiple enterotoxins. LT- (ΔeltAB) and complemented mutants of an F4+ LT+ STb+ EAST1+ ETEC strain were constructed, and the virulence of these strains was compared in gnotobiotic piglets expressing receptors for F4+ fimbria. Sixty percent of the piglets inoculated with the LT- mutant developed severe dehydrating diarrhea and septicemia compared to 100% of those inoculated with the nalidixic acid-resistant (Nalr) parent and 100% of those inoculated with the complemented mutant strain. Compared to piglets inoculated with the Nalr parent, the mean rate of weight loss (percent per hour) of those inoculated with the LT- mutant was 67% lower (P < 0.05) and that of those inoculated with the complemented strain was 36% higher (P < 0.001). Similarly, piglets inoculated with the LT- mutant had significant reductions in the mean bacterial colony count (CFU per gram) in the ileum; bacterial colonization scores (square millimeters) in the jejunum and ileum; and clinical pathology parameters of dehydration, electrolyte imbalance, and metabolic acidosis (P < 0.05). These results indicate the significance of LT to the development of severe dehydrating diarrhea and postdiarrheal septicemia in ETEC infections of swine and demonstrate that EAST1, LT, and STb may be concurrently expressed by porcine ETEC strains.


http://iai.asm.org/cgi/content/abstract/72/2/691

Type IV pili (Tfp) of gram-negative species share many characteristics, including a common architecture and conserved biogenesis pathway. Much less is known about the regulation of Tfp expression in response to changing environmental conditions. We investigated the diversity of Tfp regulatory systems by searching for the molecular basis of the reported variable expression of the Tfp gene cluster of the pathogen Actinobacillus pleuropneumoniae. Despite the presence of an intact Tfp gene cluster consisting of four genes, apfABCD, no Tfp were formed under standard growth conditions. Sequence analysis of the predicted major subunit protein ApfA showed an atypical alanine residue at position -1 from the prepilin peptidase cleavage site in 42 strains. This alanine deviates from the consensus glycine at this position in Tfp from other species. Yet, cloning of the apfABCD genes under a constitutive promoter in A. pleuropneumoniae resulted in pilin and Tfp assembly. Tfp promoter-luxAB reporter gene fusions demonstrated that the Tfp promoter was intact but tightly regulated. Promoter activity varied with bacterial growth phase and was detected only when bacteria were grown in chemically defined medium. Infection experiments with cultured epithelial cells demonstrated that Tfp promoter activity was upregulated upon adherence of the pathogen to primary cultures of lung epithelial cells. Nonadherent bacteria in the culture supernatant exhibited virtually no promoter activity. A similar upregulation of Tfp promoter activity was observed in vivo during experimental infection of pigs. The host cell contact-induced and in vivo-upregulated Tfp promoter activity in A. pleuropneumoniae adds a new dimension to the diversity of Tfp regulation.

http://iai.asm.org/cgi/content/abstract/71/12/7087

Lyme borreliosis is a multisystemic disease caused by various genospecies of the spirochete Borrelia burgdorferi. To investigate muscle involvement in the nonhuman primate (NHP) model of Lyme disease, 16 adult Macaca mulatta animals inoculated with strain N40 of B. burgdorferi sensu strictu by syringe or by tick bite or with strain Pbi of B. burgdorferi genospecies garinii by syringe were studied. Animals were necropsied while immunosuppressed on day 50 (two animals each inoculated with B. burgdorferi N40 by syringe and with B. garinii Pbi by syringe) or on day 90, 40 days after immunosuppression had been discontinued (four animals each inoculated with strain N40 by syringe, with strain N40 by tick bite, and with strain Pbi by syringe). Skeletal muscles removed at necropsy were studied by (i) microscopic examination of hematoxylin-eosin-stained sections for inflammation and tissue injury; (ii) immunohistochemical and digital image analyses for antibody and complement deposition and cellular inflammation; (iii) Western blot densitometry for the presence of antibodies; and (iv) reverse transcription-PCR for measurement of the spirochetal load or C1q (the first component of the complement cascade) synthesis. The results showed that N40 was more infectious for NHPs than Pbi. NHPs inoculated with N40 but not with Pbi developed myositis. The inflammation in skeletal muscle was more severe in NHPs inoculated with N40 by syringe than in those inoculated by tick bite. The predominant cells in the inflammatory infiltrate were T cells and plasma cells. The deposition of antibody and complement in inflamed muscles from N40-inoculated NHPs was significantly higher than that in Pbi-inoculated NHPs. The spirochetal load was very high in the two N40-inoculated NHPs examined while they were immunosuppressed but decreased to minimal levels in the NHPs when immunocompetence was restored. We conclude that myositis can be a prominent feature of Lyme borreliosis depending on the infecting organism and host immune status.


http://iai.asm.org/cgi/content/abstract/70/2/434

T-cell immunity is critical for survival of hosts infected with Toxoplasma gondii. Among the cells in the T-cell population, CD8+ T cells are considered the major effector cells against this parasite. It is believed that CD4+ T cells may be crucial for induction of the CD8+-T-cell response against T. gondii. In the present study, CD4-/− mice were used to evaluate the role of conventional CD4+ T cells in the immune response against T. gondii infection. CD4-/− mice infected with T. gondii exhibited lower gamma interferon (IFN-(gamma)) messages in the majority of their tissues. As a result, mortality due to a hyperinflammatory response was prevented in these animals. Interestingly, T. gondii infection induced a normal antigen-specific CD8+-T-cell immune response in CD4-/− mice. No difference in generation of precursor cytotoxic T lymphocytes (pCTL) or in IFN-(gamma) production by the CD8+-T-cell populations from the knockout and wild-type animals was observed. However, the mutant mice were not able to sustain CD8+-T-cell immunity. At 180 days after infection, the CD8+-T-cell response in the knockout mice was depressed, as determined by pCTL and IFN-(gamma) assays. Loss of CD8+-T-cell immunity at this time was confirmed by adoptive transfer experiments. Purified CD8+ T cells from CD4-/− donors that had been immunized 180 days earlier failed to protect the recipient mice against a lethal infection. Our study demonstrated that although CD8+-T-cell immunity can be induced in the absence of conventional CD4+ T cells, it cannot be maintained without such cells.
Hookworm infection is a major cause of iron deficiency anemia and malnutrition in developing countries. The Ancylostoma ceylanicum Kunitz-type inhibitor (AceKI) is a 7.9-kDa broad-spectrum inhibitor of trypsin, chymotrypsin, and pancreatic elastase that has previously been isolated from adult hookworms. Site-directed mutagenesis of the predicted P1 inhibitory reactive site amino acid confirmed the role of Met26 in mediating inhibition of the three target serine proteases. By using reverse transcription-PCR, it was demonstrated that the level of AceKI gene expression increased following activation of third-stage larvae with serum and that the highest level of expression was reached in the adult stage of the parasite. Immunohistochemistry studies performed with polyclonal immunoglobulin G raised against recombinant AceKI showed that the inhibitor localized to the subcuticle of the adult hookworm, suggesting that it has a potential in vivo role in neutralizing intestinal proteases at the surface of the parasite. Immunization with recombinant AceKI was shown to confer partial protection against hookworm-associated growth delay without a measurable effect on anemia. Taken together, the data suggest that AceKI plays a role in the pathogenesis of hookworm-associated malnutrition and growth delay, perhaps through inhibition of nutrient absorption in infected hosts.

Fimbriae have been shown to play an essential role in the adhesion of pathogenic gram-negative bacteria to host cells. In the enteroinvasive bacterium Yersinia pseudotuberculosis, we characterized a previously unknown 11-kb chromosomal locus involved in the synthesis of type IV pili. The locus consists of 11 open reading frames forming a polycistronic unit and encoding putative Pil proteins, PilLMNOPQRSUVW. When introduced into Escherichia coli, the Y. pseudotuberculosis operon reconstituted bundles of filaments at a pole on the bacterial surface, demonstrating that the pil locus was functional in a heterogenous genetic background. Environmental factors regulated transcription of the Y. pseudotuberculosis operon; in particular, temperature, osmolarity, and oxygen tension were critical cues. Deletion of the type IV pilus gene cluster was associated with a reduction of Y. pseudotuberculosis pathogenicity for mice infected orally. Forty-one percent of Y. pseudotuberculosis strains isolated from human or animal sources harbored the type IV pilus locus. Therefore, the pil locus of Y. pseudotuberculosis might constitute an "adaptation island," permitting the microorganism to colonize a vast reservoir.

The general concept that during infection of mice the Borrelia burgdorferi surface protein composition differs profoundly from that of tick-borne or in vitro-cultivated spirochetes is well established. Specific knowledge concerning the differences is limited because the small numbers
of spirochetes present in tissue have not been amenable to direct compositional analysis. In this report we describe novel means for studying the antigenic composition of host-adapted Borrelia (HAB). The detergent Triton X-114 was used to extract the detergent-phase HAB proteins from mouse ears, ankles, knees, and hearts. Immunoblot analysis revealed a profile distinct from that of in vitro-cultivated Borrelia (IVCB). OspA and OspB were not found in the tissues of SCID mice 17 days after infection. The amounts of antigenic variation protein VlsE and the relative amounts of its transcripts were markedly increased in ear, ankle, and knee tissues but not in heart tissue. VlsE existed as isoforms having both different unit sizes and discrete lower molecular masses. The hydrophobic smaller forms of VlsE were also found in IVCB. The amounts of the surface protein (OspC) and the decorin binding protein (DbpA) were increased in ear, ankle, knee, and heart tissues, as were the relative amounts of their transcripts. Along with these findings regarding VlsE, OspC, and DbpA, two-dimensional immunoblot analysis with immune sera also revealed additional details of the antigenic composition of HAB extracted from ear, heart, and joint tissues. A variety of novel antigens, including antigens with molecular masses of 65 and 30 kDa, were found to be upregulated in mouse tissues. Extraction of hydrophobic B. burgdorferi antigens from tissue provides a powerful tool for determining the antigenic composition of HAB.


http://iai.asm.org/cgi/content/abstract/71/7/4217

Neisseria miniature insertion sequences (nemis) are miniature DNA insertion sequences found in Neisseria species. Out of 57 elements closely flanking cellular genes analyzed by PCR, most were conserved in Neisseria meningitidis but not in N. lactamica strains. Since mRNAs spanning nemis are processed by RNase III at hairpins formed by element termini, gene sets could selectively be regulated in meningococci at the posttranscriptional level.


http://iai.asm.org/cgi/content/abstract/70/12/6987

Cryptosporidium parvum is recognized as an enteropathogen of great worldwide medical and veterinary importance, yet understanding of its pathogenesis has been hampered in part by limited knowledge of the invasion machinery of this parasite. Recently, genes containing thrombospondin type 1 (TSP1) domains have been identified in several genera of apicomplexans, including thrombospondin-related adhesive proteins (TRAPs) that have been implicated as key molecules for parasite motility and adhesion onto host cell surfaces. Previously, a large-scale random survey of the C. parvum genome conducted in our laboratory revealed the presence of multiple genomic DNA sequences with a high degree of similarity to known apicomplexan TRAP genes. In the present study, TBLASTN screening of available C. parvum genomic sequences by using TSP1 domains as queries identified a total of 12 genes possessing TSP1-like domains. All genes have putative signal peptide sequences, one or more TSP1-like domains, plus additional extracellular protein modules such as Kringle, epidermal growth factor, and Apple domains. Two genes, putative paralogs CptTSP8 and CptTSP9, contain predicted introns near their amino termini, which were verified by comparing PCR products from cDNA versus genomic DNA templates. Reverse transcription-PCR analysis of transcript levels reveals that C. parvum TSP genes were developmentally regulated with distinct patterns of expression during in vitro infection. TRAPC1, CptTSP3, and CptTSP11 were expressed at high levels during both early and late stages of infection, whereas CptTSP2, CptTSP5, CptTSP6, CptTSP8, and CptTSP9 were
maximally expressed during the late stages of infection. Only CpTSP4 was highly expressed solely at an early stage of infection.


The flagellum protein flagellin of Listeria monocytogenes is encoded by the flaA gene. Immediately downstream of flaA, two genes, cheY and cheA, encoding products with homology to chemotaxis proteins of other bacteria, are located. In this study we constructed deletion mutants with mutations in flaA, cheY, and cheA to elucidate their role in the biology of infection with L. monocytogenes. The {Delta}cheY, {Delta}cheA, and double-mutant {Delta}cheYA mutants, but not {Delta}flaA mutant, were motile in liquid media. However, the {Delta}cheA mutant had impaired swarming and the {Delta}cheY and {Delta}cheYA mutants were unable to swarm on soft agar plates, suggesting that cheY and cheA genes encode proteins involved in chemotaxis. The {Delta}flaA, {Delta}cheY, {Delta}cheA, and {Delta}cheYA mutants (grown at 24(degrees)C) showed reduced association with and invasion of Caco-2 cells compared to the wild-type strain. However, spleens from intragastrically infected BALB/c and C57BL/6 mice showed larger and similar numbers of the {Delta}flaA and {Delta}cheYA mutants, respectively, compared to the wild-type controls. Such a discrepancy could be explained by the fact that tumor necrosis factor receptor p55 deficient mice showed dramatically exacerbated susceptibility to the wild-type but unchanged or only slightly increased levels of the {Delta}flaA or {Delta}cheYA mutant. In summary, we show that listerial flaA, cheY, and cheA gene products facilitate the initial contact with epithelial cells and contribute to effective invasion but that flaA could also be involved in the triggering of immune responses.


We identified Mycobacterium tuberculosis genes preferentially expressed during infection of human macrophages using a promoter trap adapted for this pathogen. inhA encodes an enoyl-acyl carrier protein reductase that is required for mycolic acid biosynthesis (A. Quemard et al., Biochemistry 34:8235-8241, 1995) and is a major target for isoniazid (INH) in mycobacterial species (A. Banerjee et al., Science 263:227-230, 1994). Since overexpression of inhA confers INH resistance in Mycobacterium smegmatis (Banerjee et al., Science 263:227-230, 1994), we designed a promoter trap based on this gene. A library of clones, containing small fragments of M. tuberculosis DNA cloned upstream of inhA in a plasmid vector, was electroporated into M. tuberculosis, and the resulting culture was used to infect the human monocytic THP-1 cell line. Selection was made for clones surviving INH treatment during infection but retaining INH sensitivity on plates. The DNA upstream of inhA was sequenced in each clone to identify the promoter driving inhA expression. Thirteen genes identified by this method were analyzed by quantitative reverse transcription-PCR (R. Manganelli et al., Mol. Microbiol. 31:715-724, 1999), and eight of them were found to be differentially expressed from cultures grown in macrophages compared with broth-grown cultures. Several of these genes are presumed to be involved in fatty acid metabolism; one potentially codes for a unique DNA binding protein, one codes for a possible potassium channel protein, and the others code for proteins of unknown function. Genes which are induced during infection are likely to be significant for survival and growth of the pathogen; our results lend support to the view that fatty acid metabolism is essential for the virulence of M. tuberculosis.
The YadA protein is a major adhesin of Yersinia pseudotuberculosis that promotes tight adhesion to mammalian cells by binding to extracellular matrix proteins. In this study, we first addressed the possibility of competitive interference of YadA and the major invasive factor invasin and found that expression of YadA in the presence of invasin affected neither the export nor the function of invasin in the outer membrane. Furthermore, expression of YadA promoted both bacterial adhesion and high-efficiency invasion entirely independently of invasin. Antibodies against fibronectin and \( \beta1 \) integrins blocked invasion, indicating that invasion occurs via extracellular-matrix-dependent bridging between YadA and the host cell \( \beta1 \) integrin receptors. Inhibitor studies also demonstrated that tyrosine and Ser/Thr kinases, as well as phosphatidylinositol 3-kinase, are involved in the uptake process. Further expression studies revealed that \( yadA \) is regulated in response to several environmental parameters, including temperature, ion and nutrient concentrations, and the bacterial growth phase. In complex medium, YadA production was generally repressed but could be induced by addition of Mg\(^{2+} \). Maximal expression of \( yadA \) was obtained in exponential-phase cells grown in minimal medium at 37\(^{\circ} \)C, conditions under which the invasin gene is repressed. These results suggest that YadA of Y. pseudotuberculosis constitutes another independent high-level uptake pathway that might complement other cell entry mechanisms (e.g., invasin) at certain sites or stages during the infection process.

Cryptococcus neoformans, an encapsulated yeast, is a common cause of life-threatening meningoencephalitis in immunosuppressed patients. We previously observed that administration of a monoclonal antibody (MAb) to the capsular polysaccharide to mice with pulmonary infection prolonged survival and enhanced granulomatous inflammation without reducing lung CFU. To understand the mechanism of MAb action, we studied leukocyte recruitment and cytokine profiles in lungs of A/JCr mice. B lymphocytes were the predominant cell type in lung infiltrates, comprising 15 to 30% of the leukocytes. Despite alterations in histological appearance, fluorescence-activated cell sorter analysis revealed no significant difference in total numbers of lung leukocytes in MAb-treated mice and controls. Differences in the immune response to C. neoformans between MAb-treated mice and controls included (i) an increase in the percentage of granulocytes among lung leukocytes on day 14, (ii) higher macrophage surface expression of CD86 on day 28, (iii) larger amounts of IL-10 in lung homogenates at day 7, (iv) a trend toward smaller amounts of gamma interferon mRNA and protein on day 7, and (v) a smaller increase in the levels of interleukin-4 mRNA and protein on day 7. Hence, the immune responses to C. neoformans infection in the presence and absence of specific antibody were qualitatively similar, and antibody administration was associated with several subtle quantitative differences in immune response parameters that could translate into enhanced survival. MAb may function partly by down-regulating the inflammatory response and reducing host damage. Our findings demonstrate unexpected complexity in the interaction between specific MAb and other components of the host immune response.
A novel antigen that induces cross-reactive bactericidal antibodies against a number of Neisseria meningitidis strains is described. This antigen, a ~28-kDa lipoprotein called LP2086, was first observed within a complex mixture of soluble outer membrane proteins (sOMPs) following a series of fractionation, protein purification, and proteomics steps. Approximately 95 different neisserial isolates tested positive by Western blotting and PCR screening methods for the presence of the protein and the gene encoding LP2086. The strains tested included isolates of N. meningitidis serogroups A, B, C, W135, and Y, Neisseria gonorrhoeae, and Neisseria lactamica. To better understand the microheterogeneity of this protein, the 2086 genes from 63 neisserial isolates were sequenced. Two different subfamilies of LP2086 were identified based on deduced amino acid sequence homology. A high degree of amino acid sequence similarity exists within each 2086 subfamily. The highest degree of genetic diversity was seen between the two subfamilies which share approximately 60 to 75% homology at the nucleic acid level. Flow cytometry (fluorescence-activated cell sorting) analyses and electron microscopy indicated that the LP2086 is localized on the outer surface of N. meningitidis. Antiserum produced against a single protein variant was capable of eliciting bactericidal activity against strains expressing different serosubtype antigens. Combining one recombinant lipidated 2086 (rLP2086) variant from each subfamily with two rPorA variants elicited bactericidal activity against all strains tested. The rLP2086 family of antigens are candidates worthy of further vaccine development.

Legionella pneumophila, the causative agent of Legionnaires' disease, is an intracellular pathogen of amoebae, macrophages, and epithelial cells. The pathology of Legionella infections involves alveolar cell destruction, and several proteins of L. pneumophila are known to contribute to this ability. By screening a genomic library of L. pneumophila, we found an additional L. pneumophila gene, plaB, which coded for a hemolytic activity and contained a lipase consensus motif in its deduced protein sequence. Moreover, Escherichia coli harboring the L. pneumophila plaB gene showed increased activity in releasing fatty acids predominantly from diacylphospho- and lysophospholipids, demonstrating that it encodes a phospholipase A. It has been reported that culture supernatants and cell lysates of L. pneumophila possess phospholipase A activity; however, only the major secreted lysophospholipase A PlaA has been investigated on the molecular level. We therefore generated isogenic L. pneumophila plaB mutants and tested those for hemolysis, lipolytic activities, and intracellular survival in amoebae and macrophages. Compared to wild-type L. pneumophila, the plaB mutant showed reduced hemolysis of human red blood cells and almost completely lost its cell-associated lipolytic activity. We conclude that L. pneumophila plaB is the gene encoding the major cell-associated phospholipase A, possibly contributing to bacterial cytotoxicity due to its hemolytic activity. On the other hand, in view of the fact that the plaB mutant multiplied like the wild type both in U937 macrophages and in Acanthamoeba castellanii amoebae, plaB is not essential for intracellular survival of the pathogen.

http://iai.asm.org/cgi/content/abstract/70/3/1382

Listeria monocytogenes is an intracellular gram-positive human pathogen that invades eucaryotic cells. Among the surface-exposed proteins playing a role in this invasive process, internalin belongs to the family of LPXTG proteins, which are known to be covalently linked to the bacterial cell wall in gram-positive bacteria. Recently, it has been shown in Staphylococcus aureus that the covalent anchoring of protein A, a typical LPXTG protein, is due to a cysteine protease, named sortase, required for bacterial virulence. Here, we identified in silico from the genome of L. monocytogenes a gene, designated srtA, encoding a sortase homologue. The role of this previously unknown sortase was studied by constructing a sortase knockout mutant. Internalin was used as a reporter protein to study the effects of the srtA mutation on cell wall anchoring of this LPXTG protein in L. monocytogenes. We show that the srtA mutant (i) is affected in the display of internalin at the bacterial surface, (ii) is significantly less invasive in vitro, and (iii) is attenuated in its virulence in the mouse. These results demonstrate that srtA of L. monocytogenes acts as a sortase and plays a role in the pathogenicity.


http://iai.asm.org/cgi/content/abstract/71/2/801

We have recently reported Bacillus anthracis attenuated live vaccine strains efficiently expressing recombinant protective antigen (rPA) and have shown a direct correlation between the level of rPA secreted by these cells and efficacy (S. Cohen, I. Mendelson, Z. Altboum, D. Kobiler, E. Elhanany, T. Bino, M. Leitner, I. Inbar, H. Rosenberg, Y. Gozes, R. Barak, M. Fisher, C. Kronman, B. Velan, and A. Shafferman, Infect. Immun. 68:4549-4558, 2000). To isolate more potent Bacillus promoters for a further increase in the production of rPA, we developed a promoter trap system based on various gfp reporter genes adapted for use in both Bacillus subtilis and B. anthracis backgrounds. Accordingly, a B. anthracis library of 6,000 clones harboring plasmids with chromosomal B. anthracis DNA fragments inserted upstream from gfpuv was constructed. Based on fluorescence intensity, 57 clones carrying potentially strong promoters were identified, some of which were DNA sequenced. The most potent B. anthracis promoter identified (Pntr; 271 bp) was 500 times more potent than the native pagA promoter and 70 times more potent than the {alpha}-amylase promoter (Pamy). This very potent promoter was tested along with the other promoters (which are three, six, and eight times more potent than Pamy) for the ability to drive expression of rPA in either B. subtilis or B. anthracis. The number of cell-associated pre-PA molecules in B. anthracis was found to correlate well with the strength of the promoter. However, there appeared to be an upper limit to the amount of mature PA secreted into the medium, which did not exceed that driven by Pamy. Furthermore, the rPA constructs fused to the very potent promoters proved to be deleterious to the bacterial hosts and consequently led to genetic instability of the PA expression plasmid. Immunization with attenuated B. anthracis expressing rPA under the control of promoters more potent than Pamy was less efficient in eliciting anti-PA antibodies than that attained with Pamy. The results are consistent with the notion that overexpression of PA leads to severe secretion stress and have practical implications for the design of second-generation rPA-based vaccines.

Members of the genus Bartonella have historically been connected with human disease, such as cat scratch disease, trench fever, and Carrion's disease, and recently have been recognized as emerging pathogens causing other clinical manifestations in humans. However, because little is known about the antigens that elicit antibody production in response to Bartonella infections, this project was undertaken to identify and molecularly characterize these immunogens. Immunologic screening of a Bartonella vinsonii subsp. berkhoffii genomic expression library with anti-Bartonella antibodies led to the identification of the sucB gene, which encodes the enzyme dihydrolipoamide succinyltransferase. Antiserum from a mouse experimentally infected with live Bartonella was reactive against recombinant SucB, indicating the mounting of an anti-SucB response following infection. Antigenic cross-reactivity was observed with antiserum against other Bartonella spp. Antibodies against Coxiella burnetii, Francisella tularensis, and Rickettsia typhi also reacted with our recombinant Bartonella SucB. Potential SucB antigenic cross-reactivity presents a challenge to the development of serodiagnostic tests for other intracellular pathogens that cause diseases such as Q fever, rickettsioses, brucelloses, tularemia, and other bartonelloses.


http://iai.asm.org/cgi/content/abstract/73/2/1151

GNA 1870 is a novel surface-exposed lipoprotein, identified by genome analysis of Neisseria meningitidis strain MC58, which induces bactericidal antibodies. Three sequence variants of the protein were shown to be sufficient to induce bactericidal antibodies against a panel of strains representative of the diversity of serogroup B meningococci. Here, we studied the antigenic and immunogenic properties of GNA 1870, which for convenience was divided into domains A, B, and C. The immune responses of mice immunized with each of the three variants were tested using overlapping peptides scanning the entire protein length and using recombinant fragments. We found that while most of the linear epitopes are located in the A domain, the bactericidal antibodies are directed against conformational epitopes located in the BC domain. This was also confirmed by the isolation of a bactericidal murine monoclonal antibody, which failed to recognize linear peptides on the A, B, and C domains separately but recognized a conformational epitope formed only by the combination of the B and C domains. Arginine in position 204 was identified as important for binding of the monoclonal antibody. The identification of the region containing bactericidal epitopes is an important step in the design of new vaccines against meningococci.


http://iai.asm.org/cgi/content/abstract/71/9/5202

Pregnancy increases the risk of listeriosis, a systemic disease caused by Listeria monocytogenes. However, there is incomplete agreement on the reasons for this increased risk. We examined two features of listeriosis in gravid and nongravid female mice following intragastric (gavage) inoculation, namely, (i) disease severity (measured by lethality) and (ii) listerial infectivity (measured by liver and spleen colonization levels up to 120 h postinoculation). Two listerial strains of differing serotype (1/2a and 4nonb) were initially employed. Neither strain produced a
lethal infection in nonpregnant female mice (dose range, 10^6 to 10^9 CFU/mouse), and only the
4nonb strain produced lethality in pregnant mice (dose range, 10^6 to 10^8 CFU/mouse). The
4nonb strain also produced a higher level of liver and spleen colonization than the 1/2a strain
following gavage administration. (The two strains showed similar levels of colonization if
parenterally administered.) Both strains were equally capable of binding to and forming plaques
upon cultured mouse enterocytes. The ability of the 4nonb strain to produce a lethal infection in
pregnant animals did not correlate with an increased incidence or level of liver and spleen
colonization over that in nonpregnant females. However, the lethality rate did correlate well with
the rate at which embryos and their surrounding decidual covering became infected, suggesting
that intrauterine infection could be responsible for the increased disease severity in the gravid
females.

Hase, K., L. Eckmann, et al. (2002). "Cell Differentiation Is a Key Determinant of Cathelicidin LL-
37/Human Cationic Antimicrobial Protein 18 Expression by Human Colon Epithelium." Infect.
http://iai.asm.org/cgi/content/abstract/70/2/953

Antimicrobial peptides are highly conserved evolutionarily and are thought to play an important
role in innate immunity at intestinal mucosal surfaces. To better understand the role of the
antimicrobial peptide human cathelicidin LL-37/human cationic antimicrobial protein 18 (hCAP18)
in intestinal mucosal defense, we characterized the regulated expression and production of this
peptide by human intestinal epithelium. LL-37/hCAP18 is shown to be expressed within epithelial
cells located at the surface and upper crypts of normal human colon. Little or no expression was
seen within the deeper colon crypts or within epithelial cells of the small intestine. Paralleling its
expression in more differentiated epithelial cells in vivo, LL-37/hCAP18 mRNA and protein
expression was upregulated in spontaneously differentiating Caco-2 human colon epithelial cells
and in HCA-7 human colon epithelial cells treated with the cell differentiation-inducing agent
sodium butyrate. LL-37/hCAP18 expression by colon epithelium does not require commensal
bacteria, since LL-37/hCAP18 is produced with a similar expression pattern by epithelial cells in
human colon xenografts that lack a luminal microflora. LL-37/hCAP18 mRNA was not
upregulated in response to tumor necrosis factor alpha, interleukin 1{alpha} (IL-1{alpha}), gamma
interferon, lipopolysaccharide, or IL-6, nor did the expression patterns and levels of LL-
37/hCAP18 in the epithelium of the normal and inflamed colon differ. On the other hand, infection
of HCA-7 cells with Salmonella enterica serovar Dublin or enteroinvasive Escherichia coli
modestly upregulated LL-37/hCAP18 mRNA expression. We conclude that differentiated human
colon epithelium expresses LL-37/hCAP18 as part of its repertoire of innate defense molecules
and that the distribution and regulated expression of LL-37/hCAP18 in the colon differs markedly
from that of other enteric antimicrobial peptides, such as defensins.

