Abstract #2897

Discovery and characterization of driver MAPK and PI3K pathway mutations in tumors and association with drug response in cell lines

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Abstract:

The MAPK and PI3K pathways are frequently altered in human cancer and are targeted by dozens of agents in clinical trials. The efficacy of these therapies, alone or in combination, may depend on the activation status of both pathways. Next-generation sequencing of cancer exomes provides a unique opportunity to systematically survey pathway alterations in cancer. Using somatic mutation data obtained from The Cancer Genome Atlas, we sought to catalog the members of the MAPK and PI3K pathways with driver mutations, as well as the frequency of occurrence and co-occurrence of these mutations in common cancers. Furthermore, we sought to characterize the association of pathway mutation status with clinical response and with drug response in pre-clinical models.

While the MAPK and PI3K pathways were frequently altered, the frequency of single and dual pathway alteration and the altered genes varied substantially across cancer types. Predicted Gain of Function and Loss of Function mutations in MAPK pathway genes were most frequently observed in melanoma (77%), thyroid (69%), rectal (62%), colon (56%), lung adenocarcinoma (38%), and uterine cancers (24%). MAPK pathway genes were infrequently mutated in bladder (4%), breast (3%), prostate (1%), and renal cancer (1%). *KRAS*, *BRAF* and *NRAS* hotspot mutations were the most common pathway drivers. Predicted Gain of Function and Loss of Function mutations in PI3K pathway genes were most frequently observed in uterine (78%), breast cancer (36%), cervical squamous cell carcinoma (28%), glioblastoma (24%), head and neck squamous cell carcinoma (20%), squamous cell lung carcinoma (17%), and colon adenocarcinoma (17%). In contrast, PI3K pathway genes were rarely mutated in prostate cancer (1%), thyroid cancer (1%), ovarian serous adenocarcinoma (1%), and acute myeloid leukemia (0%). Hotspot mutations in *PIK3CA* as well as hotspot and deleterious mutations in *PTEN* were the most common pathway alterations. Predicted driver mutations were observed in *PIK3R1*, and *AKT1*. In uterine cancer, MAPK pathway mutations rarely occurred independently of PI3K pathway mutations while in colon and rectal adenocarcinoma, PI3K pathway mutations rarely occurred independently of MAPK pathway mutations. Co-occurring PI3K and MAPK pathway mutations in colorectal cancer led to worse overall survival compared to MAPK pathway mutations alone. Co-occurring MAPK and PI3K pathway mutations were also observed in melanoma (6%), and gastric (8%) cancers. In other cancer types one pathway was generally favored with the exception of prostate cancer, where pathway activating mutations in either the PI3K or MAPK pathway genes were uncommon.

To support the use of pre-clinical models as exhibiting responses representative of clinical populations, we assessed drug sensitivity to PI3K and MAPK pathway inhibitors in single and dual pathway activated cell lines. We integrated hybrid-capture sequencing data from the Cancer Cell Line Encyclopedia with pharmacological data from over 150 compounds. We found that MAPK and PI3K pathway mutations most significantly associated with sensitivity to MEK and PI3K/AKT/mTOR inhibitors, respectively. Notably, cell lines with co-occurring MAPK pathway and *PIK3CA* mutations were insensitive to MEK inhibitors and cell lines with co-occurring PI3K pathway and *KRAS* mutations were insensitive to PI3K inhibitors. Also, not all pathway mutations conferred equal sensitivity. For example, *BRAF* mutant cell lines were generally the most sensitive sensitive, followed by *NRAS* and *KRAS* mutant cell lines, while *NF1* and *RASA1* mutant cell lines were generally not responsive to MEK inhibitors. Taken together, our work highlights the need to consider pathways and co-occurrence in the development of standard of care and targeted therapies.