Ikebe, T., A. Wada, et al. (2002). "Dissemination of the Phage-Associated Novel Superantigen Gene speL
in Recent Invasive and Noninvasive Streptococcus pyogenes M3/T3 Isolates in Japan." Infect.
http://iai.asm.org/cgi/content/abstract/70/6/3227

In Japan, more than 10% of streptococcal toxic shock-like syndrome (TSLS) cases have been
cased by Streptococcus pyogenes M3/T3 isolates since the first reported TSLS case in 1992.
Most M3/T3 isolates from TSLS or severe invasive infection cases during 1992 to 2001 and those
from noninvasive cases during this period are indistinguishable in pulsed-field gel
electrophorograms. The longest fragments of these recent isolates were 300 kb in size, whereas
those of isolates recovered during or before 1973 were 260 kb in size. These 260- and 300-kb
fragments hybridized to each other, suggesting the acquisition of an about 40-kb fragment by the recent isolates. The whole part of the acquired fragment was cloned from the first Japanese TLS isolate, NIH1, and its nucleotide sequence was determined. The 41,796-bp fragment is temperate phage {phi}NIH1.1, containing a new superantigen gene speL near its right attachment site. The C-terminal part of the deduced amino acid sequence of speL has 48 and 46% similarity with well-characterized erythrogenic toxin SpeC and the most potent superantigen, SmeZ-2, respectively. None of 10 T3 isolates recovered during or before 1973 has speL, whereas all of 18 M3/T3 isolates recovered during or after 1992 and, surprisingly, Streptococcus equi subsp. equi ATCC 9527 do have this gene. Though plaques could not be obtained from {phi}NIH1.1, its DNA became detectable from the phage particle fraction upon mitomycin C induction, showing that this phage is not defective. A horizontal transfer of the phage carrying speL may explain the observed change in M3/T3 S. pyogenes isolates in Japan.


Using in vivo-induced antigen technology (IVIAT), a modified immunoscreening technique that circumvents the need for animal models, we directly identified immunogenic Escherichia coli O157:H7 (O157) proteins expressed either specifically during human infection but not during growth under standard laboratory conditions or at significantly higher levels in vivo than in vitro. IVIAT identified 223 O157 proteins expressed during human infection, several of which were unique to this study. These in vivo-induced (ivi) proteins, encoded by ivi genes, mapped to the backbone, O islands (OIs), and pO157. Lack of in vitro expression of O157-specific ivi proteins was confirmed by proteomic analysis of a mid-exponential-phase culture of E. coli O157 grown in LB broth. Because ivi proteins are expressed in response to specific cues during infection and might help pathogens adapt to and counter hostile in vivo environments, those identified in this study are potential targets for drug and vaccine development. Also, such proteins may be exploited as markers of O157 infection in stool specimens.


Intimin is the primary adhesin of Escherichia coli O157:H7, the most common infectious cause of bloody diarrhea in the United States and the leading cause of acute kidney failure in children who develop hemolytic uremic syndrome. Cattle are the primary reservoir of E. coli O157:H7. Indeed, most cases of E. coli O157:H7 infection in the United States occur after ingestion of contaminated undercooked hamburger or produce that had contact with bovine manure. Because intimin is required for persistent colonization of neonatal calves and adult cattle, we hypothesized that an intimin-based vaccination strategy in calves would reduce colonization of cattle with E. coli O157:H7. To test this concept in a small-animal model, we developed transgenic tobacco plant cells that express the carboxy-terminal host cell-binding domain of E. coli O157:H7 intimin. Mice were either immunized intraperitoneally with intimin expressed from the plant cells, fed transgenic plant cells, or both. Here we show that these mice generated an intimin-specific mucosal immune response when primed parenterally and then boosted orally and also exhibited a reduced duration of E. coli O157:H7 fecal shedding after challenge.

http://iai.asm.org/cgi/content/abstract/70/11/6436

Mice incapable of generating an efficient Th2 response because of functional deletion of the genes for signal transducer and activation of transcription 6 (Stat6), interleukin-4 receptor alpha chain (IL-4R{alpha}), or IL-4 plus IL-13 (IL-4/IL-13) were no more resistant than wild-type (WT) mice to airborne infection with virulent Mycobacterium tuberculosis. WT mice were able to control infection and hold it at a stationary level following 20 days of log linear M. tuberculosis growth. Likewise, infection was kept under control and was held at the same stationary level in IL-4/IL-13-/- mice but progressed to a slightly higher level in Stat6-/- and IL-4R{alpha}-/- mice. The onset of stationary-level infection in WT mice was associated with the expression of Th1-mediated immunity, as evidenced by an approximately 100- to 1,000-fold increase in the lungs in the synthesis of mRNA for IL-12, gamma interferon (IFN-{gamma}), and inducible nitric oxide synthase (NOS2) that was sustained for at least 100 days. IL-12 is essential for the induction of Th1 immunity, IFN-{gamma} is a key Th1 cytokine involved in mediation of immunity, and NOS2 is an inducible enzyme of macrophages and is needed by these cells to express immunity. In response to infection, the lungs of Stat6-/- mice showed increases in synthesis of mRNA for IL-12, IFN-{gamma}, and NOS2 similar to that seen in WT mice. In IL-4/IL-13-/- mice, however, synthesis of mRNA for IFN-{gamma} and NOS2 reached higher levels than in WT mice. These results argue against the notion that a Th2 response is partly or wholly responsible for the inability of Th1-mediated immunity to resolve infection with a virulent strain of M. tuberculosis.


http://iai.asm.org/cgi/content/abstract/72/12/7374

The role of {sigma}B in Listeria monocytogenes infection of human intestinal epithelial cells was investigated. Invasion defects associated with loss of {sigma}B paralleled those of a {Delta}inlA strain independently of the {sigma}B-dependent P2prfA promoter. Concomitantly, amounts of inlA transcript and InlA protein were significantly decreased in the {Delta}sigB strain.


http://iai.asm.org/cgi/content/abstract/70/3/1042

We investigated the role of osteopontin (OPN) in interleukin-12 (IL-12) production from peripheral blood mononuclear cells (PBMCs) stimulated with Penicillium marneffei. Kinetic studies showed that OPN synthesis preceded that of IL-12 at both mRNA and protein levels when PBMCs were cocultured with P. marneffei. Treatment with anti-OPN monoclonal antibodies (MAb) significantly suppressed IL-12 secretion. Furthermore, native OPN induced a profound level of synthesis of IL-12 from noninfected PBMCs. The major cellular source of OPN was monocytes, because depletion of CD14+ cells resulted in the abrogation of such production. We also examined the regulatory role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in OPN secretion from P. marneffei-stimulated PBMCs. Neutralizing anti-GM-CSF MAb significantly reduced OPN
secretion, and treatment with this cytokine induced OPN production from both infected and noninfected PBMCs. Finally, antagonists against the mannose receptor but not the \(\beta\)-glucan receptor almost completely abrogated the production of OPN. Our results demonstrated that OPN secreted from monocytes is involved in the production of IL-12 from PBMCs after stimulation with P. marneffei and that OPN production is regulated by GM-CSF. Our results also indicated the possible involvement of the mannose receptor as a signal-transducing receptor for triggering the secretion of OPN by P. marneffei-stimulated PBMCs.


http://iai.asm.org/cgi/content/abstract/71/11/6329

Rhodococcus equi is an opportunistic pathogen in immunocompromised humans and an important primary pathogen in young horses. Although R. equi infection can produce life-threatening pyogranulomatous pneumonia, most foals develop a protective immune response that lasts throughout life. The antigen targets of this protective response are currently unknown; however, Mycobacterium tuberculosis is a closely related intracellular pathogen and provides a model system. Based on previous studies of M. tuberculosis protective antigens released into culture filtrate supernatant (CFS), a bacterial growth system was developed for obtaining R. equi CFS antigens. Potential immunogens for prevention of equine rhodococcal pneumonia were identified by using immunoblots. The 48-h CFS contained five virulence-associated protein bands that migrated between 12 and 24 kDa and were recognized by sera from R. equi-infected foals and immune adult horses. Notably, the CFS contained the previously characterized proteins VapC, VapD, and VapE, which are encoded by genes on the R. equi virulence plasmid. R. equi CFS was also examined for the ability to stimulate a type 1-like memory response in immune horses. Three adult horses were challenged with virulent R. equi, and cells from the bronchoalveolar lavage fluid were recovered before and 1 week after challenge. In vitro stimulation of pulmonary T-lymphocytes with R. equi CFS resulted in significant proliferation and a significant increase in gamma interferon mRNA expression 1 week after challenge. These results were consistent with a memory effector response in immune adult horses and provide evidence that R. equi CFS proteins are antigen targets in the immunoprotective response against R. equi infection.


http://iai.asm.org/cgi/content/abstract/72/12/6817

The protozoan parasite Trypanosoma cruzi circulates in the blood as trypomastigotes and invades a variety of cells to multiply intracellularly as amastigotes. The acute phase leads to an immune response that restricts the proliferation of the parasite. However, parasites are able to persist in different tissues, which causes the pathology of Chagas’ disease. Natural killer (NK) cells play an important role in innate resistance to a variety of pathogens. In the present study we analyzed whether NK cells participated in the control of experimental T. cruzi infection. NK cells were depleted from C57BL/6 mice by antiasialo antibodies. This treatment caused an increased parasitemia during the acute phase, but tissue parasite burdens were not significantly altered according to quantitative real-time PCR. Our results demonstrated that NK cells were activated during the initial phase of a T. cruzi infection and exhibited a contact-dependent antiparasitic activity against extracellular parasites that was independent from perforin. Thus, NK cells limit the
propagation of the parasite by acting on circulating T. cruzi trypomastigotes.


http://iai.asm.org/cgi/content/abstract/70/1/114

Infectivity of *Anaplasma* spp. develops when infected ticks feed on a mammalian host (transmission feed). Specific *Anaplasma marginale* major surface protein 2 (MSP2) variants are selected for within the tick and are expressed within the salivary glands. The aims of this study were to determine when and where MSP2 variant selection occurs in the tick, how MSP2 expression is regulated in salivary glands of transmission-feeding ticks, and whether the number of *A. marginale* organisms per salivary gland is significantly increased during transmission feeding. The South Idaho strain of *A. marginale* was used, as MSP2 expression is restricted to two variants, SGV1 and SGV2, in Dermacentor andersoni. Using Western blot, real-time PCR, and DNA sequencing analyses it was shown that restriction and expression of MSP2 occurs early in the midgut within the first 48 h of the blood meal, when ticks acquire infection. *A. marginale* is present in the tick salivary glands before transmission feeding is initiated, but the msp2 mRNA and MSP2 protein levels per *A. marginale* organism increase only minimally and transiently in salivary glands of transmission-feeding ticks compared to that of unfed ticks. *A. marginale* numbers per tick increase gradually in salivary glands of both transmission-fed and unfed ticks. It is concluded that MSP2 variant selection is an early event in the tick and that MSP2 variants SGV1 and SGV2 are expressed both in the midgut and salivary glands. While MSP2 may be required for infectivity, there is no strict temporal correlation between MSP2 expression and the development of infectivity.


http://iai.asm.org/cgi/content/abstract/71/11/6279

The gonococcal pilus is a major virulence factor that has well-established roles in mediating epithelial cell adherence and DNA transformation. Gonococci expressing four gonococcal pilin variants with distinct piliation properties under control of the lac regulatory system were grown in different levels of the inducer isopropyl-[beta]-D-thiogalactopyranoside (IPTG). These pilin variants expressed various levels of pilin message and pilin protein in response to the level of IPTG in the growth medium. Moreover, posttranslational modifications of the variant pilin proteins were detected, including S-pilin production and glycosylation. The ratio of the modified and unmodified pilin forms did not substantially change with different levels of pilin expression, showing that these modifications are not linked to pilin expression levels. DNA transformation competence was also influenced by IPTG levels in the growth medium. Substantial increases in transformation competence over an isogenic, nonpiliated mutant were observed when limited amounts of three of the pilin variants were expressed. Immunelectron microscopy showed that when limited amounts of pilin are expressed, pili are rare and do not explain the pilin-dependent transformation competence. This pilin-dependent transformation competence required prepilin processing, the outer membrane secretin PilQ, and the twitching-motility-regulating protein PilT. These requirements show that a fully functional pilus assembly apparatus is required for DNA uptake when limited pilin is produced. We conclude that the pilus assembly apparatus functions to import DNA into the bacterial cell in a pilin-dependent manner but that extended pili are not required for transformation competence.

We used a mouse model of acute respiratory infections to investigate the role of Toll-like receptor 2 (TLR2) and TLR4 in the host response to Haemophilus influenzae. Acute aerosol exposures to wild-type strains of *H.* influenzae showed that TLR4 function was essential for TNF-α induction, neutrophil influx, and bacterial clearance. To determine how lipooligosaccharide (LOS) modifications would affect the role of TLR4 in inducing the host response, we used acute infections with an *H.* influenzae strain expressing a mutation in the htrB gene. This mutant strain expresses an LOS subunit with decreased acylation. In response to *H.* influenzae htrB infection, tumor necrosis factor alpha (TNF-α) secretion remained TLR4 dependent. But the decrease in LOS acylation made the neutrophil influx and the bacterial clearance also dependent on TLR2, as shown by the decreased host response elicited in TLR2 knockout mice compared to C57BL/6 mice. A subsequent analysis of TLR2 and TLR4 gene expression by quantitative PCR indicated that TLR4 function induces TLR2 expression and vice versa. These results indicate that some changes in the LOS subunit of *H.* influenzae can favor signaling through non-TLR4 receptors, such as TLR2. The results also indicate a close interaction between TLR4 and TLR2 that tightly regulates the expression of both receptors.


Cohesive interactions between Porphyromonas gingivalis and plaque-forming bacteria, such as Streptococcus oralis, are considered to play an important role in the colonization of *P.* gingivalis in periodontal sites. Although *P.* gingivalis fimbriae have been reported to mediate coaggregation with *S.* oralis, the *S.* oralis molecule involved has not been identified. We identified the coadhesin of *S.* oralis ATCC 9811 and purified it by affinity column chromatography. We found that the molecular mass of the purified protein was approximately 40 kDa. Dot blot and Western blot assays showed binding of the 40-kDa protein to *P.* gingivalis fimbriae. Further, turbidimetric assays showed that the coadhesin inhibited coaggregation between *P.* gingivalis and *S.* oralis in a dose-dependent manner. Analyses of the amino-terminal sequences of the protein and its lysyl endopeptidase-cleaved fragments revealed that the coadhesin was identical to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Next, we cloned the gene that encodes *S.* oralis GAPDH and found that the sequence had a high degree of homology with the sequences of GAPDHs of various bacteria, including *Streptococcus gordonii* and *Fusobacterium nucleatum*. To confirm the contribution of *S.* oralis GAPDH to the interaction with *P.* gingivalis, a recombinant GAPDH protein was generated in *Escherichia coli*; this protein bound to *P.* gingivalis fimbriae and had an inhibitory effect on coaggregation. These results suggest that *S.* oralis GAPDH functions as a coadhesin for *P.* gingivalis fimbriae. In addition, considering the high degree of homology of the GAPDHs of various bacteria, those of other plaque-forming bacteria also may contribute to the colonization of *P.* gingivalis.

The effectiveness of malaria control measures depends not only on the potency of the control measures themselves but also upon the influence of variables associated with the environment. Environmental variables have the capacity either to enhance or to impair the desired outcome. An optimal outcome in the field, which is ultimately the real goal of vaccine research, will result from prior knowledge of both the potency of the control measures and the role of environmental variables. Here we describe both the potential effectiveness of control measures and the problems associated with testing in an area of endemicity. We placed canaries with different immunologic backgrounds (e.g., naive to malaria infection, vaccinated naive, and immune) directly into an area where avian malaria, Plasmodium relictum, is endemic. In our study setting, canaries that are naive to malaria infection routinely suffer approximately 50% mortality during their first period of exposure to the disease. In comparison, birds vaccinated and boosted with a DNA vaccine plasmid encoding the circumsporozoite protein of P. relictum exhibited a moderate degree of protection against natural infection (P < 0.01). In the second year we followed the fate of all surviving birds with no further manipulation. The vaccinated birds from the first year were no longer statistically distinguishable for protection against malaria from cages of naive birds. During this period, 36% of vaccinated birds died of malaria. We postulate that the vaccine-induced protective immune responses prevented the acquisition of natural immunity similar to that concurrently acquired by birds in a neighboring cage. These results indicate that dominant environmental parameters associated with malaria deaths can be addressed before their application to a less malleable human system.


http://iai.asm.org/cgi/content/abstract/70/1/303

Bivalent recombinant strains of Mycobacterium bovis BCG (rBCG) expressing the early regulatory nef and the structural gag(p26) genes from the simian immunodeficiency virus (SIV) SIVmac251 were engineered so that both genes were cotranscribed from a synthetic operon. The expression cassette was cloned into a multicopy-replicating vector, and the expression levels of both nef and gag in the bivalent rBCG(nef-gag) strain were found to be comparable to those of monovalent rBCG(nef) or rBCG(gag) strains. However, extrachromosomal cloning of the nef-gag operon into a replicative plasmid resulted in strains of low genetic stability that rapidly lost the plasmid in vivo. Thus, the nef-gag operon was inserted site specifically into the BCG chromosome by means of mycobacteriophage Ms6-derived vectors. The resulting integrative rBCG(nef-gag) strains showed very high genetic stability both in vitro and in vivo. The in vivo expression of the heterologous genes was much longer lived when the expression cassette was inserted into the BCG chromosome. In one of the strains obtained, integrative cloning did not reduce the expression levels of the genes even though a single copy was present. Accordingly, this strain induced cellular immune responses of the same magnitude as that of the replicative rBCG strain containing several copies of the genes.


http://iai.asm.org/cgi/content/abstract/72/4/1896
The discovery that bovine peripheral lymphocytes are sensitive to Stx1 identified a possible mechanism for the persistence of infections with Shiga toxin (Stx)-producing Escherichia coli (STEC) in the bovine reservoir host. If intraepithelial lymphocytes (IEL) are also sensitive to Stx1, the idea that Stx1 affects inflammation in the bovine intestine is highly attractive. To prove this hypothesis, ileal IEL (iIEL) were prepared from adult cattle, characterized by flow cytometry, and subjected to functional assays in the presence and absence of purified Stx1. We found that 14.9% of all iIEL expressed Gb3/CD77, the Stx1 receptor on bovine lymphocytes, and 7.9% were able to bind the recombinant B subunit of Stx1. The majority of Gb3/CD77+ cells were activated CD3+ CD6+ CD8(α)+ T cells, whereas only some CD4+ T cells and B cells expressed Gb3/CD77. However, Stx1 blocked the mitogen-induced transformation to enlarged blast cells within all subpopulations to a similar extent and significantly reduced the percentage of Gb3/CD77+ cells. Although Stx1 did not affect the natural killer cell activity of iIEL, the toxin accelerated the synthesis of interleukin-4 (IL-4) mRNA and reduced the amount of IL-8 mRNA in bovine iIEL cultures. Because the intestinal system comprises a rich network of interactions between different types of cells and any dysfunction may influence the course of intestinal infections, this demonstration that Stx1 can target bovine IEL may be highly relevant for our understanding of the interplay between STEC and its reservoir host.


http://iai.asm.org/cgi/content/abstract/73/1/546

The dynamics of host-pathogen interactions have important implications for the design of new antimicrobial agents to treat chronic infections such as tuberculosis (TB), which is notoriously refractory to conventional drug therapy. In the mouse model of TB, an acute phase of exponential bacterial growth in the lungs is followed by a chronic phase characterized by relatively stable numbers of bacteria. This equilibrium could be static, with little ongoing replication, or dynamic, with continuous bacterial multiplication balanced by bacterial killing. A static model predicts a close correspondence between "viable counts" (live bacteria) and "total counts" (live plus dead bacteria) in the lungs over time. A dynamic model predicts the divergence of total counts and viable counts over time due to the accumulation of dead bacteria. Here, viable counts are defined as bacterial CFU enumerated by plating lung homogenates; total counts are defined as bacterial chromosome equivalents (CEQ) enumerated by using quantitative real-time PCR. We show that the viable and total bacterial counts in the lungs of chronically infected mice do not diverge over time. Rapid degradation of dead bacteria is unlikely to account for the stability of bacterial CEQ numbers in the lungs over time, because treatment of mice with isoniazid for 8 weeks led to a marked reduction in the number of CFU without reducing the number of CEQ. These observations support the hypothesis that the stable number of bacterial CFU in the lungs during chronic infection represents a static equilibrium between host and pathogen.


http://iai.asm.org/cgi/content/abstract/70/10/5706

The study of how bacteria respond to and obtain divalent metal ions provides insight into the regulation of virulence factors in the host environment. Regulation of metal permease operons in gram-positive bacteria may involve the binding of metal-responsive repressors to palindromic domains in their control regions. The Streptococcus parasanguis fimA operon, which encodes an
ATP-binding cassette (ABC) transporter system with sequence homology to the LraI family of metal transporters, possesses a palindromic regulatory region with high homology to that of the Streptococcus gordonii ScaR binding domain. Mapping of the promoter and regulatory regions of fimA and the divergently transcribed pepO gene, which encodes a zinc metalloendopeptidase, indicated that their promoter and regulatory elements overlap. fimA had one transcriptional start site, whereas pepO had three. Analysis of truncated versions of the pepO promoter suggested that all three transcriptional start sites are functional. Analysis of promoter activity under various environmental conditions indicated that the fimA operon promoter and the pepO promoter are not coordinately regulated. The fimA operon is responsive to changes in Mn2+ concentration, but the pepO promoter is not. A S. parasanguis fimA mutant showed a growth deficiency under conditions of limiting Mn2+. This deficiency was not alleviated by compensation with either Mg2+ or Fe3+. Wild-type S. parasanguis could take up Mn2+ and Fe3+, while the fimA mutant showed a marked reduction in this ability. These data suggested that FimA is a component of a metal transporter system capable of transporting both Mn2+ and Fe3+. FimA expression itself was shown to be responsive to Mn2+ concentration, but not to availability of Fe3+ or Mg2+.


http://iai.asm.org/cgi/content/abstract/70/10/5759

Cytolysin A (ClyA) is a pore-forming cytotoxic protein encoded by the clyA gene that has been characterized so far only in Escherichia coli. Using DNA sequence analysis and PCR, we established that clyA is conserved in the human-specific typhoid Salmonella enterica serovars Typhi and Paratyphi A and that the entire clyA gene locus is absent in many other S. enterica serovars, including Typhimurium. The gene products, designated ClyASTy and ClyASPa, show ≥90% amino acid identity to E. coli cytolysin A, ClyAEC, and they are immunogenically related. The Salmonella proteins showed a pore-forming activity and are hence functional homologues to ClyAEC. The chromosomal clyASTy gene locus was expressed at detectable levels in the serovar Typhi strains S2369/96 and S1112/97. Furthermore, in the serovar Typhi vaccine strain Ty21a, expression of clyASTy reached phenotypic levels, as detected on blood agar plates. The hemolytic phenotype was abolished by the introduction of an in-frame deletion in the clyASTy chromosomal locus of Ty21a. Transcomplementation of the mutant with a cloned clyASTy gene restored the hemolytic phenotype. To our knowledge, Ty21a is the first reported phenotypically hemolytic Salmonella strain in which the genetic determinant has been identified.


http://iai.asm.org/cgi/content/abstract/70/12/6839

It was previously demonstrated that avirulent Mycoplasma gallisepticum strain Rhhigh (passage 164) is lacking three proteins that are expressed in its virulent progenitor, strain Rlow (passage 15). These proteins were identified as the cytadhesin molecule GapA, the putative cytadhesin-related molecule CrmA, and a component of a high-affinity transporter system, HatA. Complementation of Rhhigh with wild-type gapA restored expression in the transformant (GT5) but did not restore the cytadherence phenotype and maintained avirulence in chickens. These results suggested that CrmA might play an essential role in the M. gallisepticum cytadherence process. CrmA is encoded by the second gene in the gapA operon and shares significant sequence homology to the ORF6 gene of Mycoplasma pneumoniae, which has been shown to play an accessory role in the cytadherence process. Complementation of Rhhigh with wild-type crmA resulted in the transformant (SDCA) that lacked the cytadherence and virulence phenotype.
comparable to that found in Rhigh and GT5. In contrast, complementation of Rhigh with the entire wild-type gapA operon resulted in the transformant (GCA1) that restored cytadherence to the level found in wild-type Rlow. In vivo pathogenesis trials revealed that GCA1 had regained virulence, causing airsacculitis in chickens. These results demonstrate that both GapA and CrmA are required for M. gallisepticum cytadherence and pathogenesis.


http://iai.asm.org/cgi/content/abstract/73/4/2175

Cytokine gene expression in cells migrating in afferent and efferent intestinal lymph was monitored for extended time periods in individual sheep experimentally infected with the nematode Trichostrongylus colubriformis. Animals from stable selection lines with increased levels of either genetic resistance (R) or susceptibility (S) to nematode infection were used. Genes for interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha (TNF-(alpha)), but not for IL-4, IL-10, or gamma interferon (IFN-(gamma)), were consistently expressed at higher levels in both afferent and efferent lymph cells of R sheep than in S sheep. However, only minor differences were observed in the surface phenotypes and antigenic and mitogenic responsiveness of cells in intestinal lymph between animals from the two selection lines. The IL-4 and IL-10 genes were expressed at higher levels in afferent lymph cells than in efferent lymph cells throughout the course of the nematode infection in animals of both genotypes, while the proinflammatory TNF-(alpha) gene was relatively highly expressed in both lymph types. These relationships notwithstanding, expression of the IL-10 and TNF-(alpha) genes declined significantly in afferent lymph cells but not in efferent lymph cells during infection. Collectively, the results showed that R-line sheep developed a strong polarization toward a Th2-type cytokine profile in immune cells migrating in lymph from sites where the immune response to nematodes was initiated, although the IFN-(gamma) gene was also expressed at moderate levels. Genes or alleles that predispose an animal to develop this type of response appear to have segregated with the R selection line and may contribute to the increased resistance of these animals.


http://iai.asm.org/cgi/content/abstract/71/2/647

A deletion-insertion mutation in msbB, a gene that encodes a lipid A acyltransferase, was introduced into encapsulated Neisseria meningitidis serogroup B strain NMB and an acapsular mutant of the same strain. These mutants were designated NMBA11K3 and NMBA11K3cap-, respectively. Neither lipooligosaccharide (LOS) nor lipid A could be isolated from NMBA11K3 although a number of techniques were tried, but both were easily extracted from NMBA11K3cap-. Immunoelectron microscopy using monoclonal antibody (MAB) 6B4, which recognizes the terminal Gal(beta)1-4GlcNAc of LOS, demonstrated that NMB, NMBcap-, and NMBA11K3cap-expressed LOS circumferentially, while MAB 6B4 did not bind to the surface of NMBA11K3. However, cytoplasmic staining of NMBA11K3 with MAB 6B4 was a consistent observation. Mass-spectrometric analyses demonstrated that the relative amounts of the lipid A-specific C12:0 3-OH and C14:0 3-OH present in the membrane preparations (MP) from NMBA11K3 were substantially decreased (25- and 23-fold, respectively) compared to the amount in MP from its parent strain, NMB. Western blot analyses of MP from NMBA11K3 demonstrated that the levels of porin in the
outer membrane of NMBA11K3 were also substantially decreased. These studies suggest that
the lipid A acylation defect in encapsulated NMBA11K3 influences the assembly of the lipid A and
consequently the incorporation of porin in the outer membrane.


http://iai.asm.org/cgi/content/abstract/70/2/909

Neisseria gonorrhoeae is a strict human pathogen that invades and colonizes the urogenital
tracts of males and females. Lipooligosaccharide (LOS) has been shown to play a role in
gonococcal pathogenesis. The acyl transferase MsbB is involved in the biosynthesis of the lipid A
portion of the LOS. In order to determine the role of an intact lipid A structure on the pathogenesis
of N. gonorrhoeae, the msbB gene was cloned and sequenced, a deletion and insertion mutation
was introduced into N. gonorrhoeae, and the mutant strain was designated 1291A11K3. Mass
spectrometric analyses of 1291A11K3 LOS determined that this mutation resulted in a pentaacyl
rather than a hexaacyl lipid A structure. These analyses also demonstrated an increase in the
phosphorylation of lipid A and an increase in length of the oligosaccharide of a minor species of
the msbB LOS. The interactions of this mutant with male urethral epithelial cells (uec) were
examined. Transmission and scanning electron microscopy studies indicated that the msbB
mutants formed close associations with and were internalized by the uec at levels similar to those
of the parent strain. Gentamicin survival assays performed with 1291A11K3 and 1291 bacteria
demonstrated that there was no difference in the abilities of the two strains to adhere to uec;
however, significantly fewer 1291A11K3 bacteria than parent strain bacteria were recovered from
gentamicin-treated uec. These studies suggest that the lipid A modification in the N. gonorrhoeae
msbB mutant may render it more susceptible to innate intracellular killing mechanisms when
internalized by uec.

Recktenwald, J. and H. Schmidt (2002). "The Nucleotide Sequence of Shiga Toxin (Stx) 2e-Encoding
Phage (phi)P27 Is Not Related to Other Stx Phage Genomes, but the Modular Genetic Structure

http://iai.asm.org/cgi/content/abstract/70/4/1896

In this study we determined the complete nucleotide sequence of Shiga toxin 2e-encoding
bacteriophage (phi)P27, isolated from the Shiga toxin-producing Escherichia coli patient isolate
2771/97. (phi)P27 is integrated as a prophage in the chromosomal yecE gene. This integration
generates identity segments of attl and attR sites with lengths of 11 nucleotides. The integrated
prophage genome has a size of 42,575 bp. We identified 58 open reading frames (ORFs), each
with a length of >150 nucleotides. The deduced proteins of 44 ORFs showed significant
homologies to other proteins present in sequence databases, whereas 14 putative proteins did
not. For 29 proteins, we could deduce a putative function. Most of these are related to the basic
phage propagation cycle. The (phi)P27 genome represents a mosaic composed of genetic
elements which are obviously derived from related and unrelated phages. We identified five short
linker sequences of 22 to 151 bp in the (phi)P27 sequence which have also been detected in a
couple of other lambdoid phages. These linkers are located between functional modules in the
phage genome and are thought to play a role in genetic recombination. Although the overall DNA
sequence of (phi)P27 is not highly related to other known phages, the data obtained demonstrate
a typical lambdoid genome structure.
The intracellular life of Listeria monocytogenes starts by a complex process of entry involving several bacterial ligands and eukaryotic receptors. In this work, we identified in silico from the sequence of the genome of L. monocytogenes a previously unknown gene designated lpeA (for lipoprotein promoting entry) encoding a 35-kDa protein homologous to PsaA, a lipoprotein belonging to the LraI family and implicated in the cell adherence of Streptococcus pneumoniae and related species. By constructing a mutant of L. monocytogenes in which lpeA is deleted (lpeA mutant), we show that the PsaA-like protein LpeA is not involved in bacterial adherence but is required for entry of L. monocytogenes in eukaryotic cells. In contrast to wild-type bacteria, mutant bacteria failed to invade the epithelial Caco-2 and hepatocyte TIB73 cell lines, as confirmed by confocal microscopy. The mutant bacteria rapidly penetrated in mouse bone marrow-derived macrophages. Surprisingly, lpeA mutant bacteria survive better in macrophages than do wild-type bacteria. This was correlated with a weak exacerbation of virulence of the lpeA mutant in the mouse. LpeA is therefore a novel invasin favoring the entry of L. monocytogenes into nonprofessional phagocytes but not its invasion of macrophages. This is the first report of a lipoprotein promoting cell invasion of an intracellular pathogen.


http://iai.asm.org/cgi/content/abstract/71/12/7202

A 19-kb DNA region containing genes involved in the biosynthesis of the capsule of Haemophilus influenzae serotype f (Hif) has been cloned and characterized. The Hif cap locus organization is typical of group II capsule biosynthetic loci found in other H. influenzae serotype b bacteria and other gram-negative bacteria. However, the Hif cap locus was not associated with an IS1016 element. Three new open reading frames, Fcs1, Fcs2, and Fcs3, were identified in the Hif capsule-specific region II. The chromosomal location of the Hif cap locus and the organization of the flanking sequences differed significantly from previously described division I H. influenzae serotypes.


http://iai.asm.org/cgi/content/abstract/73/2/1161

Enteropathogenic Escherichia coli (EPEC) is an important cause of diarrhea in humans. EPEC infection of cultured intestinal epithelial cells induces attaching and effacing (A/E) lesions, alters intestinal ion transport, increases paracellular permeability, and stimulates inflammation. The lack of a small-animal model has restricted in vivo studies examining EPEC-host interactions. The aim of this study was to characterize the C57BL/6J mouse as a model of EPEC infection. We have shown that EPEC can adhere to and colonize the intestinal epithelium of C57BL/6J mice. Animal weight and water intake were not altered during 10 days of EPEC infection. The proximal colon of infected mice contained semisolid stool, with stool pellets forming only in the distal colon. In contrast, the entire colon of control mice contained formed stool. Microvillous effacement and actin rearrangement, characteristic of A/E lesions, were seen in the intestine of infected mice but not control mice. Histological assessment revealed increased numbers of lamina propria
neutrophils with occasional crypt abscesses, intraepithelial lymphocytes, and goblet cells in the intestine of EPEC-infected mice. Altogether, these data suggest that the C57BL/6J mouse is susceptible to infection by EPEC and will provide a suitable in vivo model for studying the consequences of EPEC infection.


http://iai.asm.org/cgi/content/abstract/72/4/1983

Mice deficient in interleukin-2 are well suited for use as an animal model for inflammatory bowel disease. Raised under specific-pathogen-free conditions, interleukin-2-deficient mice develop an inflammatory bowel disease resembling ulcerative colitis in humans. The finding that colitis was attenuated when the mice were kept under germfree conditions implies that the resident intestinal flora is involved in the pathogenesis of colitis. The present study addresses the composition of the mucosa-associated bacterial flora in colon samples from interleukin-2-deficient mice that developed colitis. This was investigated by comparative 16S ribosomal DNA (rDNA) sequence analysis and fluorescence in situ hybridization using rRNA-targeted fluorescent probes to quantify the bacterial populations of the mucosa-associated flora. The investigations revealed distinct differences in the bacterial composition of the mucosa-associated flora between interleukin-2-deficient mice and healthy controls. Fluorescence in situ hybridization identified up to 10% of the mucosa-associated flora in interleukin-2-deficient mice as Escherichia coli, whereas no E. coli was detected in the mucosa from healthy wild-type mice. This finding was consistent with the results from comparative 16S rDNA analysis. About one-third of the clones analyzed from 16S rDNA libraries of interleukin-2-deficient mice represented Enterobacteriaceae, whereas none of the clones analyzed from the healthy controls harbored 16S rDNA from Enterobacteriaceae. The abundance of E. coli in the colonic mucosa of interleukin-2-deficient mice strongly suggests a participation in the pathogenesis of colitis in the interleukin-2-deficient mouse model for inflammatory bowel disease.


http://iai.asm.org/cgi/content/abstract/70/2/794

Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, a disease characterized by pulmonary necrosis and hemorrhage caused in part by neutrophil degranulation. In an effort to understand the pathogenesis of this disease, we have developed an in vivo expression technology (IVET) system to identify genes that are specifically up-regulated during infection. One of the genes that we have identified as being induced in vivo is ohr, encoding organic hydroperoxide reductase, an enzyme that could play a role in detoxification of organic hydroperoxides generated during infection. Among the 12 serotypes of A. pleuropneumoniae, ohr was found in only serotypes 1, 9, and 11. This distribution correlated with increased resistance to cumene hydroperoxide, an organic hydroperoxide, but not to hydrogen peroxide or to paraquat, a superoxide generator. Functional assays of Ohr activity demonstrated that A. pleuropneumoniae serotype 1 cultures, but not serotype 5 cultures, were able to degrade cumene hydroperoxide. In A. pleuropneumoniae serotype 1, expression of ohr was induced by cumene hydroperoxide, but not by either hydrogen peroxide or paraquat. In contrast, an ohr gene from serotype 1 cloned into A. pleuropneumoniae serotype 5 was not induced by cumene hydroperoxide or by other forms of oxidative stress, suggesting the presence of a serotype-specific positive regulator of ohr in A. pleuropneumoniae serotype 1.
Actinobacillus pleuropneumoniae is a strict respiratory tract pathogen of swine and is the causative agent of porcine pleuropneumonia. We have used signature-tagged mutagenesis (STM) to identify genes required for survival of the organism within the pig. A total of 2,064 signature-tagged Tn10 transposon mutants were assembled into pools of 48 each, and used to inoculate pigs by the endotracheal route. Out of 105 mutants that were consistently attenuated in vivo, only 11 mutants showed a >2-fold reduction in growth in vitro compared to the wild type, whereas 8 of 14 mutants tested showed significant levels of attenuation in pig as evidenced from competitive index experiments. Inverse PCR was used to generate DNA sequence of the chromosomal domains flanking each transposon insertion. Only one sibling pair of mutants was identified, but three apparent transposon insertion hot spots were found—an anticipated consequence of the use of a Tn10-based system. Transposon insertions were found within 55 different loci, and similarity (BLAST) searching identified possible analogues or homologues for all but four of these. Matches included proteins putatively involved in metabolism and transport of various nutrients or unknown substances, in stress responses, in gene regulation, and in the production of cell surface components. Ten of the sequences have homology with genes involved in energy metabolism, nutrient uptake and stress responses for the survival of A. pleuropneumoniae in its natural host: the pig.

Recent reports have suggested that oral vaccination of mice against Helicobacter pylori is dependent on a Th1-mediated immune response. However, oral vaccination in mice neither induces sterilizing immunity nor leads to complete protection from disease. Therefore, in this study we investigated whether a systemic subcutaneous immunization against H. pylori by using CpG oligodeoxynucleotides as a Th1 adjuvant could achieve protection in a mouse model of H. pylori infection. CpG oligodeoxynucleotides are known for their ability to induce nearly entirely Th1-biased immune responses and may be approved for human use in future. Immunization of mice with H. pylori lysate and CpG induced a strong local and systemic Th1 immune response. Despite this strong Th1 response, mice were not protected from infection with H. pylori yet had a 10-fold reduction in the number of H. pylori in the gastric mucosa compared to nonimmunized mice. Of note, reduction of the bacterial density in immunized mice was accompanied by a significantly enhanced gastritis. Hence, systemic Th1 immunization of mice, even though being able to reduce the bacterial load in the stomach, is associated with aggravated pathology.

Bordetella avium causes an upper respiratory tract disease (bordetellosis) in avian species. Commercially raised turkeys are particularly susceptible. Like other pathogenic members of the genus Bordetella (B. pertussis and B. bronchiseptica) that infect mammals, B. avium binds preferentially to ciliated tracheal epithelial cells and produces similar signs of disease. These similarities prompted us to study bordetellosis in turkeys as a possible nonmammalian model for whooping cough, the exclusively human childhood disease caused by B. pertussis. One impediment to accepting such a host-pathogen model as relevant to the human situation is evidence suggesting that B. avium does not express a number of the factors known to be associated with virulence in the other two Bordetella species. Nevertheless, with signature-tagged mutagenesis, four avirulent mutants that had lesions in genes orthologous to those associated with virulence in B. pertussis and B. bronchiseptica (bvgS, fhaB, fhaC, and fimC) were identified. None of the four B. avium genes had been previously identified as encoding factors associated with virulence, and three of the insertions (in fhaB, bvgS, and fimC) were in genes or gene clusters inferred as being absent or incomplete in B. avium, based upon the lack of DNA sequence similarities in hybridization studies and/or the lack of immunological cross-reactivity of the putative products. We further found that the genotypic arrangements of most of the B. avium orthologues were very similar in all three Bordetella species. In vitro tests, including hemagglutination, tracheal ring binding, and serum sensitivity, helped further define the phenotypes conferred by the mutations. Our findings strengthen the connection between the causative agents and the pathogenesis of bordetellosis in all hosts and may help explain the striking similarities of the histopathologic characteristics of this upper airway disease in avian and mammalian species.


http://iai.asm.org/cgi/content/abstract/71/5/2693

The early consequences of Helicobacter pylori infection and the role of bacterial virulence determinants in disease outcome remain to be established. The present study sought to measure the development of host inflammatory and immune responses and their relationship to the putative bacterial virulence factors cag pathogenicity island (cagPAI), vacA allele, and oipA in combination with bacterial colonization density in a feline model of the early stages of H. pylori infection. Gastric tissues obtained from infected and uninfected cats were evaluated for H. pylori ureB, cagPAI, vacA allele, and oipA and colonization density (urease, histology, and real-time PCR). Inflammation was assessed by measuring mRNA upregulation of gamma interferon (IFN-(gamma)), interleukin (IL)-1(alpha), IL-1(beta), IL-4, IL-6, IL-8, IL-10, and IL-12 p40 and histopathology. The mucosal immune response was characterized by morphometric analysis of lymphoid follicles and by differentiating lymphocyte populations with antibodies against surface markers. Infecting H. pylori strains were positive for vacAs1 but lacked cagPAI and an active oipA gene. Colonization density was uniform throughout the stomach. Upregulation of IFN-(gamma), IL-1(alpha), IL-1(beta), and IL-8 and increased severity of inflammatory infiltrates and fibrosis were observed in infected cats. The median number and total area of lymphoid aggregates were 5 and 10 times greater, respectively, in the stomachs of infected than uninfected cats. Secondary lymphoid follicles in uninfected cats were rare and positive for BLA.36 and B220 but negative for CD3 and CD79(alpha), whereas in infected cats they were frequent and positive for BLA.36, CD79(alpha), and CD3 but negative for B220. Upregulation of IFN-(gamma), IL-1(alpha), IL-1(beta), and IL-8 and marked hyperplasia of secondary lymphoid follicles are early consequences of H. pylori infection in cats. The response appears to be similar to that of infected people, particularly children, can develop independently of the pathogenicity factors cagPAI and oipA, and is not correlated with the degree of colonization density or urease activity.

http://iai.asm.org/cgi/content/abstract/70/8/4661

Haemophilus influenzae is a commensal and opportunistic pathogen of the human airways. A number of surface molecules contribute to colonization of the airways by *H. influenzae*, such as adhesins, including structures found in the lipooligosaccharide (LOS). A human bronchiolar xenograft model was employed to investigate the host-bacterial interactions involved in the colonization of the airway by *H. influenzae*. Differential display was used to identify *H. influenzae* mRNA that reflect genes which were preferentially expressed in the xenograft compared to growth. Eleven mRNA fragments had consistent increased expression when the bacteria grew in xenografts. On sequencing these fragments, eight open reading frames were identified. Three of these had no match in the NCBI or the TIGR database, while an additional three were homologous to genes involved in heme or iron acquisition and utilization: two of the mRNAs encoded proteins homologous to enzymes involved in LOS biosynthesis: a heptosyl transferase (*rfaF*) involved in the synthesis of the LOS core and a ketodeoxyoctonate phosphate-dependent acyltransferase (*htrB*) that performs one of the late acylation reactions in lipid A synthesis. Inoculation of human bronchiolar xenografts revealed a significant reduction in colonization capacity by htrB mutants. In vitro, htrB mutants elicited lesser degrees of cytoskeletal rearrangement and less stimulation of host cell signaling with 16HBE14o- cells and decreased intracellular survival. These results implicate acylation of *H. influenzae* lipid A as playing a key role in the organisms’ colonization of the normal airway.


http://iai.asm.org/cgi/content/abstract/73/4/2312

Mouse Paneth cells respond to bacteria and bacterial cell surface antigens by discharging secretory granules into the lumen of small intestinal crypts (T. Ayabe et al., Nat. Immunol. 1:113-118, 2000). To investigate mechanisms regulating these responses, purified surface glycolipid molecules with known acyl chain modifications and attenuated properties were tested for the ability to stimulate Paneth cell secretion. The antigens included lipopolysaccharide (LPS) from wild-type and msbB-null *Escherichia coli* and phoP-null and phoP-constitutive *Salmonella enterica* serovar *Typhimurium* strains, as well as LPS, lipid A, and lipoteichoic acid from *Pseudomonas aeruginosa* and *Listeria monocytogenes* grown in Mg2+-limited media. Measurements of total secreted protein, secreted lysozyme, and the bactericidal peptide activities of collected secretions showed that the purified antigens elicited similar secretory responses from Paneth cells in mouse crypts ex vivo, regardless of glycolipid acyl chain modification. Despite their impaired Tlr4 pathway, Paneth cells in ex vivo C3H/HeJ mouse crypts released equivalent amounts of bactericidal peptide activity in response to purified bacterial antigens, including lipid A. Thus, mouse Paneth cells respond equivalently to purified bacterial cell envelope glycolipids, regardless of functional Tlr4, the structural properties of glycolipid acyl chains, or their association with virulence in humans.

Most illnesses caused by Shiga toxin-producing Escherichia coli (STEC) have been attributed to E. coli serotype O157:H7, but non-O157 STEC infections are now increasingly recognized as public health problems worldwide. The O121:H19 serotype is being isolated more frequently from clinical specimens and has been implicated in one waterborne outbreak. We used multilocus virulence gene profiling, a PCR-based assay, to characterize the virulence gene content of 24 isolates of serotype O121:H19 and nonmotile variants. We also performed multilocus enzyme electrophoresis and multilocus sequencing to establish the clonal relatedness of O121 isolates and to elucidate the relationship of O121 to common STEC clones. The 24 isolates were found to represent a single bacterial clone, as there was no allelic variation across 18 enzyme loci among the isolates. The complete nucleotide sequence of the intimin gene differed by four substitutions from that of the epsilon (Int-ε) allele of O103:H2 strain PMK5. The typical O121 virulence gene profile was similar to the profiles of enterohemorrhagic E. coli (EHEC) clones of E. coli: it included a Shiga toxin 2 gene (stx2), two genes on the EHEC plasmid (toxB and ehxA), and the gene encoding intimin (eae). Despite the similarities, putative virulence genes distributed on O islands—large chromosomal DNA segments present in the O157:H7 genome—were useful for discriminating among STEC serotypes and the O121:H19 clone had a composite profile that was distinct from the profiles of the other major EHEC clones of pathogenic E. coli. On the basis of sequencing analysis with 13 housekeeping genes, the O121:H19 clone did not fall into any of the four classical EHEC and enteropathogenic E. coli groups but instead was closely related to two eae-negative STEC strains.


Shiga toxin (Stx) types 1 and 2 are encoded within intact or defective temperate bacteriophages in Stx-producing Escherichia coli (STEC), and expression of these toxins is linked to bacteriophage induction. Among Stx2 variants, only stx2e from one human STEC isolate has been reported to be carried within a toxin-converting phage. In this study, we examined the O91:H21 STEC isolate B2F1, which carries two functional alleles for the potent activatable Stx2 variant toxin, Stx2d, for the presence of Stx2d-converting bacteriophages. We first constructed mutants of B2F1 that produced one or the other Stx2d toxin and found that the mutant that produced only Stx2d1 made less toxin than the Stx2d2-producing mutant. Consistent with that result, the Stx2d1-producing mutant was attenuated in a streptomycin-treated mouse model of STEC infection. When the mutants were treated with mitomycin C to promote bacteriophage induction, Vero cell cytotoxicity was elevated only in extracts of the Stx2d1-producing mutant. Additionally, when mice were treated with ciprofloxacin, an antibiotic that induces the O157:H7 Stx2-converting phage, the animals were more susceptible to the Stx2d1-producing mutant. Moreover, an stx2d1-containing lysogen was isolated from plaques on strain DH5(α) that had been exposed to lysates of the mutant that produced Stx2d1 only, and supernatants from that lysogen transformed with a plasmid encoding RecA were cytotoxic when the lysogen was induced with mitomycin C. Finally, electron-microscopic examination of extracts from the Stx2d1-producing mutant showed hexagonal particles that resemble the prototypic Stx2-converting phage 933W. Together these observations provide strong evidence that expression of Stx2d1 is bacteriophage associated. We conclude that despite the sequence similarity of the stx2d1- and stx2d2-flanking regions in B2F1, Stx2d1 expression is repressed within the context of its toxin-converting phage while Stx2d2 expression is independent of phage induction.
The bacterial pathogen Citrobacter rodentium belongs to a family of gastrointestinal pathogens that includes enteropathogenic and enterohemorrhagic Escherichia coli and is the causative agent of transmissible colonic hyperplasia in mice. The molecular mechanisms used by these pathogens to colonize host epithelial surfaces and form attaching and effacing (A/E) lesions have undergone intense study. In contrast, little is known about the host's immune response to these infections and its importance in tissue pathology and bacterial clearance. To address these issues, wild-type mice and mice lacking T and B lymphocytes (RAG1 knockout [KO]) were infected with C. rodentium. By day 10 postinfection (p.i.), both wild-type and RAG1 KO mice developed colitis and crypt hyperplasia, and these responses became more exaggerated in wild-type mice over the next 2 weeks, as they cleared the infection. By day 24 p.i., bacterial clearance was complete, and the colitis had subsided; however, crypt heights remained increased. In contrast, inflammatory and crypt hyperplastic responses in the RAG1 KO mice were transient, subsiding after 2 weeks. By day 24 p.i., RAG1 KO mice showed no signs of bacterial clearance and infection was often fatal. Surprisingly, despite remaining heavily infected, tissues from RAG1 KO mice surviving the acute colitis showed few signs of disease. These results thus emphasize the important contribution of the host immune response during infection by A/E bacterial pathogens. While T and/or B lymphocytes are essential for host defense against C. rodentium, they also mediate much of the tissue pathology and disease symptoms that occur during infection.

To determine the role of endogenous interleukin-18 (IL-18) during peritonitis, IL-18 gene-deficient (IL-18 KO) mice and wild-type mice were intraperitoneally (i.p.) infected with Escherichia coli, the most common causative agent found in septic peritonitis. Peritonitis was associated with a bacterial dose-dependent increase in IL-18 concentrations in peritoneal fluid and plasma. After infection, IL-18 KO mice had significantly more bacteria in the peritoneal lavage fluid and were more susceptible for progression to systemic infection at 6 and 20 h postinoculation than wild-type mice. The relative inability of IL-18 KO mice to clear E. coli from the abdominal cavity was not due to an intrinsic defect in the phagocytosing capacity of their peritoneal macrophages or neutrophils. IL-18 KO mice displayed an increased neutrophil influx into the peritoneal cavity, but these migratory neutrophils were less activate, as reflected by a reduced CD11b surface expression. These data suggest that endogenous IL-18 plays an important role in the early antibacterial host response during E. coli-induced peritonitis.
antigenically and genetically similar organisms; however, they differ in their virulence for cattle. M. avium subsp. paratuberculosis causes a chronic intestinal infection leading to a chronic wasting disease termed paratuberculosis or Johne's disease, whereas M. avium subsp. avium causes only a transient infection. We compared the response of bovine monocyte-derived macrophages to ingestion of M. avium subsp. paratuberculosis and M. avium subsp. avium organisms by determining organism survival, superoxide and nitric oxide production, and expression of the cytokines tumor necrosis factor alpha (TNF-\{alpha\}), gamma interferon (IFN-\{gamma\}), interleukin-8 (IL-8), IL-10, IL-12, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Unlike M. avium subsp. paratuberculosis, macrophages were able to kill approximately half of the M. avium subsp. avium organisms after 96 h of incubation. This difference in killing efficiency was not related to differences in nitric oxide or superoxide production. Compared to macrophages activated with IFN-\{gamma\} and lipopolysaccharide, macrophages incubated with M. avium subsp. paratuberculosis showed greater expression of IL-10 and GM-CSF (all time points) and IL-8 (72 h) and less expression of IL-12 (72 h), IFN-\{gamma\} (6 h), and TNF-\{alpha\} (6 h). When cytokine expression by macrophages incubated with M. avium subsp. paratuberculosis was compared to those of macrophages incubated with M. avium subsp. avium, M. avium subsp. paratuberculosis-infected cells showed greater expression of IL-10 (6 and 24 h) and less expression of TNF-\{alpha\} (6 h). Therefore, the combination of inherent resistance to intracellular degradation and suppression of macrophage activation through oversecretion of IL-10 may contribute to the virulence of M. avium subsp. paratuberculosis in cattle.

**Infection, Genetics and Evolution** (2)


http://www.sciencedirect.com/science/article/B6W8B-4CXMP6N-1/2/f59e67a951becd2af84a599a8a660eca

Mucosal leishmaniasis, which is a sporadic disease in the Sudan, was shown by isoenzyme characterization and PCR to be caused by Leishmania donovani. However, it was not clear if the parasite was exactly the same strain as that causing visceral leishmaniasis (VL), or of a different strain. We utilized a new generation of molecular DNA markers, minisatellites and kinetoplast DNA, for rapid characterization of the parasite. The results show that the genotypes of some of the parasites causing VL are different from those causing mucosal leishmaniasis. The L. donovani isolates causing visceral disease, as well as post-kala-azar mucosal leishmaniasis (PKML), have been shown to possess characteristic haplotypes. However, sequencing of a portion of the cytochrome oxidase II (COII) gene indicates that the parasite that invades the oral mucosa is divergent from other parasites causing VL. It appears to possess features of a more ancestral parasite with pronounced sequence homology to L. major. This agrees with earlier studies where isolates of mucosal leishmaniasis have been shown to possess an isoenzyme profile distinct from L. donovani and a different geographical distribution, albeit often overlapping with that of L. donovani.

Shiga toxin producing Escherichia coli O103:H2/H- belong to the third most frequently isolated EHEC serotypes in Germany following isolates of O157:H7/H- and O26:H11/H-. A total of 145 respective E. coli 103 isolates from single cases of diarrhoea and haemolytic uremic syndrome (HUS) in 1997-2000 were characterised by a range of molecular subtyping methods (PFGE, P-gene profiling, ribotyping, electrotyping) and phage typing in order to analyse their genetic relatedness and the practicability for new epidemiological tracing back. All isolates cluster into a distinct EHEC subgroup and reveal a high clonal diversity together with a considerable stability. Since strains of this serotype rank up to the third most frequently isolated EHEC in Germany a large population of this serotype, and therefore, a great supply of such strains may exist in this country.

Insect Biochemistry and Molecular Biology (34)


Major proteins of honey bee (Apis mellifera) royal jelly are members of the MRJP protein family. One MRJP protein termed MRJP3 exhibits a size polymorphism as detected by SDS-PAGE. In this report we show that polymorphism of the MRJP3 protein is a consequence of the polymorphism of a region with a variable number of tandem repeats (VNTR) located at the C-terminal part of the MRJP3 coding region. We present the characterization of five polymorphic alleles of MRJP3 by DNA sequencing. By PCR analyses, at least 10 alleles of distinct sizes were found in randomly sampled bees. Studies with nurse bees from a single honeybee colony revealed both Mendelian inheritance and very high variability of the MRJP3 genomic locus. The high variability and simple detection of the MRJP3 polymorphism may be useful for genotyping of individuals in studies of the honeybee.


Long-chain neurotoxins derived from the venom of the Buthidae scorpions, which affect voltage-gated sodium channels (VGSCs) can be subdivided according to their toxicity to insects into insect-selective excitatory and depressant toxins ([beta]-toxins) and the [alpha]-like toxins which affect both mammals and insects. In the present study by the aid of reverse-phase HPLC column chromatography, RT-PCR, cloning and various toxicity assays, a new insect selective toxin designated as Bj[alpha]IT was isolated from the venom of the Judean Black Scorpion (Buthotus judaicus), and its full primary sequence was determined:

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MNYLVVICFALLMTVVESGRDAYIAKNCAVGNSYCNTECKNGAVSGCQWLGYGN
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Despite its lack of toxicity to mammals and potent toxicity to insects, Bj[alpha]IT reveals an amino acid sequence and an inferred spatial arrangement that is characteristic of the well-known scorpion [alpha]-toxins highly toxic to mammals. Bj[alpha]ITs sharp distinction between insects and mammals was also revealed by its effect on sodium conductance of two cloned neuronal VGSCs heterologously expressed in Xenopus laevis oocytes and assayed with the two-electrode voltage-clamp technique. Bj[alpha]IT completely inhibits the inactivation process of the insect para/tipE VGSC at a concentration of 100 nM, in contrast to the rat brain Nav1.2/[beta]1 which is resistant to the toxin. The above categorical distinction between mammal and insect VGSCs exhibited by Bj[alpha]IT enables its employment in the clarification of the molecular basis of the animal group specificity of scorpion venom derived neurotoxic polypeptides and voltage-gated sodium channels.


http://www.sciencedirect.com/science/article/B6T79-4BVNV0B-2/2/4d549717d457fe61d070519710e113a

Octopamine regulates multiple physiological functions in invertebrates. The biological effects of octopamine and the pharmacology of octopamine receptors have been extensively studied in the American cockroach, Periplaneta americana. This paper reports the cloning of the first octopamine receptor from Periplaneta americana. A cDNA encoding a putative 7 transmembrane receptor was isolated from the head of Periplaneta americana. The encoded protein contains 628 amino acids and has sequence similarity to other biogenic amine receptors. This protein was expressed in COS-7 cells for radioligand binding studies using the antagonist 3H-yohimbine. Competitive binding comparing biogenic amines that could potentially function as endogenous ligands demonstrated this receptor had the highest affinity for octopamine (Ki=13.3 [mu]M) followed by tyramine, dopamine, serotonin and histamine. Octopamine increased both cAMP levels (EC50=1.62 [mu]M) and intracellular concentrations of calcium through the receptor expressed in HEK-293 cells. Tyramine increased levels of both of these second messengers but only at significantly higher concentrations than octopamine. The cAMP increase by octopamine was independent of the increase in calcium. Competitive binding with antagonists revealed this receptor is similar to Lym oa1 from Lymnaea stagnalis. The data indicate that this cDNA is the first octopamine receptor cloned from Periplaneta americana and therefore has been named Pa oa1.

http://www.sciencedirect.com/science/article/B6T79-3TWYNNS-B/2/aeed34b8dad71f5485c723d6b60b28c

The cDNA coding for a Ser-protease-related protein (Scg-SPRP)1 was cloned from desert locust (Schistocerca gregaria) midgut. The derived amino acid sequence consists of 260 residues and shows strong sequence similarity to insect trypsin-like molecules. It is, however, likely that Scg-SPRP is not a proteolytically active enzyme and that it plays another physiologically relevant role, since two out of three residues which are indispensable for catalytic activity of Ser-proteases are replaced. Northern analysis revealed that the Scg-SPRP gene is expressed in midgut tissue and that this expression is strongly induced in adult female locusts. Moreover, the occurrence of the transcript (1.2 kb) fluctuates during the molting cycle and during the female reproductive cycle.