Methods:

Pathway mutation status of TCGA patients: Mutation data was integrated from TCGA. Broad mutation packager data as of the 20121024 stddata build were included as well as all DCC level 2 mutation annotation format (MAF) files as of November 15, 2012. A significant number of likely false positive mutations were identified in prostate adenocarcinoma MAF files; therefore, all calls for this disease were sourced from Compendia Bioscience's own mutation calling pipeline. The Compendia Bioscience mutation calls were made to conform to the MAF file format for integration.

Mutation classifications: In certain diseases, such as uterine corpus endometrioid cancer (UCEC) and stomach adenocarcinoma (STAD), several highly-mutated samples may dominate the overall mutation counts. Thirty-one patients with >5,000 mutations (ultra-mutators) were excluded from downstream annotation analysis. A mutation was classified as 'Recurrent' if it was observed in the same variant position in 3 or more tumor samples. A mutation was classified as 'Deleterious' if it is non-recurrent and was annotated as a frame shift insertion/deletion or nonsense mutation variant. All other mutations were classified as 'Other'. Genes were classified as 'Gain of Function' if >20% of the mutation calls within the gene were recurrent missense mutation, less than 10% of the mutations calls were deleterious, and was significantly enriched with mutations. Genes were classified as 'Loss of Function' if they contained more than 2 deleterious mutations across all samples analyzed, if >20% of mutation calls were hotspot or deleterious, and mutations were significantly enriched within the gene.

Cell line mutation and drug response data: Hybrid-capture gene sequencing data from the Cancer Cell Line Encyclopedia (CCLE) was integrated with drug response data from multiple large cell line screening panels including CCLE, Sanger cell line panel, GlaxoSmithKline (GSK) cell line panel, and OncoPanel 240 (OP240, Eurofins).

Conclusions:

- Pre-clinical models representative of clinical populations can potentially predict outcome to individual targeted therapy.
- In clinical samples, co-occurring activating mutations in MAPK and PI3K pathway genes were observed in >5% of patients in colorectal cancers, gastric cancers, endometrial cancers, melanoma, and cervical squamous cell carcinoma.
- Colorectal cancer patients with activating mutations in both PI3K and MAPK pathways had worse outcome than patients with MAPK pathway mutations alone.

Results:

Figure 1. Patient samples subjected to whole exome analysis.

Summary of patient samples initially identified for use in mutation analysis.

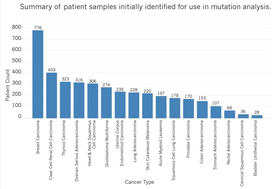


Figure 2. Compendia Bioscience mutation classification schematic.

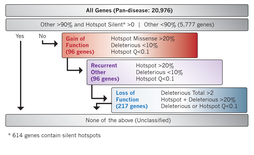


Figure 3. Pan-cancer analysis of PI3K and MAPK pathway mutations.

Mutations classified as recurrent (red) or deleterious (blue) were used to help determine Gain of Function and Loss of Function gene classifications.

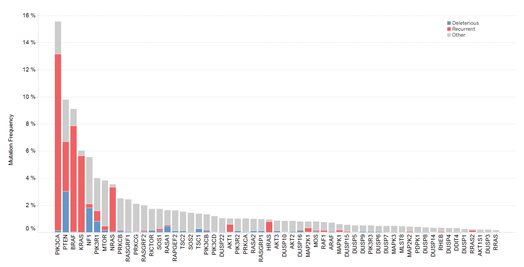
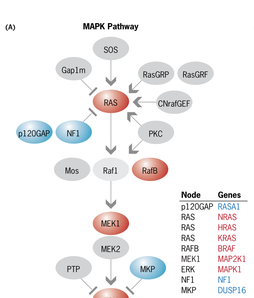
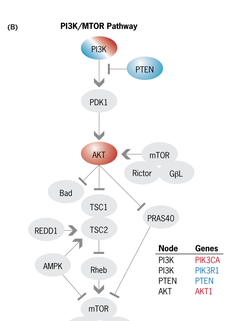


Figure 4. MAPK and PI3K pathway gene classifications.