Juvenile hormone (JH III) dependence of transcription was investigated by chemical allatectomy (precocene I) of adult females. This resulted in inhibition of vitellogenesis and in disappearance of the Scg-SPRP transcript. Expression of Scg-SPRP in precocene-treated locusts could be reinduced by additional treatment with JH III or with 20-OH-ecdysone.


http://www.sciencedirect.com/science/article/B6T79-3W2T43D-G/2/fb33e2c28ebd12288029fee4b9a417bb

Using HPLC separation, radioimmunoassay, and subsequent bioassay, we have detected the presence of an active peptide, which co-elutes with the insect myoinhibitory peptide leucomyosuppressin, in the brain of the cockroach Diploptera punctata. We have isolated a cDNA encoding the precursor for this peptide from cDNA libraries representing D. punctata brain RNA. The cDNA sequence contains an open reading frame that upon translation would result in a prepropolypeptide of 96 amino acids. Proteolytic cleavage of the predicted precursor could result in several peptides, including a 10 amino acid C-terminal peptide that would, upon modification of the NH2 and COOH-terminal amino acids, be identical to the insect FLRFamide, leucomyosuppressin. No other RFamide products are predicted to be processed from the precursor. Southern blot analysis indicates that the gene is present in the D. punctata genome in a single copy. Northern blot analysis shows that the gene is predominantly expressed as a 3.8 kb mRNA in cockroach brain. Study of the expression of the leucomyosuppressin gene in D. punctata brain, using in situ hybridization, indicates that expression occurs primarily in the pars intercerebralis of the protocerebrum, a region showing abundant FMRFamide-like immunoreactive neurosecretory cells. Immunohistochemistry and HPLC coupled to radioimmunoassay indicates that leucomyosuppressin represents a significant proportion of FMRFamide-related peptide production in the brain. However, HPLC analysis also indicates the presence of significant levels of other related peptides, demonstrating the presence of more than one FMRFamide-related gene in this insect.


http://www.sciencedirect.com/science/article/B6T79-4B4XM8C-4/2/ef01fa0ac1fa4db0a0ebd9bca8c24ef

Molecular methods have been devised for sexing Mediterranean fruit fly (medfly) individuals using minimal amounts of material from any stage of the life cycle. Molecular sexing methods are particularly valuable when material is obtained from pre-adult stages and sex identification based on morphological characters is not possible. These methods may also be useful for adult stage material in situations where only limited amounts or poorly preserved specimens are available. The sexing methods described here use the polymerase chain reaction (PCR) to amplify sequences known to originate from the sex chromosomes of this species. One method co-amplifies homologous regions of the ITS1 ribosomal DNA from both the X and Y chromosomes. Males and females are distinguished based on the restriction fragment pattern produced after digestion of the PCR products with the restriction enzyme Apol. A second method identifies males based on the positive amplification of a repetitive DNA sequence originating from the Y chromosome. Both methods are shown to be capable of establishing the sex identity of individuals using only minimal amounts of material from any stage of the life cycle.

http://www.sciencedirect.com/science/article/B6T79-3RGSWMN-D/2/a24463b492251e2e4adbac5194ce60b1

\([\alpha]\)-Amylases are important digestive enzymes in weevils that infest starchy seeds, and plants have evolved proteinaceous \([\alpha]\)-amylase inhibitors (\([\alpha]\)AI) for protection. To gain a better understanding of the interaction between weevil \([\alpha]\)-amylases and \([\alpha]\)AIs, we cloned the \([\alpha]\)-amylase cDNA of Zabrotes subfasciatus larvae. Larvae of this bruchid infest seeds of cultivated varieties of the common bean, *Phaseolus vulgaris*, although the seeds contain high levels of an \([\alpha]\)AI. The \([\alpha]\)-amylase cDNA, called ZsAmy, encodes a mature protein of 466 amino acids with a signal peptide of 17 amino acids. This protein has 50-60\% amino acid identity with the other five known insect \([\alpha]\)-amylases. Three amino acid residues known to be important for catalysis and three histidine residues involved in substrate binding are conserved in the derived amino acid sequence of ZsAmy. Expression of ZsAmy with a baculovirus vector in cultured insect cells resulted in the production of active \([\alpha]\)-amylase. \([\alpha]\)AI-1, the form of the inhibitor found in cultivated beans, does not inhibit larval or expressed bruchid \([\alpha]\)-amylase, but \([\alpha]\)AI-2, a form of the inhibitor found in certain wild bean accessions, does inhibit the larval, as well as the expressed bruchid \([\alpha]\)-amylase. These and other observations lead to the conclusion that ZsAmy encodes the major larval amylase of this bruchid species.


http://www.sciencedirect.com/science/article/B6T79-3XYG4HF-5/2/a4f9e5193c88d125cdad994ea261b30a

We identified five new serine protease cDNAs from the hemolymph of the malaria vector, *Anopheles gambiae*. All five show sequence similarity to genes thought to be involved in vertebrate or invertebrate defense responses. Sp14A, Sp14D2 and Sp22D demonstrate changes in transcript abundance in response to bacteria infections. Sp14A and Sp14D2, as well as the previously characterized Sp14D1, are induced by infection with the malaria parasite, *Plasmodium berghei*. These three proteases, along with Sp18D, are related to a group of secreted proteases that have amino-terminal clip domains and trypsin-like substrate specificity. BLAST results and phylogenetic analyses group Sp14A, Sp14D1 and Sp14D2 with the Drosophila protease EASTER, and three prophenoloxidase activating enzymes from other insects. EASTER's substrate is SPAETZLE, a ligand involved in embryogenesis but also in activating anti-microbial peptide synthesis. Their similarity to EASTER and immune inducibility suggest that one of these proteases may activate a SPAETZLE-like ligand during anti-parasite responses in mosquitoes. Alternatively, as potential prophenoloxidase activators, Sp14A, Sp14D1 or Sp14D2 may play a role in melanoctic encapsulation of *Plasmodium*.


http://www.sciencedirect.com/science/article/B6T79-49KS3H1-
The distributions of mRNAs for two cuticular proteins of Hyalophora cecropia were examined with RT-PCR and in situ hybridization. For major regions of larval and pupal cuticle, there was a strong correspondence between the type of cuticle and the predominant cuticular protein message found. Epidermal cells underlying soft cuticle had mRNA for HCCP12, with a RR-1 consensus attributed to soft cuticle, while the epidermal cells associated with hard cuticle had predominantly mRNA for HCCP66, a protein with the RR-2 consensus attributed to hard cuticle. Both messages were found in all areas of the pupal fore- and hind-wings, with modest area-specific difference in concentration being much less than differences in the relative abundance of these cuticular proteins. mRNA for HCCP12 was present in imaginal discs of feeding larvae of *H. cecropia*. Data from *Bombyx mori* available at SilkBase (http://www.ab.a.u-tokyo.ac.jp/silkbase/) revealed that imaginal discs from feeding larvae had abundant mRNA for RR-1 cuticular proteins, representing six distinct gene products. Only discs from spinning larvae had mRNAs that coded for RR-2 proteins arising from 10 distinct genes. Thus, lepidopteran wing imaginal discs can no longer be regarded as inactive in larval cuticle production.


http://www.sciencedirect.com/science/article/B6T79-416BXBB-F/2/f9576e4587290da188db31af3a0984a

Reverse transcriptase-polymerase chain reaction (PCR) was used to clone two esterase cDNAs from a diazinon-resistant field population of horn flies that expresses qualitative and quantitative differences in esterases compared with a susceptible population. The open reading frame from one of the esterase cDNAs, H[alpha]E7, exhibits substantial amino-acid identity to an esterase associated with diazinon resistance in *Lucilia cuprina*. RNA Northern blots showed that H[alpha]E7 mRNA was more abundant in the diazinon-resistant population than the susceptible population. DNA copy number analysis did not reveal major differences in H[alpha]E7 gene copy number between the two populations. The full-length cDNA to H[alpha]E7 was cloned and sequenced, and found to contain all of the highly conserved sequence elements associated with carboxyl/cholinesterases. The H[alpha]E7 homologs in diazinon-resistant strains of *L. cuprina* and *Musca domestica* have been shown to possess an amino-acid substitution conferring diazinon hydrolytic activity to the esterase enzyme. This amino-acid substitution was not found in diazinon-resistant horn flies examined by allele-specific PCR. Individual flies from the resistant field population were phenotyped as diazinon-resistant or diazinon-susceptible by topical diazinon application bioassays and total RNA isolated and hybridized to H[alpha]E7 probe in ribonuclease protection assays. H[alpha]E7 transcript was expressed at a five-fold higher level in resistant female individual flies than in susceptible female individuals.


http://www.sciencedirect.com/science/article/B6T79-47PR7BM-2C/2/6dc92f0ab396977f3fb316c9b2c9354a

The actin protein is a critical protein in eukaryotic cells. Four actin genes, constituting what appear to be a set of muscle specific actin genes, have been isolated from the genome of the oriental fruit fly *Bactrocera dorsalis*. DNA sequences have been determined for the coding as well
as 3' and 5' flanking regions for each of these genes. These genes have also been characterized in terms of RNA expression patterns, and comparisons have been made to actin genes from other species. Consistent with other actins, there is a high degree of amino acid sequence conservation in the coding regions of these genes. However, even within the coding regions codon usage patterns in the oriental fruit fly are quite different from some other well characterized species. In addition, the DNA sequences in the intermediate 3' and 5' flanking regions exhibit virtually no detectable sequence homology both within and between species. In terms of nitrons, three of the four actin genes from the oriental fruit fly described here have a single intervening sequence. Two of these genes share the same intron position with the two muscle specific actin genes act79B and act88F from Drosophila melanogaster and with one muscle specific actin gene CcA1 from the Mediterranean fruit fly, Ceratitis capitata. Another gene from the oriental fruit fly shares the same intron position as the muscle specific actin gene act57B from D. melanogaster. Such conservation of intron positioning between species is highly unusual among previously characterized actin genes. Using unique sequences found in the 3' untraslated regions, gene specific probes have also been constructed. These have been used to detect the expression patterns of individual genes in a temporal and spatial manner. Each of the four genes examined here show differential patterns of expression. The patterns indicate that all four genes are most likely to encode muscle specific actins.


http://www.sciencedirect.com/science/article/B6T79-3VNPHG5-1/2/1846ac7523f763944fb85efcfc48c36d

A putative crayfish iron-responsive element (IRE) is present in the 5'-untranslated region of the crayfish ferritin mRNA. The putative crayfish IRE is in a cap-proximal position and shares most of the structural features of the consensus IRE, but the RNA stem-loop structure contains a bulge of a guanine instead of a cytosine at the expected position, so far thought to be a hallmark of IREs. By using an electromobility shift assay this IRE was shown to specifically bind purified recombinant human iron regulatory protein 1 (IRP1) as well as a factor(s) present in a homogenate of crayfish hepatopancreas, likely to be a crayfish IRP1 homologue. With mutations in the crayfish IRE, the affinity of IRP to IRE was drastically decreased. A cDNA2 encoding an IRP1-like protein was cloned from the hepatopancreas of crayfish. This protein has sequence similarities to IRP1, and contains all the active-site residues of aconitase, two putative RNA-binding regions and a putative contact site between RNA and IRP. These results show that a crayfish IRE, lacking the bulged C, can bind IRP1 in vitro and that an IRP1-like protein present in crayfish hepatopancreas may have both aconitase and RNA-binding activities.


http://www.sciencedirect.com/science/article/B6T79-3Y2N09C-K/2/9975c340000ec4498f281e56ee869d2e

We have cloned three cDNAs from the sweet potato hornworm Agrius convolvuli that encode precursor molecules for peptides structurally related to bombyxin, an insulin-related brain secretory peptide in Bombyx mori. The Agrius bombyxin-related peptide (ABRP) cDNAs are classified into type A and B according to their sequence similarity. The prepro-ABRPs deduced from the cDNA sequences have the insulin-like domain organization of signal peptide/B chain/C peptide/A chain. The ABRP transcripts in Agrius brain were shown to locate in four pairs of
medial neurosecretory cells, the homologous group of neurosecretory cells that produce bombyxins in Bombyx brain. Genomic Southern analysis indicated the presence of multiple copies of ABRP gene in the Agrius genome. Results showed that the ABRP genes are remarkably different from the vertebrate insulin genes in the number of copy and spatial localization of the transcripts.


http://www.sciencedirect.com/science/article/B6T79-3VBSBR0-F/2/6afe567ad04ee1f0e4193ba6208b38f

The kdr and super-kdr point mutations found in the insect sodium channel gene are postulated to confer knockdown resistance (kdr) to pyrethroids. Using an allele-specific PCR assay to detect these mutations in individual horn flies, Haematobia irritans (L.), we determined the allelic frequency of the kdr and super-kdr mutations in several wild and laboratory populations. Wild populations with very similar allelic frequencies had resistance levels that ranged widely from 3- to 18-fold relative to a susceptible population. Conversely, the kdr allele frequency in a lab population with 17-fold resistance was nearly double that found in a heavily pressured wild population with 18-fold resistance. We conclude that, although the kdr mutation confers significant levels of pyrethroid resistance, a substantial component of resistance in insecticidally pressured populations is conferred by mechanisms that are PBO-suppressible. High super-kdr allele frequencies were detected in two resistant lab populations, but in wild populations with equivalent resistance the super-kdr allele frequency was very low. Interestingly, in over 1200 individuals assayed, the super-kdr mutation was never detected in the absence of the kdr mutation.


http://www.sciencedirect.com/science/article/B6T79-47PR7TS-10/2/c3fdadd2f05550c72cd9182b1ea7dd

Three degenerate primers were designed to match the most conserved regions within the DNA-binding domains of several selected members of the steroid hormone receptor family. Use of these primers in the polymerase chain reaction with cDNA from Galleria mellonella prepupae detected a 177 bp fragment that had 87% identity to the Manduca sexta gene MHR3 and 75% to the Drosophila melanogaster DHR3 gene, and therefore was named "GHR3". Screening of a Galleria penultimate instar cDNA library with this fragment yielded a cDNA clone that contained a 557 condon open reading frame, predicting a 62.3 kDa protein. The deduced amino acid sequence of GHR3 showed 92% overall identity with the MHR3 protein and 97 and 70% identity with DHR3 in the putative DNA- and ligand-binding domains, respectively. Hybridization of whole body RNA revealed high GHR3 mRNA levels during both the larval and pupal molts, coincident with the molt-inducing ecdysteroid pulses, and low or undetectable levels during the first half of the last instar. During the larval-pupal transformation, no GHR3 mRNA was found at the beginning of the stemmatal pigment retraction at the onset of the ecdysteroid rise; maximal levels were observed 4 h later, coincident with the peak ecdysteroid titer (over 2.3 [mu]g 20E equivalents/ml hemolymph). Two mRNAs (4.6 and 3.6 kb) were detected when the ecdysteroid titer was high. Injection of 2 [mu]g/gm 20E into isolated final instar larval abdomens induced the
The appearance of the 4.6 kb mRNA within 1.5 h; the mRNA level then reached maximum by 3 h and declined by 6 h. No 3.6 kb mRNA was detectable during that time. A 10-fold lower 20E dose caused only trace induction by 3 h.


http://www.sciencedirect.com/science/article/B6T79-44XM1S4-3/2/53d8b15bbe12e1c60f50005445c9daba

The aphid myrosinase gene has been elucidated using Rapid Amplification of cDNA Ends--PCR. Sequencing has shown that aphid myrosinase has significant sequence similarity (35%) to plant myrosinases and other members of glycosyl hydrolase family 1 (GHF1). The residues acting as proton donor and nucleophile, in the hydrolysis of glucosinolates by aphid myrosinase, are identified as Glu 167 and Glu 374 respectively. The equivalent residues in plant myrosinase are Gln 187 and Glu 409 and for the cyanogenic [beta]-glucosidase Glu 183 and Glu 397. Thus it would appear that the absence of a proton donor is not necessary for the hydrolysis of glucosinolates as was thought to be the case for the plant myrosinases. Aphid myrosinase appears to be more similar to animal [beta]-O-glucosidases than to plant myrosinases, as assessed by sequence similarity and phylogenetic techniques. These results strongly suggest that myrosinase activity has twice arisen from [beta]-O-glucosidases in plants and animals. Comparison of aphid myrosinase with plant myrosinase has highlighted Lys 173 and Arg 312 as possibly playing a crucial role in the hydrolysis of glucosinolates by aphid myrosinase.


http://www.sciencedirect.com/science/article/B6T79-3Y2N08T-8/2/c9a723cd78a4eab783c44022ad68e68a

The immune state of insects is defined by a set of proteins that is absent in the naive state. To explore the immune system of Trichoplusia ni in more detail we have employed a PCR differential display technique to compare the mRNA population of untreated last instar larvae to that of immunized animals. In the primary display, more than one hundred bands seemed induced upon bacterial challenge. When they were used as probes in Northern blots, 35% of these probes detected inducible mRNA species. Such probes were used to screen a cDNA library from immunized larvae. We isolated clones for T. ni homologs of cecropin A, lysozyme and attacin. One differentially expressed band hybridized to clones for BJHSP1, a hemacyanin-related protein which is hormonally up-regulated in last instar larvae; this induction is probably not related to the bacterial infection. Still other probes recognized inducible mRNAs of 1.6 and 1.0 kb. The corresponding cDNA clones did not show strong sequence homology to any known proteins. We have demonstrated the potential of this PCR technique to display both known and unknown genes specific for the immune state of whole insects against a background of genes involved in larval development.

Klaudiny, J., S. Albert, et al. (2005). "Two structurally different defensin genes, one of them encoding a novel defensin isoform, are expressed in honeybee Apis mellifera." Insect Biochemistry and Molecular Biology 35(1): 11.
Two defensins showing high mutual similarity have previously been characterized in honeybee Apis mellifera: royalisin, a peptide isolated from the royal jelly, and defensin, found in the hemolymph of bacterially infected bees. Here we show that both these peptides are encoded by the same polymorphic gene, which we termed defensin1. Besides this gene, we identified an additional defensin gene coding for a novel honeybee defensin designated defensin2. The pre-pro-peptide sequence of defensin 2 was inferred from its cDNA. Mature defensin 2 peptide shows 55.8% identity with defensin 1. Sequences of genomic loci of the two defensin genes revealed their different structure. Defensin1 possesses an exon-intron structure unique among arthropoda defensin genes. Its second intron splits exactly the common structural module of defensins from a short amidated C-terminal extension found only in hymenopteran defensins. Transcription of defensin genes in some nurse honeybees tissues was studied by RT-PCR. Both defensins are expressed in heads and thoraces. Defensin1 but not defensin2 mRNA was detected in hyphopharyngeal, mandibular and thoracic salivary glands. Immune response elements were identified by computer analysis of the promoter regions of defensin genes. Their different representation in these genes reflects presumably observed tissue-specific expression of defensins.


Three potent insecticidal peptide toxins were purified from the venom of the primitive weaving spider, Diguetia cavities. The toxins share significant homology (> 40%) in their amino acid sequences and are of related size (masses of 6371-7080 Da). In lepidopteran larvae, the toxins cause a progressive spastic paralysis, with 50% paralytic doses (PD50s) ranging from 0.38 to 3.18 nmol/g, suggesting them to be among the most potent insecticidal compounds yet described from arthropod venoms. The most potent of these toxins, DTX9.2, was cloned using a reverse transcription-polymerase chain reaction (RT-PCR). The cDNA encodes a 94 amino acid precursor which is processed to the active 56 amino acid peptide by removal of a signal and propeptide sequence. The gene encoding DTX9.2 was isolated and characterized. The transcriptional unit spans 5.5 kilobases and is segregated into five exons. DNA sequences upstream from the first exon contain a TATA box and two palindromic sequences (one with homology to a CAAT consensus) which together may constitute a functional promoter. The highly segmented gene structure observed for this small peptide suggests that a mechanism such as exon shuffling may have played a role in the evolution of this toxin family.


In many insects, semen coagulates into a mating plug at the distal part of the female’s genital tract. Mating plugs have been proposed to facilitate sperm movement or to prevent subsequent matings or sperm loss. The molecular constituents of insect mating plugs have not previously been characterized. Here we report that an abundant autofluorescent protein made by the
Drosophila melanogaster male’s ejaculatory bulb is a major constituent of the posterior region of the mating plug. Identities in size, chromosomal location and expression pattern indicate that the autofluorescent protein is PEB-me, an abundant ejaculatory bulb protein reported by Ludwig et al. [Biochem. Genet. 29 (1991) 215]. We cloned and sequenced the RNA encoding this protein. The transcript, which is male-specific and expressed only in the ejaculatory bulb, encodes a 377 a.a. predicted secreted protein with PGG repeats similar to those in homopolymer-forming proteins found in spider silk.


http://www.sciencedirect.com/science/article/B6T79-3W49339-7/2/d7b46be065a5c84815fd86690ecc2bbc

A 185-kDa silk protein (sp185) from Chironomus tentans, present in both larval and prepupal silks, contains a striking amino acid sequence motif, Cys-X-Cys-X-Cys, which occurs about every 22-26 residues. Homologous proteins have been found in Chironomus pallidivittatus (sp185) and Chironomus thummi (sp220), which apparently differ in size but are very similar in overall composition and sequence. While surveying Australasian species of Chironomus and Kiefferulus we obtained evidence for immunologically related silk proteins having similar size and amino acid composition, but noticeably less Cys. Interspecies in situ hybridization to polytene chromosomes with C. tentans and C. pallidivittatus cDNA probes indicated that each species had a related gene. One pair of C. tentans cDNA-derived primers enabled polymerase chain reaction amplification of a discrete fragment of this gene from Kiefferulus ‘cornishi’. Preliminary sequence information for this fragment confirmed the presence of an encoded Cys-X-Cys-X-Cys motif in what appeared to be a similar protein region containing less Cys. We conclude that homologs of C. tentans sp185 and its gene have been identified which may contain significant deviations in structure. Once suitable libraries are available, probes described here will be useful for selecting cDNA and genomic clones for detailed study.


The single intron of the heavy-chain fibroin gene in domesticated (Bombyx mori) and wild (B. mandarina) silk moths has a length of approximately 1000 nucleotides. It is located only 57 bp from the gene's core promoter and harbors multiple AT-rich regulatory elements that have been found to enhance the basal level of transcription in vitro. In this work, the intronic nucleotide variability among members of both Bombyx species was analyzed. The intron sequences of B. mori strains k120 and Nistari, as well as B. mandarina specimens from Japan and Korea, were obtained. This information was compared with the previously reported sequences of B. mori strains p50 and C-108, as well as an additional B. mandarina specimen collected in Japan. We found a total of 26 variant positions, including variants shared by members of both species and species-specific changes. The potential functional role of these variants was investigated by using the program MatInspector to search for putative binding sites of transcription factors within the intron. We detected a multitude of putative binding elements distributed along the entire intronic sequence. Among them, 22 correspond to protein binding domains that are known to regulate fibroin transcription. The mapping of multiple variant positions within these putative binding sequences as well as in known regulatory elements of the intron argue for functional
significance on the regulation of transcription.


http://www.sciencedirect.com/science/article/B6T79-44GHTVH-22/2/93f3c55640c9b31ee9aaf77b8602d760

The wing-deficient mutant, flugellos (fl), of the silkworm lacks four wings in the pupa and the adult, due to aberrant wing morphogenesis during metamorphosis. To elucidate the mechanisms of wing-specific deficiencies in the fl mutant, we used mRNA differential display and identified five genes abnormally expressed in the fl wing discs. Northern blot and RT-PCR analyses revealed that four genes were overexpressed, but the fifth one was not transcribed in the fl wing discs. The expression level of ribosome-associated protein p40 in the fl wing discs was elevated approximately 10 times compared to the wild-type (WT) discs. Another overexpressed gene CB10 encodes a novel wing-specific protein with a putative zinc-finger motif. Overexpression of two components of extracellular matrix, cuticle protein 18 (BMCP18) and a fibrillin-like protein AD10, may result in the abnormal wing morphogenesis in the fl mutant. In contrast, a novel member of multifunctional Ca²⁺-binding protein annexins, designated as annexin b13 (Anx b13), was expressed dominantly in the wing discs of WT but completely repressed in the fl tissues. Strong expression of Anx b13 in wing discs during the fourth and fifth instar indicates that ANX B13 plays an important role in wing morphogenesis.


http://www.sciencedirect.com/science/article/B6T79-3W2T43D-22/2/210616228cf7a5ae2bed03ea05ef2b46

The Anopheles gambiae gene encoding tryptophan oxygenase, a homolog of the Drosophila melanogaster vermilion gene, has been molecularly cloned and characterized. Unlike Drosophila, where it is X-linked, the A. gambiae gene maps to chromosome 2R, subdivision 12E, by in situ hybridization to the polytene chromosomes. Of the six introns present, four are positioned identically to those of the Drosophila homolog, one is similarly positioned, and one is novel. A 1955 nt cDNA potentially encodes a 392 amino acid protein of an estimated 45 kDa. Amino acid comparisons between the deduced protein and previously known tryptophan oxygenases revealed 74% identity between Anopheles and Drosophila, and 53% identity between Anopheles and nematode or mammalian proteins. Northern analysis detected a developmentally regulated transcript about 2 kb in length. Since this gene is known to control adult eye color in other flies, its cloning from A. gambiae provides the basis for a dominant phenotypic marker for germline transformation, one whose expression, unlike that of white, is not cell autonomous.


http://www.sciencedirect.com/science/article/B6T79-47PR7VD-6M/2/8fc0591c2fdea95bde8a942c21ccf96e
A cDNA clone containing a 921 bp open-reading frame (307 amino acids; 34 kDa) homologous to the TATA-binding protein (TBP) was isolated and sequenced from a Spodoptera frugiperda cell line that is commonly used in the baculovirus expression system. Analysis of the S. frugiperda TBP (SfTBP) sequence showed that the amino-terminal portion of SfTBP diverged significantly from that of other TBP sequences including Drosophila melanogaster whereas the carboxy-terminal sequence was highly conserved. Southern blot analysis indicated that SfTBP was encoded by a single gene in the S. frugiperda genome. Northern blot analysis indicated that steady-state levels of the 1.3 kb SfTBP transcript declined by 24 h post-infection corresponding to the time of virus-induced inhibition of host-cell transcription. Corresponding western blot analysis showed that TBP protein levels remain constant up to 72 h post-infection.


http://www.sciencedirect.com/science/article/B6T79-3T6YH4M-4/2/8124b8bd4a212f7b8a04f66995098ff

The gut of most insects is lined with a semi-permeable peritrophic membrane (or peritrophic matrix) composed of chitin, proteoglycans and proteins. Despite the probable importance of the peritrophic membrane in facilitating the digestive process and protecting insects from invasion by micro-organisms and parasites, there has been little characterization of the specific components and their interactions within this acellular structure. Here we report the characterization of an integral peritrophic membrane glycoprotein, peritrophin-48, from the larvae of the fly Lucilia cuprina, a primary agent of cutaneous myiasis in sheep. Peritrophin-48 was purified from peritrophic membrane obtained by larval culture and its location within the peritrophic membrane determined by immuno-fluorescence and immuno-gold localizations. The cDNA coding for peritrophin-48 was cloned and sequenced. The deduced amino acid sequence codes for a protein of 375 amino acids containing an amino-terminal signal sequence followed by five similar, but non-identical domains, each approximately 65-70 amino acids in length and characterised by a specific register of six cysteines. The deduced amino acid sequence shows significant similarity to two other peritrophic membrane proteins, peritrophin-95 and peritrophin-44, from the same species. A reverse transcriptase-PCR approach indicated that there are several highly related peritrophin-48 genes expressed in each individual. Reverse transcriptase-PCR also demonstrated the expression of peritrophin-48 in all three larval instars and adults but not pupae or eggs. Peritrophin-48 was expressed only by the cardia and by the larval midgut. A simple structural model of a basic unit of a type 2 peritrophic membrane is presented.


http://www.sciencedirect.com/science/article/B6T79-3VGRRV2-K/2/e83efc2d7aa503583930c0370e07bb3f0

Three major red hemoproteins, named RpSG I, II (identical with prolixin-S) and III, in the salivary glands of the blood-sucking insect, Rhodnius prolixus, show homology in N-terminal amino acid (AA) sequences, and are immunologically related. We focussed on one of these proteins, RpSG-I, in this paper. RpSG-I in fresh salivary gland extract was separated into two components (Ia and Ib) by isoelectric focussing gel electrophoresis. Absorption spectra of RpSG-Ia and Ib showed Soret peaks at 400 nm and 420 nm, respectively, suggesting that they are nitric oxide (NO)-unbound and -bound hemoproteins and function as NO-carriers. RpSG-I is stage-specific in
appearance, being absent in 3rd and 4th instar nymphs, appearing and increasing gradually in 5th (last) instar nymphs after engorgement, and present in the adult stage. We purified RpSG-I from salivary gland extract by size exclusion and ion exchange HPLCs. It is a single electrophoretic band with an absorption peak at 400 nm, representing the NO-unbound molecule. Full-size cDNA of RpSG-I was cloned by screening with a specific polyclonal antibody from a salivary gland cDNA library. Sequence analysis of RpSG-I cDNA showed an open reading frame encoding a signal peptide (23 AA) and mature protein (179 AA) of 19778 daltons. The deduced N-terminal AA sequence of the RpSG-I was identical with that of the hemoprotein reported as nitrophorin-3 (Champagne et al., 1995).


When insects molt, the exoskeleton is renewed under the controls of insect hormones via the biosynthesis and degradation of cuticle proteins. To understand the hormonal control of cuticle formation, we used the differential display method to look for stage-specific cuticle genes, and identified a novel cDNA named Bombyx mori Cuticle Protein GlyGlyTyr-repeat 1 (BMCPG1). Expression of BMCPG1 mRNA peaked sharply immediately after a pulse of ecdysteroid during the fourth molt and pre-pupal stages, concurrent with the expression of genes for FTZF1 and dopa decarboxylase. BMCPG1 was expressed only in the epidermis, but not in any other tissue. We cultured the larval epidermis and found that BMCPG1 expression is not induced by the continuous presence of ecdysteroid. Removal of ecdysteroid from the medium, which constitutes a pulse treatment, is required for the induction of BMCPG1 transcription. These results explain well the stage-specific expression of BMCPG1 by ecdysteroid in vivo. Based on its expression patterns and unique structure, we propose that BMCPG1 may be a novel component of epicuticle of B. mori, and is probably involved in cross-linking of proteins via its GGY repeats.


The intrinsic peritrophic matrix glycoprotein, peritrophin-95, from the midgut of larvae of Lucilia cuprina can only be solubilized from the matrix using strong denaturants. This suggests that the protein has a structural role in the matrix. Consistent with this is the finding that immuno-gold and immuno-fluorescence localizations of the protein showed a uniform distribution within the peritrophic matrix. RT-PCR demonstrated that expression of peritrophin-95 mRNA was restricted to the larval cardia, a small organ located in the anterior midgut from which the type 2 peritrophic matrix originates. Immuno-bLOTS and ELISAs demonstrated that the sera from sheep infested naturally or artificially with these larvae recognised peritrophin-95. This indicates that peritrophin-95 stimulates the ovine immune system during larval infestation even though the protein is firmly attached to the peritrophic matrix in the larval midgut and seemingly "concealed" from the ovine immune surveillance system. Analyses of larval regurgitated or excreted material by immuno-bLOTS, immuno-affinity purification and amino-terminal sequencing demonstrated the presence of soluble monomeric peritrophin-95. These results indicate that peritrophin-95, a candidate vaccine antigen for use in sheep is not a "concealed" antigen as previously thought. The presence of soluble peritrophin-95 in the regurgitated/excreted material from larvae suggests that this protein
may be involved in a maturation phase of peritrophic matrix production, a by-product of which is the excretion or regurgitation of soluble peritrophin-95.


http://www.sciencedirect.com/science/article/B6T79-43CH6GC-9/2/9c6ecd862d0a662a5f2ba5921b6cceb0

The peritrophic matrix lines the midgut of most insects and has important roles in digestion, protection of the midgut from mechanical damage and invasion by micro-organisms. Although a few intrinsic peritrophic matrix proteins have been characterised, no direct homologues of any of these proteins have been found in other insect species, even closely related species, suggesting that the peritrophic matrix proteins show considerable sequence divergence. We now report the identification of the cDNA and genomic DNA sequences of a Chrysomya bezziana homologue of the Lucilia cuprina intrinsic peritrophic matrix protein, peritrophin-48. The gene for C. bezziana peritrophin-48 spans 1315 bp and consists of three exons (65, 560 and 690 bp, respectively) separated by introns of 566 and 72 bp. The transcriptional start site, identified by a consensus of cDNA clones and primer extension analysis, is probably located 58 bp upstream from the start codon. However, there may be multiple start sites for transcription. Two potential TATA boxes and a consensus arthropod transcription initiator are located within 134 bp of sequence upstream of the putative transcriptional start site suggesting that this region contains the gene promoter. Immuno-fluorescence localization demonstrated that C. bezziana peritrophin-48 was localised to the larval peritrophic matrix. Protein fold recognition analysis indicated structural similarities between peritrophin-48 and wheatgerm lectin. As wheatgerm lectin binds chitin, this result suggested that C. bezziana peritrophin-48 may also bind chitin, a constituent of the peritrophic matrix. Chitin binding studies with a recombinant peritrophin-48 protein confirmed that it binds chitin. A Drosophila melanogaster homologue of peritrophin-48 encoded in an EST and a genomic sequence was also identified. The pairwise percentage identities of the deduced amino acid sequences for the peritrophin-48 homologues from the three higher Dipteran species were relatively low, ranging between 32 and 42%. Despite this sequence variability, the predicted structure of these proteins, dictated by five domains, each containing a characteristic distribution of six cysteines, was strictly conserved. It is concluded that considerable sequence variation can be tolerated in this protein because of the constraints imposed on the structure of the protein by an extensive disulphide bonded framework.


http://www.sciencedirect.com/science/article/B6T79-4967CHH-1/2/6b1c6d10c779a20de62c765d3cda7f1a

The post-integration behavior of insect gene vectors will determine the types of applications for which they can be used. Transposon mutagenesis, enhancer trapping, and the use of transposable elements as genetic drive systems in insects requires transposable elements with high rates of remobilization in the presence of transposase. We investigated the post-integration behavior of the Mos1 mariner element in transgenic Aedes aegypti by examining both germ-line and somatic transpositions of a non-autonomous element in the presence of Mos1 transposase. Somatic transpositions were occasionally detected while germ-line transposition was only rarely observed. Only a single germ-line transposition event was recovered after screening 14,000 progeny. The observed patterns of transposition suggest that Mos1 movement takes place
between the S phase and anaphase. The data reported here indicate that Mos1 will be a useful vector in Ae. aegypti for applications requiring a very high degree of vector stability but will have limited use in the construction of genetic drive, enhancer trap, or transposon tagging systems in this species.


http://www.sciencedirect.com/science/article/B6T79-42M1D7K-5/2/53b67de498c43f389992b54ae3d46389

Muscle fatty acid binding protein (FABP) is a major cytosolic protein in flight muscle of the desert locust, Schistocerca gregaria. FABP expression varies greatly during development and periods of increased fatty acid utilization, but the molecular mechanisms that regulate its expression are not known. In this study, the gene coding for locust muscle FABP was amplified by PCR and cloned, together with 1.2 kb of upstream sequence. The sequence coding for the 607 bp cDNA is interrupted by two introns of 12.7 and 2.9 kb, inserted in analogous positions as the first and third intron of the mammalian homologues. Both introns contain repetitive sequences also found in other locust genes, and the second intron contains a GT-microsatellite. The promoter sequence includes a canonical TATA box 24 bp upstream of the transcription start site. The upstream sequence contains various potential myocyte enhancer sequences and a 160 bp segment that is repeated three times. In database searches in the genome database of Drosophila melanogaster, a gene with the same gene organization and promoter structure was identified, likely the dipteran homologue of muscle FABP. Upstream of both insect genes, a conserved 19 bp inverted repeat sequence was detected. A similar but reverse palindrome is also present upstream of all mammalian heart FABP genes, possibly representing a novel element involved in muscle FABP expression.


http://www.sciencedirect.com/science/article/B6T79-3TXSR1F-7/2/ce39bc5978279c7d2f2a1b7b61f06d7c

FABP is the most abundant cytosolic protein in developed flight muscle of adult locusts, but it is completely absent in nymphs. During the two weeks following adult ecdysis, FABP rises to 18% of the total soluble proteins. Its mRNA increases steadily up to day 8, before it gradually declines until reaching a low concentration at day 15, which remains constant for the rest of the animal's life. Using a PCR primer combination specific for a 597 bp sequence of intron 1, we have developed a reverse transcription PCR assay to quantify the amount of primary transcript present in muscle tissue at various ages. The FABP gene is not transcribed prior to metamorphosis; its primary transcript rises rapidly during the first two days of adult life, and remains close to maximal until day 5. Subsequently its level rapidly declines, ultimately reaching values of less than 0.02% of the maximal level. The correlation between primary transcript, mRNA and FABP content were analyzed by modeling transcription, translation and degradation with computer modeling software. The computer simulation is in good agreement with the experimentally obtained data, suggesting that the control of FABP expression in locust flight muscle occurs predominantly at the level of transcription initiation.

http://www.sciencedirect.com/science/article/B6T79-3YF4B3R-8/2/b5c0907dc4e249f0298ef46357e3ec52

A cDNA encoding acetylcholinesterase (AChE, EC 1.1.1.7) was cloned from a cDNA library constructed from an insecticide-susceptible strain of Colorado potato beetle, Leptinotarsa decemlineata (Say). The complete amino acid sequence of AChE deduced from the cDNA consisted of 29 residues for the putative signal peptide and 600 residues for the mature protein with a predicted molecular weight of 67,994. Northern blot analysis of poly(A) RNA showed an approx 13.1-kb transcript. The mature protein sequence had 57 and 61% of amino acid residues identical to those of Drosophila melanogaster and Anopheles stephensi, respectively, and produced a remarkably similar hydropathy profile when compared to those of the two dipterous species. The three residues (Ser, Glu and His) that putatively form the catalytic triad and the six Cys that form intra-subunit disulfide bonds were completely conserved when compared to the other seven AChEs from a broad range of animal species reported to date. Other properties of the deduced protein of AChE, including molecular weight and amino acid composition, agreed well with those of a previously reported study on the purified AChE from the same insect species. All these features firmly established that the cloned cDNA encodes AChE in Colorado potato beetle.

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Sixty strains of Gram-negative, anaerobic, rod-shaped bacteria from human sources initially assigned to Leptotrichia buccalis (n=58) and Leptotrichia pseudobuccalis' (n=2) have been subjected to polyphasic taxonomy. Full-length 16S rDNA sequencing, DNA-DNA hybridization, RAPD, SDS-PAGE of whole-cell proteins, cellular fatty acid analysis and enzymic/biochemical tests supported the establishment of four novel Leptotrichia species from this collection, Leptotrichia goodfellowii sp. nov. (type strain LB 57T=CCUG 32286T=CIP 107915T), Leptotrichia hofstadii sp. nov. (type strain LB 23T=CCUG 47504T=CIP 107917T), Leptotrichia shahii sp. nov. (type strain LB 37T=CCUG 47503T=CIP 107916T) and Leptotrichia wadei sp. nov. (type strain LB 16T=CCUG 47505T=CIP 107918T). Light and electron microscopy showed that the four novel species were Gram-negative, non-spore-forming and non-motile rods. L. goodfellowii produced arginine dihydrolase, (beta)-galactosidase, N-acetyl-(beta)-glucosaminidase, arginine arylamidase, leucine arylamidase and histidine arylamidase. L. shahii produced (alpha)-arabinosidase. L. buccalis and L. goodfellowii fermented mannose and were (beta)-galactosidase-6-phosphate positive. L. goodfellowii, L. hofstadii and L. wadei were (beta) -haemolytic. L. buccalis fermented raffinose. With L. buccalis, L. goodfellowii showed 3(middle dot)8-5(middle dot)5 % DNA-DNA relatedness, L. shahii showed 24(middle dot)5-34(middle dot)1 % relatedness, L. hofstadii showed 27(middle dot)3-36(middle dot)3 % relatedness and L. wadei showed 24(middle dot)1-35(middle dot)9 % relatedness. 16S rDNA sequencing demonstrated that L. hofstadii, L. shahii, L. wadei and L. goodfellowii each formed individual clusters with 97, 96, 94 and 92 % similarity, respectively, to L. buccalis.


http://ijs.sgmjournals.org/cgi/content/abstract/52/6/1929

To further investigate the diversity of micro-organisms capable of conserving energy to support growth from dissimilatory Fe(III) reduction, Fe(III)-reducing micro-organisms were enriched and isolated from subsurface sediments collected in Oyster Bay, VA, USA. A novel isolate, designated T118T, was recovered in a medium with lactate as the sole electron donor and Fe(III) as the sole electron acceptor. Cells of T118T were Gram-negative, motile, short rods with a single polar flagellum. Strain T118T grew between pH 6.7 and 7.1, with a temperature range of 4-30 °C. The optimal growth temperature was 25 °C. Electron donors utilized by strain T118T with Fe(III) as the sole electron acceptor included acetate, lactate, malate, propionate, pyruvate, succinate and benzoate. None of the compounds tested was fermented. Electron acceptors utilized with either acetate or lactate as the electron donor included Fe(III)-NTA (nitrilotriacetic acid), Mn(IV) oxide, nitrate, fumarate and oxygen. Phylogenetic analysis demonstrated that strain T118T is most closely related to the genus Rhodoferax. Unlike other species in this genus, strain T118T is not a phototroph and does not ferment fructose. However, phototrophic genes may be present but not expressed under the experimental conditions tested. No Rhodoferax species have been reported to grow via dissimilatory Fe(III) reduction. Based on these physiological and phylogenetic differences, strain T118T (=ATCC BAA-621T=DSM 15236T) is proposed as a novel species, Rhodoferax ferrireducens sp. nov.


Five strains of halophilic, Gram-negative marine bacteria (KMM 3809T, KMM 3814, KMM 3815, KMM 3817 and KMM 3818) were isolated from sediments collected from Chazhma Bay, Sea of Japan. Phylogenetic 16S rRNA gene sequence-based analysis placed these bacteria in a clade within the genus Marinobacter in the{gamma} -Proteobacteria. KMM 3809T showed highest 16S rRNA gene sequence similarity of 97.3 % to Marinobacter litoralis and 96.9 % to Marinobacter hydrocarbonoclasticus and Marinobacter aquaeolei. DNA-DNA hybridization between the five isolates was at the conspecific level (94-96 %) and that among the closest phylogenetic neighbours ranged from 45 to 62 %. The new organisms were susceptible to polymyxin. Predominant fatty acids were C16: 0, C16: 1{omega}9c, C16: 1{omega}7c and C18: 1{omega}9c. Phylogenetic evidence, along with phenotypic and genotypic characteristics, showed that the bacteria constituted a novel species of the genus Marinobacter. The name Marinobacter excellens sp. nov. is proposed for this species, with the type strain KMM 3809T (=CIP 107686T).


The term flexispira' refers to micro-organisms with a particular morphology: fusiform-shaped with helical periplasmic fibrils and bipolar tufts of sheathed flagella. Two flexispira taxa have been formally named, Helicobacter bilis and Helicobacter trogontum, a third named species is Helicobacter aurati and eight additional 16S rRNA sequence-based flexispira taxa have been described by Dewhirst et al. (Int J Syst Evol Microbiol 50, 1781-1787, 2000) and given the
provisional designation Helicobacter sp. flexispira taxa 1-5, 7, 8 and 10. In the present study, seven gastric or intestinal flexispira isolates from seven Finnish pigs originating from different farms were characterized. Morphologically, all these porcine isolates had typical flexispira morphology. Analysis of the 16S rDNA sequences of five isolates showed that they were most closely related to the sequences of flexispira taxa 4 and 5 and H. trogontum (taxon 6), but less closely related to taxa 1-3 and 8, H. bilis and H. aurati. Phenotypic characterization, analysis of RFLPs of 16S and 23S rDNAs and SDS-PAGE profiles revealed that all of the porcine isolates, reference strains of flexispira taxa 1, 4 and 5 and the type strain of H. trogontum (ATCC 700114T) had highly related characteristics that differed from those of the reference strains of taxa 2, 3 and 8 and H. bilis. Furthermore, a high DNA-DNA binding rate was found, in dot-blot hybridization studies, between the Finnish porcine strains, taxa 1, 4 and 5 reference strains and H. trogontum ATCC 700114T. In conclusion, polyphasic characterization of novel porcine flexispira isolates and previously described taxa 1, 4 and 5 reference strains showed that they all belong to a validly described species, H. trogontum, and that the taxonomy of known flexispiras is less complicated than proposed on the basis of 16S rDNA sequence analysis.


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The taxonomic status of 64 strains of whorl-forming Streptomyces (formerly Streptoverticillium) species was re-evaluated and strains were reclassified on the basis of their phenotypes, DNA-DNA hybridization data and partial sequences of gyrB, the structural gene of the B subunit of DNA gyrase. These strains, which consisted of 46 species and eight subspecies with validly published names and 13 species whose names have not been validly published [including 10 strains examined by the International Streptomyces Project (ISP)], were divided into two groups, namely typical and atypical whorl-forming Streptomyces species, based on their phenotypes and gyrB gene sequences. The typical whorl-forming species (59 strains) were divided into six major clusters of three or more species, seven minor clusters of two species and five single-member clusters, based on the threshold value of 97 % gyrB sequence similarity. Major clusters were typified by Streptomyces abikoensis, Streptomyces cinnamoneus, Streptomyces distallicus, Streptomyces griseocarneus, Streptomyces hiroshimensis and Streptomyces netropsis. Phenotypically, members of each cluster resembled each other closely except for the S. distallicus cluster, which was divided phenotypically into the S. distallicus and Streptomyces stramineus subclusters, and the S. netropsis cluster, which was divided into the S. netropsis and Streptomyces eurocidicus subclusters. Strains in each minor cluster closely resembled each other phenotypically. DNA-DNA relatedness between the representative species and others in each major cluster and/or subcluster, and between strains in the minor clusters, was >70 %, indicating that the major clusters and/or subclusters and the minor clusters each comprise a single species. It was concluded that 59 strains of typical whorl-forming Streptomyces species consisted of the following 18 species, including subjective synonym(s): S. abikoensis, Streptomyces ardis, Streptomyces blastmyceticus, S. cinnamoneus, S. eurocidicus, S. griseocarneus, S. hiroshimensis, Streptomyces lilacinus, Streptomyces luteoreticuli", Streptomyces luteosporeus, Streptomyces mashuensis, Streptomyces mobaraensis, Streptomyces morookaense, S. netropsis, Streptomyces orinoci, S. stramineus, Streptomyces thioluteus and Streptomyces viridiflavus.

Among a large collection of South African dairy isolates, a novel Chryseobacterium taxon (DNA group 3) was previously delineated by a polyphasic taxonomic study (Hugo et al., Syst Appl Microbiol 22, 586-595, 1999). In the present paper, this taxon is further characterized using 16S rDNA sequencing, fatty acid methyl ester analysis and a comparative phenotypic analysis, resulting in the proposal of a novel species, Chryseobacterium joostei sp. nov. (type strain Ix 5aT=LMG 18212T=CCUG 46665T).


Four light-yellow-pigmented, Gram-negative, short-rod-shaped, non-motile isolates were obtained from enrichment culture during degradation of the thallus of the brown alga Fucus evanescens. The isolates studied were chemo-organotrophic, alkalitolerant and mesophilic. Polar lipids were analysed and phosphatidylethanolamine was the only phospholipid identified. The predominant cellular fatty acids were 15: 0, i15: 0, a15: 0, i15: 1 and 15: 1(n-6). The DNA G+C contents of the four strains were 34-34.4 mol%. The level of DNA relatedness of the four isolates was conspecific (88-98 %), indicating that they belong to the same species. The 16S rDNA sequence of strain KMM 3553T was determined. Phylogenetic analysis revealed that KMM 3553T formed a distinct phyletic line in the phylum Bacteroidetes, class Flavobacteria in the family Flavobacteriaceae and that, phylogenetically, this strain could be placed almost equidistant from the genera Gelidibacter and Psychroserpens (16S rRNA gene sequence similarities of 94 %). On the basis of significant differences in phenotypic and chemotaxonomic characteristics, it is suggested that the isolates represent a novel species in a new genus; the name Formosa algae gen. nov., sp. nov. is proposed. The type strain is KMM 3553T (=CIP 107684T).


Two whitish yellow, Gram-positive, non-motile, aerobic bacteria were isolated from enrichment culture during degradation of the thallus of the brown alga Fucus evanescens. The bacteria studied were chemo-organotrophic, mesophilic and grew well on nutrient media containing up to 15 % (w/v) NaCl. The DNA G+C content was 61 mol%. The two isolates exhibited a conspecific DNA-DNA relatedness value of 98 %, indicating that they belong to the same species. A comparative analysis of 16S rRNA gene sequences revealed that strain KMM 3637T formed a distinct phyletic lineage in the genus Brevibacterium (family Brevibacteriaceae, class Actinobacteria) and showed the highest sequence similarity (about 97 %) to Brevibacterium casei. DNA-DNA hybridization experiments demonstrated 45 % binding with the DNA of B. casei DSM 20657T. Physiological and chemotaxonomic characteristics (meso-diaminopimelic acid in the peptidoglycan, major cellular fatty acids 15: 0ai and 17: 0ai) of the bacteria studied were consistent with the genomic and phylogenetic data. On the basis of the results of this study, a novel species, Brevibacterium celere sp. nov., is proposed. The type strain is KMM 3637T (=DSM 15453T=ATCC BAA-809T).

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Two marine bacterial strains, KMM 3823T and KMM 3836, isolated from a sipuncula (Phascolosoma japonicum), a common inhabitant of Troitsa Bay in the Gulf of Peter the Great (Sea of Japan), were studied. Comparative 16S rRNA gene sequence-based phylogenetic analysis placed these bacteria into a separate branch of the Gammaproteobacteria within members of the genus Shewanella. KMM 3823T showed the highest similarity (96% of Shewanella fidelis). The DNA G+C contents of the two strains studied were 43 mol%. The level of DNA homology between these two strains was conspecific (93%), indicating that they represent a single genospecies. These organisms were greenish-brown, Gram-negative, polarly flagellated, facultatively anaerobic, mesophilic (temperature range 4-30 °C), neutrophilic, haemolytic and were able to degrade elastin, gelatin and DNA. They were susceptible to ampicillin, carbenicillin, gentamicin and kanamycin. The predominant fatty acids were characteristic for shewanellas: 13: 0-i, 15: 0-i and 16: 1(n-7); up to 6% of eicosapentaenoic fatty acid, 20: 5(n-3), was produced during growth at 28 °C. Phylogenetic evidence, confirmed by DNA hybridization and phenotypic characteristics revealed that the two bacteria studied constitute a new species, Shewanella waksmanii sp. nov., the type strain of which is KMM 3823T (=CIP 107701T=ATCC BAA-643T).


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Aster yellows (AY) group (16SrI) phytoplasmas are associated with over 100 economically important diseases worldwide and represent the most diverse and widespread phytoplasma group. Strains that belong to the AY group form a phylogenetically discrete subclade within the phytoplasma clade and are related most closely to the stolbur phytoplasma subclade, based on analysis of 16S rRNA gene sequences. AY subclade strains are related more closely to their culturable relatives, Acholeplasma spp., than any other phytoplasmas known. Within the AY subclade, six distinct phylogenetic lineages were revealed. Congruent phylogenies obtained by analyses of tuf gene and ribosomal protein (rp) operon gene sequences further resolved the diversity among AY group phytoplasmas. Distinct phylogenetic lineages were identified by RFLP analysis of 16S rRNA, tuf or rp gene sequences. Ten subgroups were differentiated, based on
analysis of rp gene sequences. It is proposed that AY group phytoplasmas represent at least one novel taxon. Strain OAY, which is a member of subgroups 16SrI-B, rpI-B and tufI-B and is associated with evening primrose (Oenothera hookeri) virescence in Michigan, USA, was selected as the reference strain for the novel taxon Candidatus Phytoplasma asteris. A comprehensive database of diverse AY phytoplasma strains and their geographical distribution is presented.


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Five Aeromonas strains (848TT, 93M, 431E, 849T and 869N), which were isolated from bivalve molluscs and were recognized previously by numerical taxonomy as members of an unknown Aeromonas taxon, were subjected to a polyphasic taxonomic study. DNA-DNA hybridization experiments showed that DNA of strain 848TT was <70 % similar (27-45 %) to that of the type/reference strains of the current Aeromonas hybridization groups (HGs), but 93 % similar to that of strain 93M. The DNA G+C content of the five strains ranged from 59{middle dot}0 to 59{middle dot}4 mol%. 16S rRNA gene sequence analysis confirmed that the strains belonged to the genus Aeromonas and showed high similarity to Aeromonas encheleia. Amplified fragment length polymorphism fingerprinting clustered the novel strains in a homogeneous group with low genotypic relatedness to other Aeromonas species. Useful phenotypic features for differentiating the five isolates from other Aeromonas species include their negative reactions in tests for indole production, lysine decarboxylase, gas from glucose and starch hydrolysis. From the results of this study, the name Aeromonas molluscorum sp. nov. is proposed for these strains, with the type strain 848TT (=CECT 5864T=LMG 22214T).


http://ijs.sgmjournals.org/cgi/content/abstract/53/6/1725

The chloroplast genes of dinoflagellates are distributed among small, circular dsDNA molecules termed minicircles. In this paper, we describe the structure of the non-coding region of the psbA minicircle from Symbiodinium. DNA sequence was obtained from five Symbiodinium strains obtained from four different coral host species (Goniopora tenuidens, Heliofungia actiniformis, Leptastrea purpurea and Pocillopora damicornis), which had previously been determined to be closely related using LSU rDNA region D1/D2 sequence analysis. Eight distinct sequence blocks, consisting of four conserved cores interspersed with two metastable regions and flanked by two variable regions, occurred at similar positions in all strains. Inverted repeats (IRs) occurred in tandem or twin' formation within two of the four cores. The metastable regions also consisted of twin IRs and had modular behaviour, being either fully present or completely absent in the different strains. These twin IRs are similar in sequence to double-hairpin elements (DHEs) found
in the mitochondrial genomes of some fungi, and may be mobile elements or may serve a functional role in recombination or replication. Within the central unit (consisting of the cores plus the metastable regions), all IRs contained perfect sequence inverses, implying they are highly evolved. IRs were also present outside the central unit but these were imperfect and possessed by individual strains only. A central adenine-rich sequence most closely resembled one in the centre of the non-coding part of Amphidinium operculatum minicircles, and is a potential origin of replication. Sequence polymorphism was extremely high in the variable regions, suggesting that these regions may be useful for distinguishing strains that cannot be differentiated using molecular markers currently available for Symbiodinium.


http://ijs.sgmjournals.org/cgi/content/abstract/54/4/1063

A moderately thermophilic and alkaliphilic bacillus, which had been reported and designated BLx (Haruta et al., 2002), was isolated from a semi-continuous decomposing system of kitchen refuse. Cells of strain BLxT were strictly aerobic, rod-shaped, motile and spore forming. The optimum temperature and pH for growth were approximately 50 °C and pH 8-9. Strain BLxT was able to grow at NaCl concentrations from 0 to 7 %, with optimum growth at 0 % NaCl. The predominant menaquinone was MK-7, and the major fatty acid was iso-C15: 0. Phylogenetic analysis showed that strain BLxT was positioned in an independent lineage within the cluster that includes the genera Virgibacillus and Lentibacillus in Bacillus rRNA group 1. Strain BLxT exhibited 16S rDNA similarity of 92 to 94 % to Virgibacillus species and 92 % to Lentibacillus salicampi. Phenotypic, chemotaxonomic and phylogenetic analyses supported the classification of strain BLxT in a novel genus and species. Cerasibacillus quisquiliarum gen. nov., sp. nov. is proposed on the basis of phenotypic, chemotaxonomic and phylogenetic data. The type strain is BLxT (DSM 15825T=IAM15044T=KCTC 3815T).


http://ijs.sgmjournals.org/cgi/content/abstract/53/6/2019

The family Vibrionaceae is considered to be one of the most diverse and well-studied groups of bacteria. Here, evolution is assessed within the Vibrionaceae to determine whether multiple origins of eukaryotic associations have occurred within this diverse group of bacteria. Analyses were based on a large molecular dataset, along with a matrix that consisted of 100 biochemical and restriction digest characters. By using direct optimization methods to analyse both datasets individually and in combination, a total-evidence cladogram has been produced, which supports the hypothesis that several important symbionts (both mutualistic and pathogenic) within the Vibrionaceae are not monophyletic. This leads us to consider that symbiosis (and subsequently, associations with Eukarya) has evolved multiple times within the Vibrionaceae lineage.