MAPK **(A)** and PI3K **(B)** pathway genes were classified by Compendia Bioscience as Gain of Function (red) and Loss of Function (blue).



Node	Genes
p120GAP	RASA1
RAS	NRAS
RAS	HRAS
RAS	KRAS
RAFB	BRAF
MEK1	MAP2K1
ERK	MAPK1
NF1	NF1
MKP	DUSP16



Node	Genes
PI3K	PIK3CA
PI3K	PIK3R1
PTEN	PTEN
AKT	AKT1

Figure 5. Co-occurrence and mutual exclusivity of MAPK and PI3K pathway mutations in 17 cancer types.

Frequency of MAPK and PI3K pathway mutation co-occurrence and mutual exclusivity across 17 cancer types **(A)** and a patient strip plot of colon adenocarcinoma patients that have both MAPK and/or PI3K pathway aberrations **(B)**, including Gain of Function mutations (pink) and amplifications (red). Only somatic hotspot missense and deleterious mutations in genes classified as Gain or Loss of Function were aggregated at the pathway level. In colon adenocarcinoma, patients with Gain of Function mutations in *PIK3CA* had significantly co-occurring Gain of Function mutations in *KRAS* (*p*-value=9.03E-05 determined by Fisher's exact test).

Cancer types represented in the pie charts are as follows: BLCA=bladder carcinoma, BRCA=breast carcinoma, CESC=cervical squamous cell carcinoma, COAD=colon adenocarcinoma, GBM=glioblastoma multiforme, HNSC=head and neck squamous cell carcinoma, KIRC=clear cell renal cell carcinoma, LAML=acute myeloid leukemia, LUAD=lung adenocarcinoma, LUSC=squamous cell lung carcinoma, OV=ovarian serous adenocarcinoma, PRAD=prostate adenocarcinoma, READ=rectal adenocarcinoma, SKCM=cutaneous melanoma, STAD=stomach adenocarcinoma, THCA=thyroid carcinoma, UCEC=uterine corpus endometrioid carcinoma.

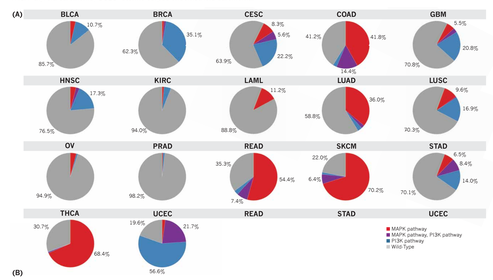


Figure 6. Colorectal cancer patients with co-occurring MAPK and PI3K pathway mutations exhibit decreased overall survival.

Only somatic hotspot missense and deleterious mutations in genes classified as Gain or Loss of Function were aggregated at the pathway level. Six colorectal cancer patients were also positive for PI3K pathway mutations; no patient events were reported within that group.

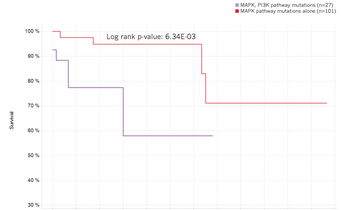


Table 1. MAPK pathway mutations associate with sensitivity to MAPK pathway inhibitors *in vitro*.

MAPK pathway mutations were assessed for association with drug sensitivity to various MAPK pathway inhibitors. Categorical calls for gene mutations in MAPK pathway genes and drug sensitivity were used to generate p-values and odds ratios (Fisher's exact test). Moderately responsive cell lines were excluded from association analyses.

Drug	Target	Cell Line Panel	p-value	Odds Ratio
PD-0325901	MEK	CCLE	2.00E-23	18.3
GSK1120212	MEK	GSK	1.30E-14	35.4
PD-0325901	MEK	Sanger	5.40E-13	17.8
CI-1040	MEK	OP240	9.30E-12	23.9
AZD6244	MEK	CCLE	2.00E-10	9.7
AZD6244	MEK	Sanger	4.80E-08	21.6
RDEA119	MEK	Sanger	7.80E-08	21.0
AZ628	RAF	Sanger	1.70E-06	34.8
PLX4720	RAF	CCLE	2.00E-06	27.7
CI-1040	MEK	Sanger	2.10E-05	10.6
AZ628	RAF	Settleman	3.40E-05	3.8
RAF265	RAF	CCLE	6.40E-05	3.1

Figure 7. MAPK pathway mutations associate with MEK inhibitor sensitivity *in vitro*.

PD-0325901 sensitivity and MAPK pathway biomarker associations. Cell lines were ranked by increasing PD-0325901 IC50 (μM), and colored by MAPK pathway mutation status (**A**, mutant=yellow, wild type=blue) and colored by PD-0325901 sensitivity status (**B**, pink=sensitive, green=resistant, gray=moderate).

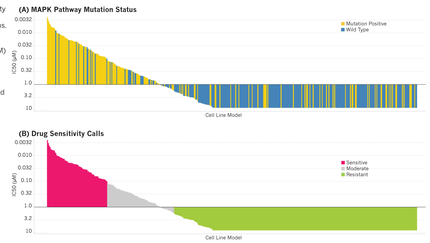


Table 2. Individual MAPK pathway mutations exhibited differential sensitivity to MEK inhibition.

Individual MAPK pathway aberrations were assessed for significant association with sensitivity to the MEK inhibitor, PD-0325901. Categorical calls for gene mutations in Gain of Function and Loss of Function MAPK pathway genes and categorical drug sensitivity calls were used to calculate p-values and odds ratios (Fisher's exact test).

Pathway gene	Mutation type	Association	p-value	Odds Ratio
BRAF	Gain of Function	Sensitivity	1.13E-15	18.44
KRAS	Gain of Function	Sensitivity	2.53E-03	2.55
NRAS	Gain of Function	Sensitivity	5.91E-07	7.32
HRAS	Gain of Function	Sensitivity	5.00E-03	.
NF1	Loss of Function	-	2.93E-01	-
RASA1	Loss of Function	-	2.55E-01	-

Figure 8. Individual MAPK pathway mutations were differentially sensitive to MEK inhibition.

Cell lines were ranked by increasing PD-0325901 IC50 (μM) and colored by individual MAPK pathway gene mutation status (mutant=yellow, wild type=blue), BRAF mutant vs. wild type (**A**), *NRAS* mutant vs. wild type (**B**), *KRAS* mutant vs. wild type (**C**), *HRAS* mutant vs. wild type (**D**), *NF1* mutant vs. wild type (**E**), and *RASA1* mutant vs. wild type (**F**).

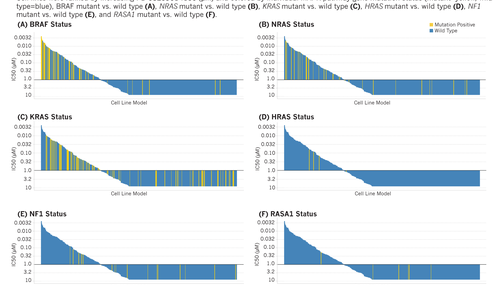


Figure 9. In MAPK pathway mutant cell lines, PIK3CA mutations predict resistance to MEK inhibition.

MAPK pathway mutant positive cell lines were ranked by increasing PD-0325901 IC50 (μM). In the context of MAPK pathway mutant cell lines, PIK3CA mutations (**A**) were associated with resistance to the MEK inhibitor, PD-0325901 (p-value<0.05). MAPK pathway mutant cell lines had co-occurring TP53 mutations (**B**) that spanned the sensitivity profile.