Strain H2-LRT, a 5-18 μm long and 0.7 μm wide filamentous, mesophilic, moderately halophilic, non-motile hydrogenotrophic methanogen, was isolated from marine sediment of Aarhus Bay, Denmark, 1 m below the sediment surface. On the basis of 16S rRNA gene comparison with sequences of known methanogens, strain H2-LRT could be affiliated to the genus Methanobacterium. The strain forms a distinct line of descent within this genus, with Methanobacterium oryzae (95.9% sequence identity) and Methanobacterium bryantii (95.7% sequence identity) as its closest relatives. The 16S rRNA-based affiliation was supported by comparison of the mcrA gene, which encodes the α-subunit of methyl-coenzyme M reductase. Strain H2-LRT grew only on H2/CO2. The DNA G+C content is 34.9 mol%. Optimum growth temperature was 45 °C. The strain grew equally well at pH 7 and 8. No growth or methane production was observed below pH 5 or above pH 9. Strain H2-LRT grew well within an NaCl concentration range of 100 and 900 mM. No growth or methane production was observed at 1 M NaCl. At 50 mM NaCl, growth and methane production were reduced. Based on 16S rRNA gene sequence analysis, the isolate is proposed to represent a novel taxon within the genus Methanobacterium, namely Methanobacterium aarhusense sp. nov. The type strain is H2-LRT (=DSM 15219T=ATCC BAA-828T).


This study analysed the usefulness of rpoB gene sequences as an alternative phylogenetic and/or identification marker for vibrios. The rpoB sequences suggest that the genus Vibrio is polyphyletic. The high heterogeneity observed within vibrios was congruent with former polyphasic taxonomic studies on this group. Photobacterium species clustered together and apparently nested within vibrios, while Grimontia hollisae was apart from other vibrios. Within the vibrios, Vibrio cholerae and Vibrio mimicus clustered apart from the other genus members. Vibrio
harveyi- and Vibrio splendidus-related species formed compact separated groups. On the other hand, species related to Vibrio tubiashii appeared scattered in the phylogenetic tree. The pairs Vibrio coralliilyticus and Vibrio neptunius, Vibrio nereis and Vibrio xuii and V. tubiashii and Vibrio brasiliensis clustered completely apart from each other. There was a correlation of 0.58 between recA and 16S rDNA pairwise similarities. Strains of the same species have at least 94% recA sequence similarity. recA gene sequences are much more discriminatory than 16S rDNA. For 16S rDNA similarity values above 98% there was a wide range of recA similarities, from 83 to 99%.


http://intimm.oupjournals.org/cgi/content/abstract/14/6/545

We studied the expression of a human macrophage lectin specific for galactose/N-acetylgalactosamine (hMGL) during macrophage differentiation. The expression of hMGL during the in vitro differentiation induced by human serum was examined by immunostaining and Western blotting with a specific mAb, MLD-1, as well as with RT-PCR analysis. hMGL was detected on cells at an intermediate stage of differentiation. These cells were round, slightly larger in size (12.7 +/- 0.2 (micro)m) than monocytes (9.8 +/- 0.1 (micro)m) and expressed the macrophage marker CD14, but lacked the dendritic cell marker CD1a. The highest levels of expression occurred after 2-4 days of culture. At this time point, MLD-1 prominently stained 20-40% of the cells. Monocytes cultured for 16 h or fully differentiated monocyte-derived macrophages were negative or weak for hMGL expression. Similar transient expression was also observed during granulocyte macrophage colony stimulating factor- or macrophage colony stimulating factor-dependent macrophage differentiation. The lectin was characterized as a functional endocytic receptor for glycosylated macromolecules, since the uptake of carbohydrate polymers was partially inhibited by the addition of MLD-1. The distribution of hMGL+ cells in normal human skin was found by immunostaining to be mainly in the upper dermis distant from vascular structures. More than 90% of the hMGL+ cells were double stained with anti-CD68 mAb and constituted [-]20% of the CD68+ cells. We suggest that the dermal hMGL+ cells are a subset of differentiated cells derived from monocytes and that hMGL is a unique marker for cells at an intermediate stage of macrophage differentiation.


http://intimm.oupjournals.org/cgi/content/abstract/15/8/1017

The vitamin A metabolite, retinoic acid (RA), affects Th1 and Th2 development. The effect is partly exerted through the modulation of antigen-presenting cell functions, but it remains unclear whether RA directly exerts its effect on T cells to influence Th1/Th2 development. To clarify this problem, we used two experimental systems with isolated T cells in vitro. In one system, isolated
CD4+CD8+ thymocytes differentiated into Th1 and Th2 by two transient stimulations with defined combinations of ionomycin and phorbol myristate acetate followed by treatment with IL-2 and IL-4 and/or IL-12. In the second system, functional differentiation was induced in purified naive CD4 T cells from DO-11.10 TCR-transgenic and RAG-2-deficient mice with cytokines and antibodies to CD3 and CD28. In both systems, all-trans-RA at 1 nM concentrations suppressed Th1 development, but enhanced Th2 development. 9-cis-RA elicited similar effects. The optimal enhancement of Th2 development in the second system, however, was achieved with a delayed addition of RA. The presence of RA during the initial stimulation period often suppressed Th2 development. The RA receptor (RAR) antagonists, LE540 and LE135, but not the retinoic X receptor (RXR) antagonist, PA452, inhibited the effect of RA on Th1/Th2 development. Accordingly, the RAR agonists, Am80 and Tp80, but not the RXR agonists, HX600 and TZ335, mimicked the effect of RA. The RXR agonists enhanced the effect of the RAR agonists only slightly, if at all. These results indicate that, via RAR, RA directly suppresses Th1 development and directly enhances Th2 development with its timely addition.


http://intimm.oupjournals.org/cgi/content/abstract/14/1/79

We have previously identified a locus on mouse chromosome 15 (eae2) that regulates susceptibility to experimental autoimmune encephalomyelitis in a cross between the susceptible strain B10.RIII and the resistant strain RIIIS/J. In an effort to verify the protective effect from having two RIIIS/J alleles at eae2, the resistant locus was bred into the susceptible strain in homozygous form. However, the expected effect was not as clear as in the original study. This might be due to an epistatic effect conferred by several unidentified genes in the genome of the resistant strain or due to the environment by genotype interactions, possibly overcoming the effect of protective alleles at eae2. To further the genetic understanding in this disease, a genome-wide linkage screening approach was employed on an F2 intercross that carried the protective allele at eae2 in homozygous form while the rest of the genome segregated between the B10.RIII and RIIIS/J strains as in the original investigation. In the present study we find one region on chromosome 7, not previously identified in this strain combination, that affects the disease at significant logarithm of the odds score and six regions showing suggestive evidence for linkage to disease phenotypes.


http://intimm.oupjournals.org/cgi/content/abstract/15/1/109

Mannose-binding lectin (MBL) is a C-type lectin involved in the first line of host defense and it requires MBL-associated serine proteases (MASP) for activation of the lectin complement pathway (LCP). Recently we reported that human ficolins, L-ficolin/P35 and H-ficolin/Hakata antigen, as well as MBL activate the LCP in association with MASP. We investigated in vitro expression of complements of the lectin complement pathway in several cell lines. Out of 17 cell lines tested using RT-PCR, a human glioma cell line, T98G, expressed high levels of H-ficolin/Hakata antigen, MASP1 and MASP3 mRNAs. Similar results were obtained in four other glioma lines. In addition, mRNAs for C1r, C1s, C2, C3, C4, C5 and C6 were also detected in T98G cells, but very low amount of mRNAs for C1q and MBL. MBL mRNA was seen in two of the other glioma cell lines. An ELISA of culture supernatants showed that T98G cells secreted a
considerable amount of MASP-1 and MASP-3 proteins. SDS-PAGE and immunoblotting analyses showed the secreted H-ficolin/Hakata antigen, MASP-1 and MASP-3 to be 34, 81 and 105 kDa in size respectively, similar to their serum counterparts. Since the glioma cells used are derived from astrocytes, this suggests that human astrocytes may be a source of some components of the LCP in the brain.


Maturation of dendritic cells (DC) serves a deterministic role in the link between innate and adaptive immunity, constituting a checkpoint with regard to whether responses from the lymphocyte compartment shall be raised and what class of response is needed to protect the host against invading pathogens. Since DC have not been shown to possess mechanisms such as gene recombination or somatic mutation for generating a diverse repertoire of antigen-recognition receptors, it is unlikely that these leukocytes can intrinsically respond to all conceivable molecules present in our environment. In the present study, we have therefore determined how mediators of the inflammatory response regulate global gene transcription in DC. The data represent an extensive and time-ordered reprogramming of the DC during their course of maturation, involving genes encoding proteins that regulate responses of both innate cells and lymphocytes. This transcriptional reorganization may reflect the effect of in vivo released inflammatory mediators induced by endogenous or pathogenic stimulation.


Peyer's patch follicle-associated epithelium (FAE) regulates intestinal antigen access to the immune system in part through the action of microfold (M) cells which mediate transcytosis of antigens and microorganisms. Studies on M cells have been limited by the difficulties in isolating purified cells, so we applied TOGA mRNA expression profiling to identify genes associated with the in vitro induction of M cell-like features in Caco-2 cells and tested them against normal Peyer's patch tissue for their expression in FAE. Among the genes identified by this method, laminin (beta)3, a matrix metalloproteinase and a tetraspan family member, showed enriched expression in FAE of mouse Peyer's patches. Moreover, the C. perfringens enterotoxin receptor (CPE-R) appeared to be expressed more strongly by UEA-1+ M cells relative to neighboring FAE. Expression of the tetraspan TM4SF3 gene and CPE-R was also confirmed in human Peyer's patch FAE. Our results suggest that while the Caco-2 differentiation model is associated with some functional features of M cells, the genes induced may instead reflect the acquisition of a more general FAE phenotype, sharing only select features with the M cell subset.

MHC class II expression defects have been evidenced in several human tumor cell lines originating from lung cancers or retinoblastoma. Accordingly, the mouse adenocarcinoma and fibrosarcoma cell lines, RAG and L(tk-), do not express I-A and I-E molecules even when treated with IFN-\(\gamma\). Here we show that fusion of both cell lines restores the inducible expression of MHC class II, thereby demonstrating that they present different and recessive alterations outside the MHC class II locus. CIITA, the MHC class II transactivator, controls the tissue-specific expression of MHC class II genes and creates the architecture of the transcriptional complex that binds to the MHC class II gene promoters. In L(tk-) cells, C2ta transcripts, expressed from the gene encoding CIITA, were indeed detected in severely limited amounts, with a defect in C2ta transcription initiation. In agreement we show here that the L(tk-) cell line does not express the CIITA protein. In contrast, in the RAG cell line, C2ta transcripts were expressed at normal levels, from the proper initiation site. The nucleotide sequencing of the CIITA cDNA from RAG did not reveal any mutation. However, the CIITA protein was not detected. These data evidence a new type of defect in a MHC class II-defective tumor cell line, as we show here that the alteration in the RAG cells occurs downstream of C2ta transcription. The RAG mutation might therefore reside in the C2ta transcript nuclear export or translation, or in the stability of the CIITA protein.


http://intimm.oupjournals.org/cgi/content/abstract/14/10/1085

Cytotoxic lymphocytes, NK cells and CD8+ T cells play a pivotal role in the host defense. To reveal the biological function of these cells through establishing a comprehensive gene expression profile, serial analysis of gene expression was performed in human peripheral blood NK cells and CD8+ T cells. In total, 85,848 tags corresponding to >20,000 different transcripts were sequenced. The genes expressed abundantly in these libraries mostly consisted of genes encoding MHC class I and molecules related to protein synthesis. Among gene transcripts which related to cytotoxicity, granulysin, perforin, granzyme B and (alpha)-defensin 1 were highly expressed in NK cells. Resting CD8+ T cells did not express the genes related to cytotoxicity, but expressed abundantly the genes encoding chemokines, tumor necrosis factor family. When CD8+ T cells were sorted into naive, memory and effector subsets based on the expression of CD45RA and CD27, perforin and granzyme B were expressed in the CD45RA+CD27- effector subset. (alpha)-Defensin 1, one of the selectively expressed genes in NK cells, induced migration of naive CD8+CD45RA+CD27+ T cells, but not memory CD8+CD45RA-CD27+ or effector CD8+CD45RA+CD27- T cells. Furthermore, treatment with IL-15, a stimulator of NK cell development, differentiation, survival and cytotoxicity, rapidly enhanced the expression of (alpha)-defensin 1 in NK cells. The identification of the genes preferentially expressed in NK and CD8+ T cell subsets may give important insights into the functions of these cells against virus infection and in tumor immunity.


http://intimm.oupjournals.org/cgi/content/abstract/14/6/599

In order to explore the role of gp130-linked signal transduction in the differentiation and maturation of dendritic cells (DC), the mAb, B-S12, an agonist of gp130, was used for the activation of gp130 on DC. The effects of cytokines and of anti-gp130 mAb on the proliferation of DC, and their expression of IL-12 and CD80 (B7-1) by DC were evaluated. DC differentiating from peripheral blood mononuclear cells did not express the IL-6 receptor (alpha) chain, but expressed gp130. Anti-gp130 mAb promoted the proliferation of DC, induced by IL-4 and granulocyte
macrophage colony stimulating factor (GM-CSF), by up-regulating the GM-CSF receptor on DC. DC induced by gp130 mAb and cytokines expressed DC-derived CC chemokine, as measured by RT-PCR. Induced DC also stimulated strong proliferation of autologous T cells in mixed lymphocyte reaction since an up-regulated expression of IL-12 and CD80 (B7-1) was observed in DC activated by anti-gp130 mAb. Thus, gp130 signal transduction is important for the differentiation and maturation of DC.


http://intimm.oupjournals.org/cgi/content/abstract/15/9/1073

T cells recognizing myelin basic protein (MBP) are potentially involved in the pathogenesis of multiple sclerosis (MS). In vivo clonal expansion of MBP-reactive T cells in MS may relate in part to dysfunction of peripheral regulatory mechanisms, including the anti-idiotypic immune network. In this study, we examined anti-idiotypic immune responses and the functional properties of anti-idiotypic T cells in patients with MS and healthy controls using TCR peptides corresponding to a CDR3 sequence motif preferentially expressed among T cells recognizing the 83-99 immunodominant peptide of MBP in some patients with MS. The study demonstrated that anti-idiotypic T cells could be induced in vitro by 8mer and 15mer peptides containing the CDR3 motif in MS patients and healthy controls respectively. The estimated precursor frequency of the anti-idiotypic T cells was slightly reduced in MS patients compared to control subjects. The obtained anti-idiotypic T cells recognizing the 15mer TCR peptide were found to express the CD4 phenotype, produce predominantly IL-10 and inhibit the proliferation of autologous T cells recognizing the immunodominant peptide of MBP. Anti-idiotypic T cells induced by the 8mer TCR peptide were predominantly CD8+ cytotoxic T cells and exhibited cytotoxic activity against autologous MBP-specific T cells expressing the CDR3 sequence. When added in primary culture, both TCR peptides had a significant inhibitory effect on the T cell responses to the immunodominant peptide of MBP. The findings suggest that anti-idiotypic immune responses can be activated by selected TCR peptides and may play an important role in the in vivo regulation of MBP-reactive T cells.

International Hepatology Communications (2)


http://www.sciencedirect.com/science/article/B6T7D-4F6DWMP-3S/2/80e313b577a18c72574340ee9ca6f671

We determined quantitative values of serum hepatitis C virus RNA by branched DNA amplification assay in 52 consecutive patients with chronic hepatitis C immediately before high-dose treatment with interferon-alpha. Thirty-four out of 52 patients had >106.3 ([ap]2 x 106) equivalents/ml of viral genomes. Only three (8.8%) out of these 34 were long-term responders, while 16 (88.9%) out of 18 patients with 6.3 equivalents/ml of viral genomes were long-term
responders (P 6.3 equivalents/ml and 10 (90.9%) out of these 11 had long-term response, while only one (3.2%) out of 31 patients with >106.3 equivalents/ml was a long-term responder (P < 0.001). By determining HCV-RNA levels in serum, we could thus predict long-term responsiveness to high-dose interferon therapy in 47 (90.4%) out of 52 chronic hepatitis C patients, and 40 (95.2%) out of 42 patients with genotype II hepatitis C virus. Our results indicate that quantitative measurement of hepatitis C viral genomes in pretreatment serum by the simple branched DNA amplification assay is an excellent method for predicting long-term responsiveness to interferon treatment.


http://www.sciencedirect.com/science/article/B6T7D-4F6DWMP-3C/2/cc0c722792d6cf3729d77800932971fe

The effect of interferon on hepatitis C virus RNA levels in the liver was studied by polymerase chain reaction in chronic hepatitis C patients who were enrolled into a pilot study with short-term interferon treatment. Among 17 patients treated with interferon [alpha] or [beta] (168-560 M.U. in total), eight were 'long-term responders' as defined by sustained normal serum alanine aminotransferase levels for more than 12 months, while the other nine were 'nonresponders' with abnormal alanine aminotransferase levels. In eight 'long-term responders', both the plus and minus strands (replicative intermediate) of hepatitis C virus RNA could not be detected in the liver after interferon treatment whereas eight of nine 'non-responders' retained the minus strand or both strands of hepatitis C virus RNA in the liver. Serum hepatitis C virus RNA was no longer detected in seven of nine 'non-responders' or in all 'long-term responders' at the end of treatment, but it reappeared in 'non-responders' with the elevation of serum alanine aminotransferase levels, which excluded serum hepatitis C virus RNA as a prognostic marker for sustained alanine aminotransferase normalization after interferon treatment. Our results indicate that the disappearance of hepatitis C virus RNA including the minus strand RNA from the liver is a predictive marker for good prognosis in chronic hepatitis C patients after interferon treatment. It is of great use to determine the levels of hepatitis C virus RNA in the liver to know the prognosis of interferon-treated patients who maintain sustained normal serum alanine aminotransferase levels and undetectable serum hepatitis C virus RNA after interferon treatment.

International Immunopharmacology (10)


http://www.sciencedirect.com/science/article/B6W7N-44F6DJV-7/2/5c7706d087732867371db915883d0256

An in vitro model of multi-step activation, in which cells of macrophage lineage are driven sequentially through inflammatory, primed, and fully activated states, was employed to assess for cannabinoid receptor expression. Murine and rat peritoneal macrophages, murine RAW264.7 and
P388D1 macrophage-like cells, and neonatal rat brain cortex microglia expressed the cannabinoid receptor type 2 (CB2) differentially in relation to cell activation. The CB2 was undetectable in resident peritoneal macrophages, present at high levels in thioglycolate-elicited inflammatory and interferon gamma (IFN[gamma])-primed peritoneal macrophages, and detected at significantly diminished levels in bacterial lipopolysaccharide (LPS)-activated peritoneal macrophages. A comparable pattern of differential expression of the CB2 was noted for murine macrophage-like cells and neonatal rat brain cortex microglia. The cannabinoid receptor type 1 (CB1) was not detected in peritoneal macrophages or murine macrophage-like cells regardless of cell activation state but was present in neonatal rat microglia at low levels. These results indicate that levels of the CB2 in cells of macrophage lineage undergo major modulatory changes in relation to cell activation. Furthermore, since inflammatory and primed macrophages express the highest levels of CB2, the functional activities of macrophages when in these respective states of activation may be the most sensitive to the action of cannabinoids.


Herbal medicines are increasingly being utilized to treat a wide variety of disease processes. Aqueous extract from the root of Platycodon grandiflorum A. DC (Campanulaceae), Changkil (CK), is reported to have antitumor and immunomodulatory activities; however, the mechanism underlying its therapeutic effect is not known. In the present study we examined the effects of CK on the release of nitric oxide (NO) and tumor necrosis factor-[alpha] (TNF-[alpha]), and on the gene expression of iNOS and TNF-[alpha] in mouse macrophages. CK elicited a dose-dependent increase in NO and TNF-[alpha] production in cultured macrophages. CK significantly affected secretion at concentrations of more than 5 [mu]g/ml, and its maximum effect was at concentration of 100 [mu]g/ml. Reverse transcription polymerase chain reaction showed that increases in NO and TNF-[alpha] secretion were due to an increase in inducible NO synthase mRNA and TNF-[alpha] mRNA, respectively. Transient expression assays with NF-[kappa]B binding sites linked to the luciferase gene revealed that CK-induced increase of inducible NO synthase mRNA and TNF-[alpha] mRNA were mediated by the NF-[kappa]B transcription factor complex. These results demonstrate that CK stimulates NO and TNF-[alpha] release and is able to upregulate iNOS and TNF-[alpha] expression through NF-[kappa]B transactivation and this may be a mechanism whereby this herbal medicine elicits its therapeutic effects.


RNA oligonucleotides termed External Guide Sequence (EGS) and RNAi have been described that target specific gene expression by site-specific cleavage of mRNA. EGS serve as an RNA catalyst or ribozyme by directing bound mRNA to the ubiquitous cellular enzyme RNAse P. We describe an EGS targeting human interleukin (IL)-4 receptor [alpha] mRNA, an important cytokine receptor in the pathogenesis of asthma and allergic disease expressed in pulmonary tissues. This EGS was designed to explore pulmonary delivery of catalytic RNA oligonucleotides as a novel therapy in asthma and other atopic diseases. Inhaled DNA oligonucleotides termed Respirable Antisense OligoNucleotide Sequences (RASONS) are selectively internalized in lung tissues in a
complex with endogenous lipid surfactants present in normal lung and can alter pulmonary gene expression. Potential applications of inhaled RNA oligonucleotides in therapy of pulmonary and related systemic diseases are discussed.


http://www.sciencedirect.com/science/article/B6W7N-4C56GF5-1/2/be7b06c26f4abdaecb359814cf9b8f05

Signaling via the endogenous arylhydrocarbon receptor (AHR) affects proliferation, differentiation, function and gene expression of thymocytes. In the present study, we show that treatment of mouse fetal thymus lobes in organ culture (FTOC) with AHR ligands results in (a) a drastic decrease in the emigration of thymocytes in terms of numbers and types of cells, and (b) preferential emigration of CD4-CD8- (DN) cells expressing CD44v7- and CD44v10-containing isoforms on the cell surface. Moreover, a higher level of transcripts of various other CD44 variant isoforms (CD44v) could be detected by RT-PCR in emigrants from fetal thymi exposed to either AHR-agonist during culture. Expression of CD44v9-10-containing isoforms could be exclusively detected in DN thymic emigrants. Thus, signaling via AHR by ligands alters CD44v expression patterns in a thymocyte subpopulation. Furthermore, emigration could be decreased by the addition of anti-panCD44 antibodies to TCDD-treated FTOCs, suggesting a role for CD44 in emigration.

Fujimoto, Y., H. Iwagaki, et al. "Involvement of prostaglandin receptors (EPR2-4) in in vivo immunosuppression of PGE2 in rat skin transplant model." International Immunopharmacology In Press, Uncorrected Proof http://www.sciencedirect.com/science/article/B6W7N-4FN2K5W-1/2/49c6b1dd24658860d5e4539a96878d9a

BackgroundProstaglandin E2 (PGE2) is known to modulate immune responses and is widely viewed as a general immunosuppressant. There have been recognized four receptors for PGE2 (EP1-EP4 receptor) so far, and EP2 and EP4 receptors are mainly involved in the immunosuppressive effect of PGE2 in vitro. In the present study we examined the in vivo immunosuppressive effects of selective EP receptor agonists using a high-responder rat skin transplantation model.Materials and methodsSkin allografts from ACI donors were transplanted onto LEW recipients. Agents were injected everyday between day 0 and day 5 after skin transplantation at the dose of 300 [μg/kg subcutaneously. Survival of the skin allograft, histological changes and changes of the intragraft cytokine expressions were analyzed using the reverse transcription polymerase chain reaction (RT-PCR). We also assessed the mixed lymphocyte reaction (MLR) assay using splenocytes.ResultsPGE2 significantly prolonged allograft survival (18.8 [plus-or-minus sign] 1.5 days) compared with untreated control (14.8 [plus-or-minus sign] 0.8 days). EP2R + EP3R + EP4R agonists also prolonged allograft survival (18.0 [plus-or-minus sign] 1.0 days) although EP3R agonist or EP2R + EP4R agonists alone failed (15.5 [plus-or-minus sign] 0.7, 15.4 [plus-or-minus sign] 1.3 days, respectively). RT-PCR analysis in the skin grafts demonstrated IL-10 up-regulation and IFN-[gamma] down-regulation in all groups except untreated control and EP2R agonist-treated groups. MLR was significantly reduced in groups of EP2R + EP4R agonists, EP2R + EP3R + EP4R agonists and PGE2, compared with untreated control.ConclusionsThe effect of PGE2 to prolong the survival of skin transplant requires the action of a combination of three receptors, i.e., EP2 + EP3 + EP4.

http://www.sciencedirect.com/science/article/B6W7N-43XNVWD-7/2/a405c8af0b83f350bafcd1176eca470

Many polysaccharides isolated from plants are considered to be biological response modifiers and have been shown to enhance various immune responses in vivo and in vitro. Here, we demonstrate that polysaccharide isolated from the radix of Platycodon grandiflorum (PG) has a unique mode of immunostimulation with regard to its cell-type specificity. PG was found to markedly increase polyclonal IgM antibody production and the proliferation of B cells, and to activate iNOS transcription and NO production in macrophages. Moreover, the intraperitoneal administration of PG in mice resulted in increased IgM antibody production in B cells, which were immunized by using T-dependent antigen sheep red blood cells (sRBCs). However, PG did not affect the proliferation of T cells, the IL-2 expression of Th1 cells, or the IL-4 expression of Th2 cells. Although PG and lipopolysaccharide (LPS) had a similar mode of action in B cells and macrophages, they were differentiated by the fact that PG-induced cellular activation was not inhibited by polymyxin B, a specific inhibitor of LPS. Anti-CD19 or anti-CD79b antibody blocked B cell proliferation and anti-CD14 or anti-CD11b antibody decreased macrophage NO production, indicating the possible cellular binding sites of PG. Our results demonstrate that PG is a specific activator of B cells and macrophages but not of T cells, and suggest that PG is quite distinct from other well-known immunostimulants, such as lentinan and schizophyllan, which mainly act upon macrophages and T cells.


http://www.sciencedirect.com/science/article/B6W7N-48342TC-1/2/cb01711e3e2dbe29e676251ba0d851ed

We investigated the immunomodulatory activity of polysaccharide isolated from the root of Acanthopanax koreanum (AK) at the cellular level. AK directly increased B cell proliferation and antibody production, but did not affect the expression of IL-2, IFN-[gamma] or IL-4 by T cells, or T cell proliferation in vitro. Since AK cannot penetrate cells due to its large molecular mass, B cell activation may be caused by the surface binding of AK to B cell-specific receptors. The role of TLR4 as an AK receptor was shown by the fact that AK activity in B cells from C3H/HeJ mice, which are known to have a defective Toll-like receptor (TLR)-4, was found to be reduced compared with that in control cells from C3H/HeN mice. AK activity was also reduced by antibodies blocking TLR2, TLR4, CD19 or CD79b, but not by an antibody blocking CD38, which suggests AK receptor profiling in B cells. Two main differences between AK and lipopolysaccharide (LPS) were observed. First, LPS activity was inhibited by antibodies to either TLR2 or TLR4, but not by antibodies to CD19, CD79b or CD38. Another was that LPS-induced B cell proliferation was inhibited by polymyxin B (PMB), a specific inhibitor of LPS, whereas AK activity was not affected. Taken together, our results demonstrate that AK directly activates B cells, but not T cells, and suggest that AK has a broader receptor profile than LPS in B cells.

We investigated the mechanism of the immunomodulatory action of polysaccharide (ASP) isolated from a cell culture of Acanthopanax senticosus. ASP was found to directly increase the proliferation and differentiation of B cells, and the cytokine production of macrophage, but not the proliferation and cytokine production of T cells. Since ASP cannot penetrate the cell membrane due to its large molecular mass, such cellular activation may be caused by the surface binding of ASP to receptors expressed on B cells and macrophages. The possibility that TLRs, which are known to be involved in immune-related responses, may be the receptor(s) of ASP was investigated. The immunomodulating activities of ASP on the B cells and macrophages of C3H/HeJ mice, expressing a defective toll-like receptor (TLR)-4, were decreased versus the corresponding cells from C3H/HeN mice. In addition, the activities of ASP on B cells and macrophages were significantly reduced by treating the cells with antibodies to TLR4 and TLR2 prior to ASP, suggesting that both of them are the possible receptors of ASP. The ligation of TLRs induced by ASP was able to activate mitogen-activated protein kinases (MAPKs), such as Erk1/2, p38 and JNK, and the transcription factor NF-κB. Although ASP was shown to activate the TLR signaling cascades in the same manner as lipopolysaccharide (LPS), these two could be differentiated by the finding that polymyxin B (PMB), a specific inhibitor of LPS, did not significantly affect the activities of ASP on B cells and macrophages. Taken together, our results demonstrate that ASP, isolated from a cell culture of A. senticosus, activates B cells and macrophages by interacting with TLRs and leading to the subsequent activation of mitogen-activated protein kinases and NF-κB.


Interleukin-12 (IL-12) is a heterodimeric cytokine that enhances immune responses to bacterial, parasitic, and viral pathogens, and leads to tumor regression in animal models. For this reason, the use of IL-12 as a vaccine adjuvant and as a therapeutic agent for the treatment of cancer is being investigated. Unfortunately, the extreme toxicity of this molecule observed during clinical trials has limited its use. This toxicity correlates with increased IFN-γ expression, decreased glucose levels, and altered histological responses in the spleen and duodenum. In this study, we show that intranasal (i.n.) delivery of IL-12 is a less toxic route of inoculation compared to the commonly employed subcutaneous route. When delivered i.n., IL-12 induces less systemic IFN-γ production and fewer pathological tissue changes, yet is efficacious, as indicated by enhanced CD3+ T cell activation and increased production of Th1-associated immunoglobulins (i.e., serum IgG2a). Thus, IL-12 can be delivered safely and effectively by the i.n. route, a finding which may allow IL-12 to fulfill its clinical potential.


Herein we report mechanisms whereby Flt3 ligand (FL) augments steady state T cell activity in addition to the expansion of dendritic cells (DCs). We demonstrate that in vivo administration of
FL increases the frequency and absolute number of effector/memory T cells and preferentially expands T cells that express a type-1 cytokine phenotype. In addition, FL enhances T cell proliferative responses to Concanavalin A that directly correlated with increased frequencies in effector/memory T cells and expansion of lymphoid-derived (type 1) DCs (DC1s). Together, these data demonstrate that mechanisms of FL-induced T cell regulation include not only the expansion of DC subsets, but also the preferential expansion of type 1-effector/memory T cell populations, and suggest multiple mechanisms of action for FL as a vaccine adjuvant and as a therapeutic modality.

International Journal of Biochemistry (2)


http://www.sciencedirect.com/science/article/B73GR-47F1F5V-8D/2/27ed9fbbd44295dfde577f2a70dbca30

1. A DNA fragment encoding the [beta] subunit of bovine inhibin was amplified using the polymerase chain reaction and was cloned in plasmids pUC8 and pUR291. 2. Cultures of Escherichia coli TG2 harbouring pKDK37, a pUR291-derived recombinant plasmid, produced a novel protein with a molecular weight of 130,000 corresponding to a [beta]-galaetosidase-inhibin [beta] fusion protein. 3. The fusion protein was purified from inclusion bodies by solubilization in 8 M urea followed by an ion-exchange and gel permeation chromatography. 4. Analysis by immunoblotting and competitive radioimmuno assay revealed that the fusion protein was recognized by a monoclonal antibody raised against a chemically synthesized peptide for amino acid residues from +82 to +114 of the [beta] subunit of the bovine inhibin thereby confirming its identity.


http://www.sciencedirect.com/science/article/B73GR-47F1T6V-FX/2/4a48790111eb36afac91fe8e538cf337

1. The polymerase chain reaction has been used to amplify specifically the cDNA coding for the secreted form of ovine trophoblast protein-one from a preparation of total cellular RNA extracted from sheep embryos removed from ewes 16 days after mating. 2. Cloning and sequencing of the amplified cDNA revealed two new sequence variants: SPW49 having 93% similarity with deduced amino acid sequences from published cDNA data, and SPW27 a variant coding for a deleted form of ovine trophoblast protone-one. 3. The gene for ovine trophoblast protein-one is intronless. 4. This study provides further evidence for the existence of an ovine trophoblast protein-one gene family. 5. Both variants contain a potential N-glycosylation site not apparent in published sequences for ovine trophoblast protein-one.

http://www.sciencedirect.com/science/article/B6T16-40GHY30-6/2/88ebcb0e484e4939d8353a4ec0175863

It has been suggested that a Q/R (Gln192Arg) polymorphism of paraoxonase (PON) might be associated with the predisposition to coronary artery disease (CAD). Therefore, we studied the human paraoxonase gene (PON1) polymorphism in Turkish patients with CAD by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). This polymorphism was determined in 96 CAD patients and in 105 control subjects. The frequencies of the QQ, QR, and RR genotypes were found as 36.5, 52.0, and 11.5% in CAD patients and 48.6, 41.0, and 10.4% in control subjects, respectively. The QR genotype was the most common in the patient group, whereas the QQ genotype was more frequent in individuals without CAD. Frequency of the R allele was higher among CAD patients compared to controls (38.5% versus 31%). However, neither the genotype nor the allele distribution of the Gln92Arg polymorphism of PON1 was statistically significantly different between the two groups (P>0.05). Although both systolic and diastolic blood pressure levels were slightly higher in patients with the QQ genotype, there was no differences in regard to age, sex, serum triglyceride, total cholesterol or high-density lipoprotein cholesterol among CAD patients with different PON1 Gln192Arg genotypes. In summary, our results suggest that no association exists between the Gln192Arg polymorphism of paraoxonase and CAD in Turkish patients.


http://www.sciencedirect.com/science/article/B6T16-4BC2MBR-C/2/a228bdd12892566add0bd43079db64d4

Background: Abnormalities of collagen and elastic fibers were found in floppy mitral valves (FMV). Urokinase-plasminogen activator (PLAU) was suggested to be involved in the pathogenesis of elastin and collagen degradation in arterial aneurysm. The role of PLAU genetic variant in mitral valve prolapse (MVP) has not been studied. We, therefore, performed a case-controlled study investigating the possible relation between the PLAU gene polymorphisms and risk of MVP in Taiwan Chinese. Methods: We studied 100 patients with MVP diagnosed by echocardiography and 106 age- and sex-matched normal control subjects. The T4065C and T3995C polymorphisms of the PLAU gene were identified by polymerase chain reaction (PCR)-based restriction analysis. Results: There was a significant difference in either the genotype distribution or allelic frequencies between MVP cases and controls for PLAU gene T4065C polymorphism (P=0.0001 and 0.0002, respectively). An odds ratio for risk of MVP associated with PLAU T4065C TC genotype was 6.03 (95% confidence interval 2.11-14.83). An odds ratio for risk of MVP associated with PLAU T4065C T allele was 4.99 (95% confidence interval 1.93-12.91). There was no significant difference in either the genotype distribution or allelic frequencies between MVP cases and controls for PLAU T3995C polymorphism. Further categorization of the MVP patients into mild and severe subgroups revealed no statistical difference between these two subgroups for PLAU T4065C and T3995C polymorphisms. Conclusions: This study shows that patients with MVP have a higher frequency of PLAU T4065C TC genotype and T allele that supports a role of the PLAU T4065C polymorphism in determining the risk of MVP among the Chinese population in Taiwan.

http://www.sciencedirect.com/science/article/B6T16-414NWXF-D/2/db7443b01ae41493013ce530ba9b798b

To further investigate the immunological mechanisms involved in Takayasu's arteritis, we analyzed the T-cell receptor (TCR) V[gamma] and V[delta] gene usage by infiltrating [gamma][delta] T-cells and the expression of costimulatory molecules B7-1, B7-2, CD40, CD27 ligand (CD27L), CD30L, OX40L in the arterial tissue of a patient with Takayasu's arteritis. We found that the repertoires of TCR V[gamma] as well as V[delta] gene transcripts of the infiltrating cells were restricted as compared with those of peripheral blood lymphocytes from a patient with Takayasu's arteritis. This strongly suggests that [gamma][delta] T-cells as well as [alpha][beta] T-cells, as we previously reported, were specifically involved in the pathogenesis of Takayasu's arteritis. We also found that B7-1, B7-2, CD40, CD27L, CD30L, and OX40L were expressed in the arterial tissue, suggesting the roles for these costimulatory molecules in T-cell-mediated vascular injury in Takayasu's arteritis. Our findings strongly support the involvement of T-cell-mediated immunological mechanisms in the pathogenesis of Takayasu's arteritis.


http://www.sciencedirect.com/science/article/B6T16-472BNT6-9/2/1d2261c603199b29384bcfa515528c4

Background: Proinflammatory cytokines such as tumor necrosis factor [alpha] (TNF-[alpha]), interleukin (IL)-6, and IL-8 have been implicated in myocardial injury following cardiopulmonary bypass (CPB). However, little evidence is currently available to directly confirm such a relationship. We have previously documented that a newly discovered 'four and a half LIM-only protein 2' (FHL2) is exclusively expressed in myofibres. We hypothesized that the upregulation of FHL2 is proportional to the degree of myocardial injury and investigated the myocardial expression of FHL2(793,565),(890,579)(797,579),(889,593)(800,593),(890,607)(802,607),(891,619) together with these cytokine messenger RNAs (mRNAs) during clinical CPB.

Methods: Intermittent hypothermic blood cardioplegia was used in all patients. Atrial myocardial biopsies were obtained immediately at the onset and at the end of CPB in 33 consecutive patients undergoing valvular or coronary artery surgery. TNF-[alpha], IL-6, and IL-8 mRNA expressions in these myocardial samples were determined by semi-quantitative reverse transcription-polymerase chain reaction. Myocardial FHL2 expression was determined by Western blot analysis. Serum levels of the MB isoenzyme of creatine kinase (CK-MB) and cardiac troponin-I (cTnI) before surgery and 24 h after the end of CPB were also measured. Results: The duration of aortic crossclamping and CPB was 70+-33 and 99+-37 min, respectively. No elevated myocardial TNF-[alpha] mRNA expression was found after CPB. IL-6 mRNA expressions were detected in 14 pairs of the myocardial biopsies and were elevated in 11 (33%) post-CPB biopsies. Similarly, IL-8 mRNA expressions were detected in 19 pairs of samples and were elevated in 14 (42%) post-CPB biopsies. Among the 17 pairs of biopsies with positive FHL2 expression, FHL2 levels were increased in 11 (33%) post-CPB samples. Moreover, the elevated FHL2 expression was associated with an increase in IL-6 (P=0.018) and IL-8 (P=0.024) mRNA expression after CPB. Postoperative CK-MB and cTnI levels were significantly higher in patients with myocardial FHL2 expressions than those without (CK-MB, 13.5+-2.3 vs. 6.5+-0.8 ng/ml, P=0.022; cTnI, 10.7+-2.0 vs. 3.5+-0.6 ng/ml, P=0.0013). Conclusions: Our findings demonstrate for the first time that both IL-6 and IL-8 mRNAs are upregulated in human cardiac myocytes following CPB and these cytokines may be involved in myocardial ischemia-reperfusion injury, as
reflected by their association with an increased expression of FHL2.

International Journal of Developmental Neuroscience  (7)


http://www.sciencedirect.com/science/article/B6T01-4D4PP9T-3/2/cf76390d52240a34a2a4d68d345b799f

The molecular mechanisms of [beta]-amyloidogenesis in sporadic Alzheimer's disease are still poorly understood. To reveal whether aging-associated increases in brain oxidative stress and inflammation may trigger onset or progression of [beta]-amyloid deposition, a transgenic mouse (Tg2576) that express the Swedish double mutation of human amyloid precursor protein (APP) was used as animal model to study the developmental pattern of markers of oxidative stress and APP processing. In Tg2576 mouse brain, cortical levels of soluble [beta]-amyloid (1-40) and (1-42) steadily increased with age, but significant deposition of fibrillary [beta]-amyloid in cortical areas did not occur before postnatal age of 10 months. The slope of increase in cerebral cortical [beta]-secretase (BACE1) activities in Tg2576 mice between ages of 9 and 13 months was significantly higher as compared to that of the [alpha]-secretase, while the expression level of BACE1 protein and mRNA did not change with age. The activities of superoxide dismutase and glutathione peroxidase in cortical tissue from Tg2576 mice steadily increased from postnatal age 9-12 months. The levels of cortical nitric oxide, and reactive nitrogen species demonstrated peak values around 9 months of age, while the level of interleukin-1[beta] steadily increased from postnatal month 13 onwards. The developmental temporal coincidence of increased levels of reactive nitrogen species and antioxidative enzymes with the onset of [beta]-amyloid plaque deposition provides further evidence that developmentally and aging-induced alterations in brain oxidative status exhibit a major factor in triggering enhanced production and deposition of [beta]-amyloid, and potentially predispose to Alzheimer's disease.


http://www.sciencedirect.com/science/article/B6T01-485H1WS-2/2/38cdab6e701af6a3e801a0a4f2eaf96d

Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3) are structurally related survival and differentiation factors for distinct sets of peripheral and central neurons. We previously reported that BDNF and NGF gene expression are differentially regulated in mouse L929 fibroblasts. Here we examine expression of these three neurotrophins in human fibroblasts. Northern blots detected BDNF and NT-3 mRNAs in fibroblasts derived from lung (WI-38), calvarium and foreskin. WI-38 cells and foreskin fibroblasts expressed 1.6 kb as well as 4 kb BDNF mRNAs whereas only the smaller BDNF mRNA was detected in calvarium fibroblasts. NGF mRNA was present in foreskin and calvarium but not lung fibroblasts. In WI-38 cells serum treatment increased levels of BDNF mRNA within 2 hr. Cycloheximide did not inhibit the increase.
Treatment with 12-O-tetradecanoyl phorbol-13-acetate (TPA) transiently suppressed BDNF mRNA. Treatment with both serum and TPA first stimulated and then transiently suppressed BDNF mRNA. TPA and/or serum did not significantly affect BDNF mRNA in calvarium fibroblasts. These results show that human fibroblasts derived from different tissues express and regulate neurotrophin genes differentially.


The actions of the neurotrophins are mediated through specific receptors. Nerve growth factor (NGF), the prototypic neurotrophin, binds to receptors of both high and low affinity. A protein 75 kDa in size (p75NGFR) binds NGF, as well as brain-derived neurotrophic factor and neurotrophin 3, with low affinity. Recent investigations suggest that this protein may also be a component of the high affinity NGF receptor complex. To study gene expression of the p75NGFR molecule, we used a sensitive reverse transcription-polymerase chain reaction (RT-PCR) assay to measure levels of its messenger RNA (mRNA) in small samples of total RNA. The assay is based on using a shortened p75NGFR cRNA as an internal RNA standard to control for variability in reverse transcription and polymerase chain amplification. We measured p75NGFR mRNA levels in the rat cerebellum during ontogeny to further study the transient developmental increase in receptor gene expression known to occur in this brain region during the early postnatal period. We found that p75NGFRmRNA levels were most abundant at postnatal day 2, and then declined to lower levels throughout postnatal development and in the adult. Northern blot analysis of the same total RNA samples used in our RT-PCR assay verified that p75NGFR expression is highest in the early postnatal period. These results confirm those of previous studies accomplished with much larger amounts of RNA using ribonuclease protection or northern blot assays. The use of an RT-PCR assay that utilized an internal standard also controls against changes in RNA complexity which can affect the measurement of message abundance across developmental stages. These results again suggest that p75NGFR may play an important role in the postnatal development of cerebellum in rats.


In this report, we describe the effect of nerve growth factor (NGF) on the transcriptional expression of voltage-dependent Ca2+ channel [alpha]1 subunits, i.e., [alpha]1A, [alpha]1B, [alpha]1C, [alpha]1D, and [alpha]1E in rat pheochromocytoma (PC12) cells. Using reverse transcriptase-coupled polymerase chain reaction (RT-PCR) and class-specific Ca2+ channel oligonucleotide probes, messenger RNA levels were measured and compared to Histone H3.3 transcript which remained relatively constant over the duration of NGF treatment. Although no statistically significant differences in P-type ([alpha]1A) Ca2+ channel transcript levels were observed, N-type ([alpha]1B) Ca2+ channel transcript levels increased 50% over control values (P value [alpha]1C and [alpha]1D transcripts with [alpha]1C decreasing steadily to ~50% of control (P value [alpha]1E Ca2+ channel transcripts were detected in PC12 cells. For comparison, PC12 cells were also treated with
another differentiative growth factor, i.e., basic fibroblast growth factor (bFGF) and a nondifferentiative growth factor epidermal growth factor (EGF). In contrast to NGF, bFGF and EGF treatment had no inhibitory effect on L-type ([alpha]1C and [alpha]1D) channel transcript levels after 3 days. Like NGF, EGF treatment had no statistically significant effect upon P-type ([alpha]1A) transcript levels but increased in a biphasic manner following bFGF treatment. Presynaptic-associated [alpha]1B (N-type) Ca[2+] channel transcripts were observed decreased following EGF treatment (2 days) while L-type [alpha]1C transcripts decreased after 7 days (P value 2) channel transcripts, while L-type ([alpha]1C and [alpha]1D) Ca[2+] channel transcripts appear to be down regulated.


http://www.sciencedirect.com/science/article/B6T01-42Y117P-5/2/58874e96c26cce71597431c24303e7cc

Changes in the metabolic activity within the brain of patients suffering from Alzheimer's disease (AD) were investigated and compared with biochemical alterations in the hippocampus induced by fimbria/fornix transection in the rat. The deafferentation of the hippocampus results in a degeneration of cholinergic septo-hippocampal terminals accompanied by a persistent decrease of choline acetyltransferase (ChAT) and acetylcholine esterase (AChE) activities similar to the cholinergic malfunction in AD. In the animal model the [3H]-cytochalasin B binding to the glucose transporters was elevated up to the day 7 after surgery as was the activity of the phosphofructokinase (PFK) on day 3. A reactive astrogliosis could be evidenced by the upregulation of glial fibrillary acidic protein (GFAP). An increase of the PFK activity was also found in AD being accompanied by enhanced level of GFAP as well. A higher concentration of mRNA for all three isoenzymes of PFK was shown by reverse transcription (RT)-real time polymerase chain reaction (PCR) amplification. However, the pattern of PFK isoenzyme proteins and mRNAs did neither change in diseased human nor in the lesioned rat brain. The activities of the mitochondrial enzymes pyruvate dehydrogenase complex (PDHC) and cytochrome c oxidase (CO) were diminished in the lesioned rat hippocampus on day 7 as well as in AD brain. Subcellular fractionation showed that the activity of these enzymes was affected in the synaptosomal as well as in the extrasynaptosomal mitochondria indicating a loss of neuronal input and also a vulnerability of intrinsic hippocampal neurons and/or non-neuronal cells. The recovery of the mitochondrial enzyme activity in the animal model at later post lesion intervals may be the result of compensatory responses of surviving cells or of sprouting of other non-affected inputs. It is concluded that common metabolic mechanisms may underlie the concurrent degenerative and repair processes in the denervated hippocampus and the diseased Alzheimer brain.


http://www.sciencedirect.com/science/article/B6T01-3PFC5W0-10/2/60ac13b42314526097b6efabcaa0980c

The growth and differentiation of olfactory sensory neurons are regulated tightly. We had shown previously, by immunohistochemistry, that transforming growth factor-[alpha] (TGF-[alpha]) and epidermal growth factor (EGF) receptor are present in the olfactory epithelium of untreated adult rats and that TGF-[alpha] is a potent mitogen of olfactory epithelium in vitro. Expression of EGF receptor and TGF-[alpha] was detected primarily in horizontal basal cells and supporting cells but
rarely in globose basal cells, which suggested that EGF receptor is not a likely candidate for the mitotic regulator of sensory neurons. In order to expand the search for candidate regulators, we have now examined other members of the EGF family of receptors and ligands. By utilizing reverse transcriptase-polymerase chain reaction (RT-PCR) methodology, we have detected the messenger RNA encoding the protein of the neu gene (p185neu) and Neu differentiation factor (NDF) isoforms in the olfactory mucosa. Immunohistochemical localization of p185neu and NDF indicates expression of these proteins in the olfactory epithelium of adult rats in regions where globose basal cells and immature sensory neurons are found, as well as in the ensheathing cells of the olfactory nerve. The presence of neu and NDF transcripts in the olfactory tissue and the localization of their encoded polypeptides to proliferative regions of the epithelium suggest involvement of these gene products in the regulated proliferation/differentiation of the sensory neurons.


http://www.sciencedirect.com/science/article/B6T01-3PFC5W0-T/2/b169d7a80af5560330831ad15c555a9f

Voltage-gated sodium channels are responsible for the initial depolarizing phase of the action potential. In hippocampal neurons cultured from trisomy 16 (Ts16) mice (a model for Down's syndrome), the maximum inward conductance mediated by these channels was reduced 47% relative to control diploid neurons. This reduced conductance was reflected in a 35% decrease in binding of radiolabeled saxitoxin, a sodium channel-specific ligand, indicating expression of fewer channels in these neurons. The mRNAs encoding the \([\alpha]\) and \([\beta]1\) subunits were, however, present at the same levels in Ts16 neurons and control diploid neurons. Thus, the altered regulation of voltage-gated sodium channels in Ts16 neurons is apparently a post-transcriptional event and possible mechanisms are discussed.


http://www.sciencedirect.com/science/article/B6T7M-405SVBF-2/2/31f3c0b2e1a05f95f721d57d6cbfc8a

Objective: Thalassemia is a highly prevalent genetic disorder in Taiwan. The major goal of this study was to present a feasible protocol for the prenatal diagnosis of thalassemia. Method: Prenatal investigation of thalassemia was performed on 57 at-risk cases at the Mackay Memorial Hospital, Taipei, Taiwan. We developed a method using polymerase chain reaction (PCR) and high-throughput DNA sequencing to detect mutations. All diagnoses were confirmed after delivery. Result: Prenatal testing revealed 16 normal fetuses, 24 [\([\alpha]\)]-thal-1 carriers, eight Hb Bart's hydrops fetalis, seven [\([\beta]\)]-thalassemia minor, and two [\([\beta]\)]-thalassemia major fetuses. No false-positive or false-negative cases were found during the postnatal follow-ups. Conclusion: The results of this study indicate that prenatal diagnosis of thalassemia syndromes in Taiwan is
successful with the use of a rapid and accurate molecular method.

International Journal of Infectious Diseases (2)


Objectives: To investigate the incidence and epidemiology of non-multiresistant methicillin-resistant Staphylococcus aureus (nmMRSA) infection in south-east Queensland, Australia. Study design: A retrospective survey was done of hospital records of all patients who had non-multiresistant MRSA isolated at Ipswich Hospital (a 250-bed general hospital, 40 km south-west of Brisbane, Queensland, Australia) between March 2000 and June 2001. Laboratory typing of these isolates was done with antibiogram, pulsed-field gel electrophoresis, bacteriophage typing and coagulase gene typing. Results: There were 44 infections caused by nmMRSA. Seventeen infections (39%) occurred in patients from the south-west Pacific Islands (predominantly Samoa, Tonga and New Zealand). Laboratory typing showed that the isolates in Pacific Islanders were Pacific Island strains, and 16/17 of these infections were community acquired. Twenty-three infections (52%) occurred in Caucasians. Eleven of the isolates from Caucasians (48%) were a new predominantly community-acquired strain that we have termed the 'R' pulsotype, nine (39%) were Pacific Island strains, and three (13%) were health care institution-associated strains. Four infections occurred in patients who were not Caucasians or Pacific Islanders. Overall, 34 of all 44 infections (77%) were community acquired. Conclusions: Non-multiresistant MRSA infection, relatively frequently observed in Pacific Islanders in south-east Queensland, is now a risk for Caucasians as well, and is usually community acquired. Clinicians should consider taking microbiological specimens for culture and antimicrobial susceptibility testing in patients with suspected staphylococcal infections who are not responding to empirical therapy with [beta]-lactam antibiotics.


Background: Atypical serum neutralizing antibody responses to prototype strains of Puumala viruses in some patients with hemorrhagic fever with renal syndrome (HFRS) have long suggested the existence of other hantaviruses in the Balkans. Objective: To determine the presence of arvicolid rodent-borne Puumala-like hantaviruses in Yugoslavia. Materials and Methods: Using reverse transcript-polymerase chain reaction, Tula virus RNA was amplified from lung tissues of a European pine vole (Pitymys subterraneus) captured in 1987, following an outbreak of HFRS in the Cacak region of Serbia-Yugoslavia. Results: Sequence analysis of the entire coding region of the S segment and a 948-nucleotide region of the G2 glycoprotein-encoding M segment revealed divergence of approximately 14% from Tula virus strains harbored
by European common voles (Microtus arvalis) captured in Central Russia and the Czech Republic. However, nearly complete identity was found in the corresponding deduced amino acid sequences. Moreover, phylogenetic trees constructed by the maximum parsimony and neighbor-joining methods indicated that this Pitymys-borne hantavirus shared a common ancestry with other Tula virus strains. Conclusions: The data demonstrate that Pitymys subterraneus also serves as a rodent reservoir of Tula virus in Serbia-Yugoslavia. To what extent this represents virus spillover from Microtus arvalis warrants further investigation.

International Journal of Mass Spectrometry (2)


http://www.sciencedirect.com/science/article/B6VND-4BYN5ST-4/2/e96b5a055bbd58b64738489981388eb5

A 53-base pair region on the long arm of chromosome 22 was amplified using PCR with 7-deaza-modified deoxynucleotides. Increased amplification efficiency was achieved by doubling the concentration of the modified deoxynucleotide triphosphates. Incorporation of 7-deaza purines has been previously shown to selectively eliminate fragmentation pathways during gas-phase sequencing of nucleic acids by sustained off-resonance irradiation collision-induced dissociation (SORI-CID) and infrared multiphoton dissociation. However, 7-deaza analogs result in significant duplex stability precluding interrogation of the single-stranded species by tandem mass spectrometry. Herein, we demonstrate the use of lambda exonuclease to successfully overcome this problem and are able to obtain single-stranded PCR products containing 7-deaza adenine and guanine nucleobases. Mass accuracy was used as our metric to determine complete incorporation of 7-deaza residues in PCR products>15 kDa; averatide) chemical formula developed herein which was based on the relative frequencies of AT and GC base pairs in the human genome. Single-stranded PCR products were fragmented using SORI-CID and as expected, cleavage at the 7-deaza modified sites was not observed. Collectively, this integrated approach can facilitate top-down sequencing of PCR products by a variety of tandem mass spectrometry methods.


http://www.sciencedirect.com/science/article/B6VND-4F31R2G-1/2/96bc83d6dec9dffe8429ca692be52a08

In this work, we describe a strategy for the detection and characterization of microorganisms associated with a potential biological warfare attack or a natural outbreak of an emerging infectious disease. This approach, termed TIGER (Triangulation Identification for the Genetic Evaluation of Risks), relies on mass spectrometry-derived base composition signatures obtained from PCR amplification of broadly conserved regions of the microbial genome(s) in a sample. The sample can be derived from air filtration devices, clinical samples, or other sources. Core to this
approach are "intelligent PCR primers" that target broadly conserved regions of microbial genomes that flank variable regions. This approach requires that high-performance mass measurements be made on PCR products in the 80-140 bp size range in a high-throughput, robust modality. As will be demonstrated, the concept is equally applicable to bacteria and viruses and could be further applied to fungi and protozoa. In addition to describing the fundamental strategy of this approach, several specific examples of TIGER are presented that illustrate the impact this approach could have on the way biological weapons attacks are detected and the way that the etiologies of infectious diseases are determined. The first example illustrates how any bacterial species might be identified, using Bacillus anthracis as the test agent. The second example demonstrates how DNA-genome viruses are identified using five members of Poxviridae family, whose members includes Variola virus, the agent responsible for smallpox. The third example demonstrates how RNA-genome viruses are identified using the Alphaviruses (VEE, WEE, and EEE) as representative examples. These examples illustrate how the TIGER technology can be applied to create a universal identification strategy for all pathogens, including those that infect humans, livestock, and plants.