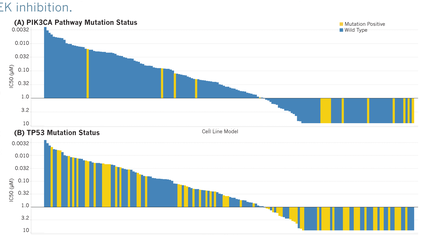


Table 3. PI3K pathway mutations associate with sensitivity to PI3K pathway inhibitors.

PI3K pathway mutations were assessed for association with drug sensitivity to various PI3K pathway inhibitors. Categorical calls for gene mutations in PI3K pathway genes and drug sensitivity were used to generate p-values and odds ratios (Fisher's exact test). Moderately responsive cell lines were excluded from association analyses.

Drug	Target	Cell Line Panel	p-value	Odds Ratio
AKT inhibitor VIII	AKT	Sanger	5.70E-04	25.3
GDC0941	PI3K	Sanger	7.03E-04	5.2
GSK690693	AKT	GSK	1.72E-03	3.9
TGX-221	PI3KCB	GSK	1.62E-02	3.4
Temsirolimus	MTOR	OP240	1.71E-02	3.7
AZD0530	SRC	Sanger	2.46E-02	4.6
MK-2206	AKT	Sanger	2.67E-02	4.2

Figure 10. PI3K pathway mutations associate with PI3K inhibitor sensitivity.

GDC0941 sensitivity and PI3K pathway biomarker associations. Cell lines were ranked by increasing GDC0941 IC50 (μM) and colored by PI3K pathway mutation status (**A**, mutant=yellow, wild type=blue) and colored by GDC0941 sensitivity status (**B**, pink=sensitive, green=resistant, gray=moderate).

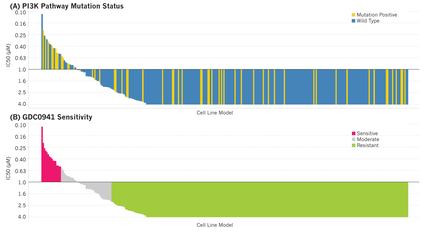


Table 4. Individual PI3K pathway mutation associations with PI3K inhibitor sensitivity.

Individual PI3K pathway aberrations were assessed for significant association with sensitivity to the PI3K inhibitor, GDC0941. Categorical calls for gene mutations in PI3K pathway genes and categorical drug sensitivity calls were used to calculate p-values and odds ratios (Fisher's exact test).

Pathway gene	Association	p-value	Odds Ratio
PIK3CA	Sensitivity	6.64E-02	2.96
PTEN	Sensitivity	3.49E-02	3.98
AKT1	Sensitivity	1.15E-04	.
PIK3R1	-	7.12E-01	-

Figure 11. Individual PI3K pathway mutation associations with GDC0941 sensitivity.

Cell lines were ranked by increasing GDC0941 IC50 (μM) and colored by individual PI3K pathway gene mutation status (mutant=yellow, wild type=blue). *PIK3CA* (**A**) and *PTEN* (**B**) mutations were associated with sensitivity while cell lines with mutations in *PIK3R1* (**C**) were not associated with sensitivity. One cell line had an *AKT1* mutation (**D**) and was sensitive to GDC0491.

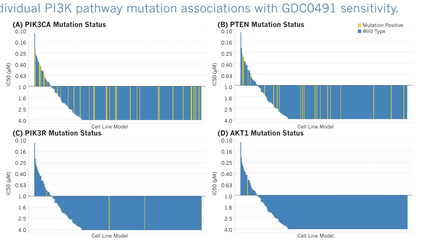


Figure 12. A large fraction of PI3K pathway mutant cell lines resistant to PI3K pathway inhibition had co-occuring MAPK pathway mutations.

PI3K pathway mutant positive cell lines were ranked by increasing GDC0941 IC50 (μM). 36% of PI3K pathway mutant cell lines resistant to GDC0941 also had co-occurring MAPK pathway mutations.