**International Journal of Medical Microbiology**(3)


http://www.sciencedirect.com/science/article/B7GW0-4FFN4SH-1/2/d567cd93b9ba7d876d68da29e1ec4566

Intestinal infections due to shiga toxin-producing Escherichia coli bacteria (STEC) reveal a broad range of clinical symptoms and a large scale of virulence properties of the respective pathogens. The question whether all STEC variants or only a particular group of them need to be considered for clinical and epidemiological purposes was answered throughout this study. Using the PCR technique for the identification of 25 different virulence-associated genes, 266 E. coli strains belonging to 81 different E. coli serotypes from various clinical origins were investigated. A great genetic diversity of the virulence properties and a broad range of virulence marker combinations have been identified. However, distinct virulence marker combinations (e.g. Stx2/LEE/pO157 as well as Stx2dac/pO113) were found to be associated with the same notified clinical symptoms (e.g. HUS). Such an association speaks either for the "shiga toxin-only concept" or for several redundant, but clinically or epidemiologically important virulence properties.


http://www.sciencedirect.com/science/article/B7GW0-4F97397-4/2/a190909fba2cd1d814a1c80e2c231c69

Multilocus sequence typing (MLST) has become the gold standard for typing of a variety of bacterial and fungal micro-organisms. Others recently reported the successful use of the tiling DNA array technology to sequence-type Staphylococcus aureus. We now evaluated microarrays
based on polymorphism-directed oligonucleotide design for typing of Neisseria meningitidis. The rationale behind this approach was to minimize the number of microarray probes by exploiting the comprehensive knowledge of polymorphisms combined in the Neisseria MLST website. Initial experiments using model oligonucleotides of 28-32 base-pairs in length revealed that the hybridization protocols used were highly specific. However, despite of several optimization steps, the rate of misidentification of oligonucleotides remained >1.8% in consecutive validation experiments using arrays representing the genetic diversity at three MLST loci. We assume that the high density of polymorphic sites and the extensive GC-content variations at N. meningitidis MLST loci hinder the successful implementation of MLST microarrays based on polymorphism-directed oligonucleotide design.


A DNA fingerprinting method for the characterization of Legionella pneumophila serogroup 1 strains was established. This method was based on the DNA extraction using Chelex 100 and subsequent PCR analysis using primers under conditions of low stringency. Sixteen single primers were tested for the typing of the 10 epidemiologically unrelated reference strains of L. pneumophila serogroup 1 as well as patient isolates and environmental strains isolated from the water system of a hospital where patients with legionellosis were treated. In addition, a combination of two primers (Lpm-1 and Lpm-2) originally established for the specific detection of Legionella strains was tested. The PCR results were compared with two further subtyping methods, i.e. monoclonal antibody analysis and pulsed-field gel electrophoresis. The type strains Philadelphia 1, Knoxville 1, Allentown 1, Benidorm 0303E, Bellingham 1, and France 5811 could be distinguished clearly in experiments using all of the primers. Depending on the primer used, Heysham 1 and Oxford 4032E showed different DNA profiles. The strains Olda and Camperdown 1 were nearly indistinguishable. In contrast, the analysis by PFGE and MAb subtyping revealed distinct types for all 10 reference strains. The discrimination of the patient isolates from two suspected cases of nosocomial legionellosis and environmental isolates was not possible with the 16 single primers used in the study. However, the PCR assay with the combination of Lpm-1 and Lpm-2 as well as the PFGE and MAb analysis were able to differentiate distinct types. The use of the sequence-specific primers under low-stringency annealing conditions allowed both simultaneous gene detection as well as epidemiological typing of Legionella strains.


Split hand/split foot malformation (SHFM), which typically appears as lobster-like limb
malformation, is a rare clinical condition caused by a partial deletion of chromosome 7q. Hearing impairment sometimes accompanies syndromic SHFM cases; a case of inner and middle ear malformation with SHFM is described in this report. We conducted a genetic evaluation of this patient and found a deleted region that overlaps a previously reported locus of SHFM as well as a DFN11 locus that can cause nonsyndromic hearing impairment by autosomal recessive inheritance.


http://www.sciencedirect.com/science/article/B6T7V-44J3TM5-1/2/d470d1b9367bb208852c1e37e1afde26

Objective: We applied mutation screening in seven cochlear implant users to identify those persons with GJB2-related deafness to determine whether etiology of deafness was predictive of speech performance after implantation. Methods: Direct sequence of GJB2 was conducted over seven cochlear implant users with prelingual hearing impairment and their speech, language and cognitive performance was examined. Results: The three persons with GJB2-related deafness had a mean vocabulary of 1243 words compared to a mean vocabulary of 195 words in the four children with GJB2-unrelated deafness, although the number of patients examined here was limited. The developmental quotient (DQ) of cognitive ability also was higher in those children with GJB2-related deafness. Conclusions: These preliminary results suggest that better speech performance after cochlear implantation may be observed in persons with GJB2-related deafness. In the future, detailed phenotypic studies and mutation screening for non-syndromic hearing loss may play an important role in the preoperative assessment of prelingually-deafened children.


http://www.sciencedirect.com/science/article/B6T7V-40HV0MB-4/2/c83cf79ccccdebb895f07a64d4a5c6ca

Apoptosis -- programmed death of a cell -- is a natural mechanism that controls the number of cells in an organism. Neoplastic cells as many types of normal cells, may be the subject of spontaneous apoptosis as well as they may be induced by anti-neoplastic factors. Neoplastic cells' resistance to drugs is often correlated with impossible induction of apoptosis in those cells. Though the process of apoptosis is not fully explained, a possible involvement of many genes in regulation of this process is indicated. One of them is bcl-2 gene and its product -- bcl-2 protein, which has the property of apoptosis process inhibition and stimulation of a cell towards outliving (survival). Increased expression of bcl-2 gene is present in many neoplastic cells and it suggests a possible pathogenic role of bcl-2 gene in oncogenesis. In this paper the expression of bcl-2 gene in the cells of papilloma in larynx is defined in six children operated in the Department of Paediatric Otolaryngology of Medical School in Lublin. Papillomas of larynx are neoplasm's of particular resistance to treatment. Complete, cellular RNA was isolated with Chomczynski and Sacchi method using guanidine thiocyanate. Gene expression was defined with the method of reverse transcription by cDNA synthesis and amplification of bcl-2 gene fragment with specific oligonucleotides in reverse transcriptase polymerase chain reaction (RT-PCR). The products were identified on agarose gel. Expression of bcl-2 gene in the investigated cells of laryngeal papilloma was confirmed in all the children. The presence of bcl-2 gene product in these cells may be the cause of apoptosis inhibition and stimulation of cells proliferation of the neoplasm.

http://www.sciencedirect.com/science/article/B6T7X-429XX63-11/2/5904ecc7be6e656ac7b9b9d1c5dbd85a

Purpose: To investigate the correlation between tumor potential doubling time, Tpot, and mutations in the p53 gene, TP53, and the potential of these parameters to predict outcome of head and neck cancer patients treated with radiotherapy.

Methods and Materials: Data from two independent studies on Tpot and TP53 mutations were combined, including 58 patients with squamous cell carcinoma of the head and neck. Tpot was estimated on biopsies obtained 6-9 h after infusion of iododeoxyuridine by combined flow cytometry and immunohistology. TP53 mutations were detected using DGGE and sequenced. All patients received primary radiotherapy alone.

Results: The predictive value of Tpot alone was of borderline significance. However, in TP53 wild-type tumors, Tpot was a strong predictor of outcome, whereas Tpot in TP53 mutant tumors failed to provide any information. Tpot and TP53 were not associated with nodal control; however, there was a strong relationship with control in the T-position, disease-specific survival, and overall survival.

Conclusion: Tpot can be a relevant parameter for predicting outcome of radiotherapy in head and neck cancer but only in the subset of patients without mutations in the p53 gene.


http://www.sciencedirect.com/science/article/B6T7X-456FCPG-G2/82b7662dcb07c93ecb17549b923b9b6: The objective of this study was twofold: first, to identify patients with locally advanced breast cancer (LABC) who achieve a pathological response to a preoperative regimen of concurrent paclitaxel and radiation; and second, to explore associations between molecular markers from the original tumors and pathological response.

Patients with previously untreated LABC were eligible to receive a regimen of preoperative concurrent paclitaxel, 30 mg/m2 twice a week for a total of 8 weeks, and radiation delivered Weeks 2-6, 45 Gy at 1.8 Gy per fraction to the breast, ipsilateral axilla, and supraclavicular nodes. At mastectomy, pathologic findings were classified as pathological complete response (pCR) = no residual invasive cells in the breast and axillary contents; pathological partial response (pPR) = presence of neu and p53 overexpression. Estrogen receptor (ER), HER2/neu, metablastin, [beta]-tubulin III and IV, microtubule-associated protein-4 (MAP-4), bcl-2, bax, and cyclooxygenase-2 (COX-2) gene expression were measured using real-time quantitative polymerase chain reaction (PCR). A total of 36 patients had pretreatment biopsies and were evaluable for the analysis of the association of molecular markers with pathological response.

Pathological response in the mastectomy specimen was achieved in 12 of these 36 patients (33%). Only HER2/neu and ER gene expression were found to be significantly associated with the extent of pathological response to the regimen, i.e., tumors with low HER2/neu gene expression and negative estrogen receptors were more likely to respond to the tested regimen (p
= 0.009 and p = 0.006, respectively). Conversely, p53 protein expression measured by IHC did
not appear to be associated with pathological response (p = 0.67). Further studies in LABC
should assess whether patient selection for treatment based on the original tumor molecular
characteristics could affect their chance to achieve a pathological response.

canal carcinoma to radiation, 5-fluorouracil, and mitomycin C." International Journal of Radiation
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http://www.sciencedirect.com/science/article/B6T7X-3WPPR1F-G/2/13deea8a953cdf3fc8532a5af65bf90

Purpose: To determine, retrospectively, the status of the bp 609 mutation in the DT-diaphorase
gene in anal canal carcinoma patients who have undergone radical radiotherapy with concurrent
5-fluorouracil (5-FU) and mitomycin C (MMC), to determine the relationship of the mutant form of
the gene to treatment outcomes.Methods and Materials: Paraffin blocks of pretreatment tumor
biopsies were obtained on 49 patients who underwent treatment with curative intent using
radiation, infusional 5-FU and bolus MMC from January 1991 to December 1993. DNA was
extracted and subjected to polymerase chain reaction (PCR) analysis using primers that
encompassed the bp 609 C to T mutation. Restriction endonuclease cleavage with Hinf 1 and gel
electrophoresis were used to determine the polymorphism status of each patient.Results: DNA of
46 patients was successfully amplified. The 46 patients were distributed as follows: 26 (56.5%)
C/C--homozygous wildtype, 18 (39%) T/C--heterozygous, and 2 (4.5%) T/T--homozygous mutant.
Eleven of 46 patients had suffered treatment failure. The status of the bp 609 polymorphism in
this group was 5 (45.5%) C/C, 5 (45.5%) C/T, and 1 (9%) T/T.Conclusion: In this series, there
was not an overrepresentation of the mutant allele in patients with treatment failure, suggesting
that the bp 609 alteration is not a strong determinant of treatment outcome.

Hara, T., M. Omura-Minamisawa, et al. (2005). "Bcl-2 inhibitors potentiate the cytotoxic effects of
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http://www.sciencedirect.com/science/article/B6T7X-4F9MJMX-10/2/c06daa2860c8ff5c7a200e5688e866fc8

PurposeBcl-2, an inhibitor of apoptosis frequently shows elevated expression in human tumors,
thus resulting in resistance to radiation therapy. Therefore, inhibiting Bcl-2 function may enhance
the radiosensitivity of tumor cells. Tetrocarcin A (TC-A) and bcl-2 antisense oligonucleotides
exhibit antitumor activity by inhibiting Bcl-2 function and transcription, respectively. We
investigated whether these antitumor agents would enhance the cytotoxic effects of radiation in
tumor cells overexpressing Bcl-2.Methods and materialsWe used HeLa/bcl-2 cells, a stable Bcl-2-
expressing cell line derived from wild-type HeLa (HeLa/wt) cells. Cells were incubated with TC-A
and bcl-2 antisense oligonucleotides for 24 h after irradiation, and cell viability was then
determined. Apoptotic cells were quantified by flow cytometric assay. ResultsThe HeLa/bcl-2 cells
were more resistant to radiation than HeLa/wt cells. At concentrations that are not inherently
cytotoxic, both TC-A and bcl-2 antisense oligonucleotides increased the cytotoxic effects of
radiation in HeLa/bcl-2 cells, but not in HeLa/wt cells. However, in HeLa/bcl-2 cells, additional
treatment with TC-A in combination with radiation did not significantly increase
apoptosis. Conclusions The present results suggest that TC-A and bcl-2 antisense
oligonucleotides reduce radioresistance of tumor cells overexpressing Bcl-2. Therefore, a
combination of radiotherapy and Bcl-2 inhibitors may prove to be a useful therapeutic approach
for treating tumors that overexpress Bcl-2.

http://www.sciencedirect.com/science/article/B6T7X-41KP2BW-7/2/d5db3e0e7cacc14ba0180c41eb9b7d67

Purpose: The dose intensity of radiotherapy (RT) used in cancer treatment is limited in rare individuals who display severe normal tissue reactions after standard RT treatments. Novel predictive assays are required to identify these individuals prior to treatment. The mechanisms responsible for such reactions are unknown, but may involve dysfunction of genes involved in the sensing and response of cells to DNA damage. The breast cancer susceptibility genes BRCA1 and BRCA2 are implicated in DNA damage repair and the control of genome stability. The purpose of this study was to determine if clinical radiation hypersensitivity is related to mutations of the BRCA1 and BRCA2 genes. Such information is of potential use in the clinical management of BRCA mutation carriers and their families.

Methods and Materials: Twenty-two cancer patients who developed severe normal tissue reactions after RT were screened for mutations of BRCA1 and BRCA2, using various methods including protein truncation testing, direct DNA sequencing, and a PCR-based BRCA1 exon 13 duplication test.

Results: No mutations were detected in the 22 patients tested, despite screening for the majority of commonly described types of mutations of BRCA1 and BRCA2.

Conclusion: These early results suggest that genes other than BRCA1 and BRCA2 probably account for most cases of clinical radiation hypersensitivity, and that screening for mutations of BRCA1 and BRCA2 is unlikely to be useful in predicting response to radiotherapy. However, it has not been excluded that some BRCA1 or BRCA2 heterozygotes might experience unexpected RT toxicity; further BRCA mutation screening on radiation sensitive individuals is warranted.


http://www.sciencedirect.com/science/article/B6T7X-485FW6G-10/2/e3891cbbdee00d24abe8f6e7f93328d7a

Background: Spontaneous apoptosis has been shown to predict tumor response to radiochemotherapy in rectal cancer in vivo. It remains to be elucidated, however, which genetic profile determines whether a tumor is more or less prone to apoptosis. Recently, a novel member of the inhibitor of apoptosis protein family, designated survivin, was identified. We investigated the impact of surviving expression on tumor cell apoptosis in three colorectal cell lines of different intrinsic radiosensitivities.

Methods and materials: Survivin protein expression was measured by Western blot analysis, and survivin mRNA expression by quantitative TaqMan reverse transcription polymerase chain reaction, both in untreated cell and after irradiation with 2 and 8 Gy. The expression profile was then correlated to spontaneous and radiation-induced apoptosis (Tunel-Assay, DAPI-staining) in three colorectal cell lines of low (SW 480), intermediate (HCT-15), and high radiosensitivity (SW 48), as determined by the colony-forming assay.

Results: In vitro analysis revealed higher spontaneous and higher radiation-induced apoptosis rates in the radiosensitive line (SW 48), as compared with the more resistant line (SW 480). In Western blot analysis and in TaqMan analysis, SW 480 was characterized by a higher spontaneous expression and a pronounced induction of survivin 48 h after irradiation, whereas survivin expression was low when untreated and not increased after irradiation in the most radiosensitive line SW 48. HCT-15 was intermediate, both with respect to the level of survivin mRNA and protein expression.

Conclusion: The inverse correlation of survivin-expression with spontaneous
and radiation-induced apoptosis suggests that survivin is an important inhibitor of apoptosis in colorectal cancer cell lines. Analysis of survivin mRNA or protein expression may therefore provide predictive information on radio- and chemoresistance of individual colorectal tumors.


http://www.sciencedirect.com/science/article/B6T7X-44HTB4T-S/2/ce74ea381334016e77cab26c3ece9d20

Purpose: The aim of this study was to investigate the efficacy of combination therapy of ionizing radiation (IR) and adenoviral p53 gene therapy and to evaluate its molecular mechanisms.Methods and Materials: Two human prostate cancer cell lines, DU145 and PC-3 cells, containing different types of p53 gene mutations, were investigated. The recombinant adenovirus vector containing the wild-type p53 gene (Ad5CMV-p53) was used for this study. Cells were irradiated (in 0, 2, 4, and 6 Gy, 300 cGy/min) and after 12 h of irradiation, the cells were infected with various doses of Ad5CMV-p53 (0-40 multiplicity of infection [MOI]). Cytotoxicity was determined by clonogenic assay. The molecular mechanisms were evaluated by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), apoptotic cell detection, and cell cycle analysis.Results: The cell growth inhibition in DU145 (p53-mutated) cells by IR was strongly enhanced by additional Ad5CMV-p53 infection in a viral dose-dependent manner. In DU145 cells, IR alone induced minimal p53 mRNA expression. However, IR combined with Ad5CMV-p53 infection stimulated significant increase in p53 mRNA expression supplemented with Bax and p21 mRNA expressions. In PC-3 (p53-null), IR induced Bax and p21 mRNA expression, while the combination effects were observed in p53, Bax, and p21 mRNA expression. Apoptotic cell deaths were rarely observed after IR alone (DU145: 3%, PC-3: 5%). However, after combination therapy, the proportion of apoptotic cells greatly increased (sevenfold in DU145 cells, and twice in PC-3 cells). G1 cell cycle arrest was observed after Ad5CMV-p53 infection and the combination in both cell lines.Conclusion: In this study, we demonstrated that the combination of IR and Ad5CMV-p53 gene therapy resulted in remarkable synergistic effects in human prostate cancer cells. This combination therapy could be one of the optimal treatment strategies for radioresistant prostate cancer.


http://abstracts.iovs.org/cgi/content/abstract/45/5/686

Purpose: The purpose was two-fold: 1) to develop economical quantitative PCR (real time) assays for mouse retinal genes expression that include matched primers/reference cDNA sets, and 2) to produce a companion database with practical information on reaction products and conditions. Methods: Several hot start Taq polymerases were compared for robustness with different primer sets. The gene expression Q-PCR kit development consisted of three parts: 1) computed evaluation of primer sets for intron/exon boundaries, literature errors, or custom
design, 2) cloning of custom primers matched template cDNAs into pGemT Easy vector for use as standards, controls, and probes, and 3) experimental evaluation of annealing temperature ranges for each assay. Results: We have developed a collection of Q-PCR assays with companion standards and database for several retina specific genes. Those assays that have been developed are rhodopsin, PDE subunits alpha, beta, and gamma, blue opsin, green opsin, beta-actin, GAP-DH, Flt3, Fiz1, and NRL. We have found that custom assay conditions based on AmpliTaq Gold offers an economical advantage over pre-made kits. Conclusions: Q-PCR primer sets with matching standard cDNA were developed for measuring the expression of several retinal genes for use in the Eye Research Institute and will soon be available to the eye research community.

http://www.iovs.org/cgi/content/abstract/44/4/1657

PURPOSE. To describe the phenotype of an autosomal dominant macular dystrophy and identify the chromosomal locus. METHODS. Eleven members of a five-generation, nonconsanguineous British family were examined clinically and also underwent automated perimetry, electrodiagnostic testing, fundus fluorescein angiography, and fundus autofluorescence imaging. Blood samples were taken for DNA extraction and linkage analysis was performed. RESULTS. The phenotype is characterized by bull's-eye macular dystrophy first evident in the first or second decade of life. There is mild visual impairment, central scotomata, and electrophysiological testing indicates that most affected individuals have disease confined to the central retina but older subjects have more widespread rod and cone abnormalities, demonstrated by flash ERG. Genetic linkage analysis established linkage to chromosome 4 at p15.2-16.3 with a maximum lod score of 3.03 at a recombination fraction of 0.00 for marker D4S391. The locus for this autosomal dominant macular dystrophy lies between flanking markers D4S3023 and D4S3022, and overlaps the Stargardt 4 locus. CONCLUSIONS. A new locus was identified for a bull's-eye macular dystrophy on the short arm of chromosome 4.

http://www.iovs.org/cgi/content/abstract/44/10/4347

PURPOSE. To systematically explore changes in gene expression in the retina of monkeys with laser-induced glaucoma and to validate the microarray data on eyes with experimental glaucoma. METHODS. Glaucoma was induced in the right eye of four monkeys by repeated argon laser photocoagulation of the trabecular meshwork. The left eye served as the control. Retinas were isolated from glaucomatous and control eyes 30 days after photocoagulation. Gene expression changes were analyzed by human microarray chips which displayed a total of 9182 elements including Expression Sequence Tag (EST) clones. Changes in the expression of some genes were further confirmed by real-time PCR analysis. Immunohistochemical studies to examine protein expression of some gene products were also done for several genes that showed up- or downregulation by the microarray analysis. RESULTS. Two eyes with mild glaucoma and two with severe glaucoma were produced. In the mild and severe glaucomatous retina, the number of upregulated genes was 45 and 18, and the number of downregulated genes was 17 and 21, respectively. The number of genes that were up- or downregulated was 0.7% of all the genes examined. The real-time PCR analysis confirmed expression changes of some genes found in the microarray analysis. Ceruloplasmin was one of the upregulated genes, and it was found by
immunohistochemical analyses to be expressed in Muller cells. CONCLUSIONS. Gene expression profiles in laser-induced glaucomatous monkey retinas were determined, and only a very small population of genes was up- or downregulated in glaucomatous eyes. Upregulation of ceruloplasmin protein was found in the Muller cells.


http://www.iovs.org/cgi/content/abstract/43/6/1870

PURPOSE. The Emory mouse is a well-characterized model for age-onset cataract. The purpose of the present study was to identify differentially expressed genes between pre- and postcataract Emory mouse lenses. METHODS. Eyes were extracted from Emory mice at 3 weeks (precataract) and 7.5 months (postcataract) of age, and lenses were dissected. Lens RNA was compared for gene expression differences by RT-PCR differential display, and transcripts exhibiting altered levels of gene expression were cloned and identified by sequencing. The levels of two transcripts were further evaluated by RT-PCR in 3-week- and 7.5-month-old lenses and the remainder of the eye. The same transcripts were also measured in lenses from three non-Emory mouse strains (FVB/N, 129Sv, and CD1) ages 4 weeks to 11.5 months. RESULTS. Three transcripts were identified as exhibiting altered levels of gene expression between 3-week- and 7.5-month-old Emory mouse lenses. These encoded {alpha}A-crystallin (decreased), {beta}A3/A1-crystallin (decreased), and adhesion-related kinase (ARK) receptor tyrosine kinase (increased). Decreased {alpha}A-crystallin and increased ARK expression were not detected in lenses isolated from three non-Emory mouse strains of similar age. Increased expression of ARK was not detected between 3-week- and 7.5-month-old Emory mouse eye nonlens tissues. CONCLUSIONS. The present data confirm that expression of the {alpha}A-crystallin gene is decreased in cataract in the Emory mouse lens relative to age-matched control lenses and they provide evidence for cataract- and lens-specific upregulation of the ARK receptor tyrosine kinase in the Emory mouse.


http://abstracts.iovs.org/cgi/content/abstract/45/5/1071

Purpose: Primary intraocular lymphoma (PIOL) is a diffuse large B cell lymphoma (DLBCL) in which malignant lymphoid cells invade the retina, vitreous, or optic nerve head, with or without concomitant central nervous system involvement. The bcl-2 t(14;18) translocation brings the bcl-2 gene, an anti-apoptosis gene found on chromosome 18, under the control of the IgH promoter, located on chromosome 14, leading to Bcl-2 expression. This translocation is found in 85% of follicular non-Hodgkin's lymphomas (FL) and 28% of diffuse large B-cell lymphomas (DLBCL). Sixty percent of Bcl-2 breakpoints are located at the major breakpoint region (Mbr) in the 3' non-coding part of the third exon. The next most frequent location for translocations (10-25%) is the minor cluster region (mcr) located 20 kb downstream of the gene. The purpose of this study was to examine the distribution of these bcl-2 breakpoints in PIOL. Methods: Polymerase chain reaction (PCR) was performed on DNA extracted from microdissected PIOL cells. The Mbr was analyzed in 69 patients, and the mcr was analyzed in 67 patients. The PCR-amplifiable mixture contained microdissected DNA, 4 pmol 32P-labeled sense primer of Mbr, 5'-TGA GAG AGT TGC TTT ACG TGG CCT-3' or mcr, 5'-GAC TCC TTT ACG TGC TGG TAC C-3', 5 pmol antisense primer (CFW1), 5'-ACC TGA GGA GAC GGT GAC GGT TTT AC-3', 10 nmol dNTP, 5 nmol MgCl2, and 0.5 U AmpliTaq Gold Enzyme in a final volume of 10 {micro}L. The PCR reaction for the Mbr was performed as follows: 94{degrees}C for 9 min, then 40 cycles of 94{degrees}C x 45 sec,
55\( ^\circ \)C x 45 sec, and 72\( ^\circ \)C x 1 min, followed by 72\( ^\circ \)C for 7 min. The PCR reaction for the mcr was performed as follows: 94\( ^\circ \)C for 9 min, then 35 cycles of 94\( ^\circ \)C x 1 min, 55\( ^\circ \)C x 2 min, and 72\( ^\circ \)C x 2 min, followed by 72\( ^\circ \)C for 7 min. The amplified DNA was separated on a 3% agarose gel. The gel was then stained with ethidium bromide and autoradiographed. Results: 37/69 (54\%) PIOL patients expressed the t(14;18) translocation at the Mbr. 15/67 (22\%) expressed the translocation at the mcr. Of these patients, 14/15 (93\%) also positive for the Mbr, indicating some overlap of the breakpoint regions, while 1/15 (7\%) was positive only at the mcr. Conclusion: These results are similar to those seen in FL and DLBCL, indicating that this translocation also plays a role in PIOL pathogenesis. Furthermore, this translocation is used as a marker for diagnosis and monitoring of FL, although its role in prognosis and treatment selection for FL and DLBCL remains unclear. This study lays a foundation for future studies aimed at exploring the role of Bcl-2 expression in clinical presentation, treatment response, relapse, and survival in patients with PIOL.


http://abstracts.iovs.org/cgi/content/abstract/46/5/3465

Purpose: Primary intraocular lymphoma (PIOL) is typically a diffuse large B cell lymphoma of the retina, vitreous, or optic nerve head. The bcl-2 t(14;18) translocation brings the bcl-2 gene, an anti-apoptosis gene, under the control of the IgH promoter. This study examined the role of this translocation in PIOL survival and relapse. Methods: From 1991 to 2003, the NEI received ocular specimens from 72 patients with PIOL. Detailed clinical information was available for 23 patients. In order to detect the bcl-2 t(14;18) translocation at the major and minor break points, PCR was performed on DNA from microdissected PIOL cells from vitreous samples. The PCR-amplifiable mixture contained microdissected DNA, 32P-labeled sense primer for Mbr, 5'-TTAGAGAGTTGCTTTACGTGCGCTT-3' or mcr, 5'-GACTCCTTTACGTGCTGGTACC-3', antisense primer (CFW1), 5'-ACCTGAGGAGACGGTGACCAGGGT-3', dNTPs, MgCl2, and AmpliTaq Gold Enzyme. The outcome variables of interest included length of survival and relapse. Analysis of survival was made from two-sided Wald statistics from Cox regression analysis using age as a covariate. Relapse was determined by two-sided Wald statistics from the logistic regression analysis using age as a covariate. Results: The clinical course was obtained for 23 patients diagnosed with PIOL. The mean follow up was 29 months. 61\% (14/23) had CNS involvement. 77\% received radiation, while 61\% received methotrexate based therapy (systemic, intravitreal, and intrathecal). Other systemic chemotherapies included vincristine, thiopeta, procarbazine, dexamethasone, Promace, and CHOP. Relapse occurred in 10 (43\%) patients. The bcl-2 t(14;18) translocation was detected in 14 (61\%) of specimens. Patients who were positive for the translocation were significantly younger (58.9 years vs. 73.9 years) (p = 0.001). After adjusting for age, no statistically significant difference was found in survival (HR = 2.33; p = 0.478) or relapse (OR = 1.28; p = 0.840) between patients who did or did not have the bcl-2 t(14;18) translocation. Conclusions: Studies have found conflicting roles for the bcl-2 t(14;18) translocation in systemic follicular and diffuse large B-cell lymphomas. Our study did not find an role for the bcl-2 t(14;18) translocation in determining length of survival or likelihood of disease relapse in PIOL, although it is interesting to note that patients with the bcl-2 t(14;18) translocation were significantly younger. This may suggest a role for the translocation in accelerating disease presentation and progression.