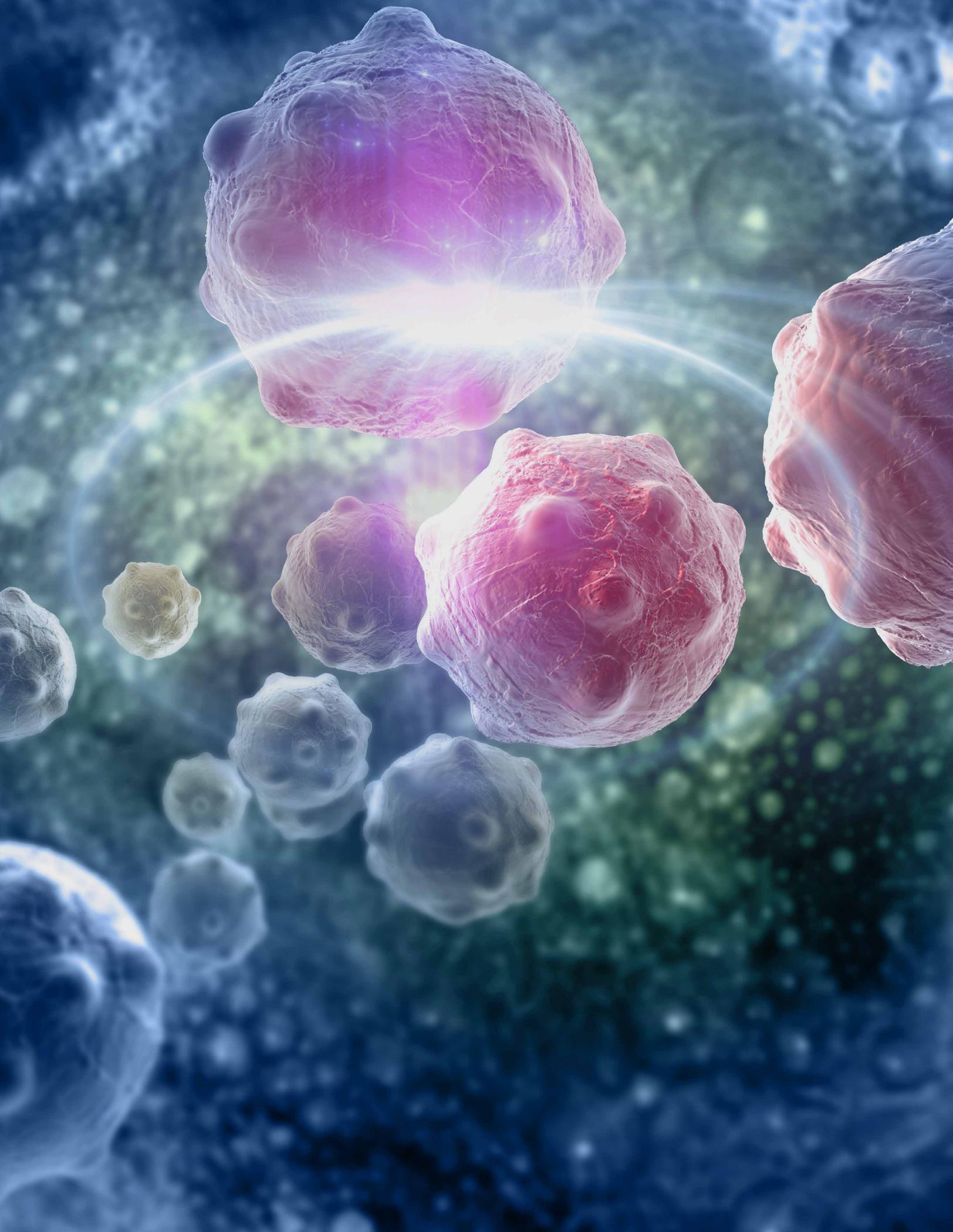


CANCER CELL CULTURE BASICS HANDBOOK



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General introduction to cancer biology

Cancer is a leading cause of death worldwide and has become a major public health issue in developed countries [1]. Cancer development is a multistep process, during which cells accumulate genetic abnormalities, especially in oncogenes and tumor suppressor genes, contributing to uncontrolled proliferation. These abnormalities provide several growth advantages. Indeed, the transformation

from normal cell to tumor cell frequently involves mutations in the cell genome. Hanahan and Weinberg described six key changes that occur during the transformation from a normal cell to a tumor cell; these features may be considered hallmarks of cancer [2]. Since then, four additional hallmarks and characteristics have been proposed [3] (Figure 1).

Cancer hallmarks

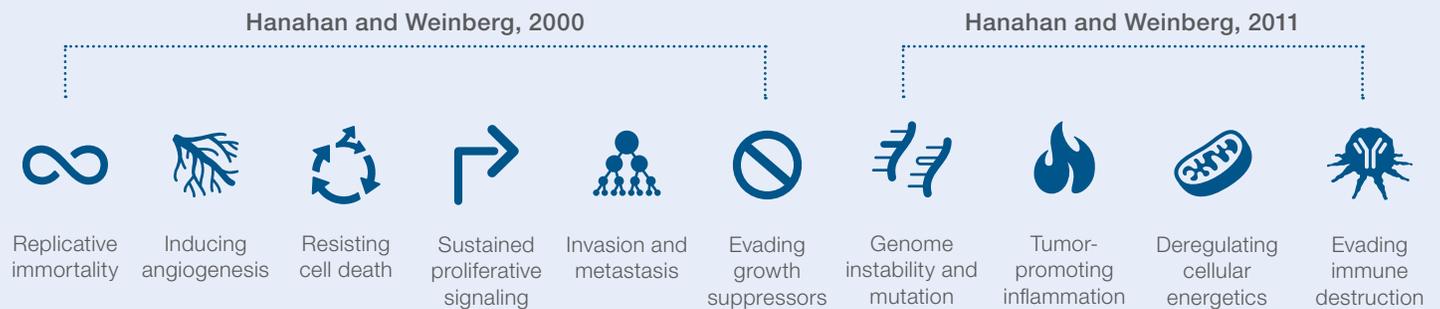
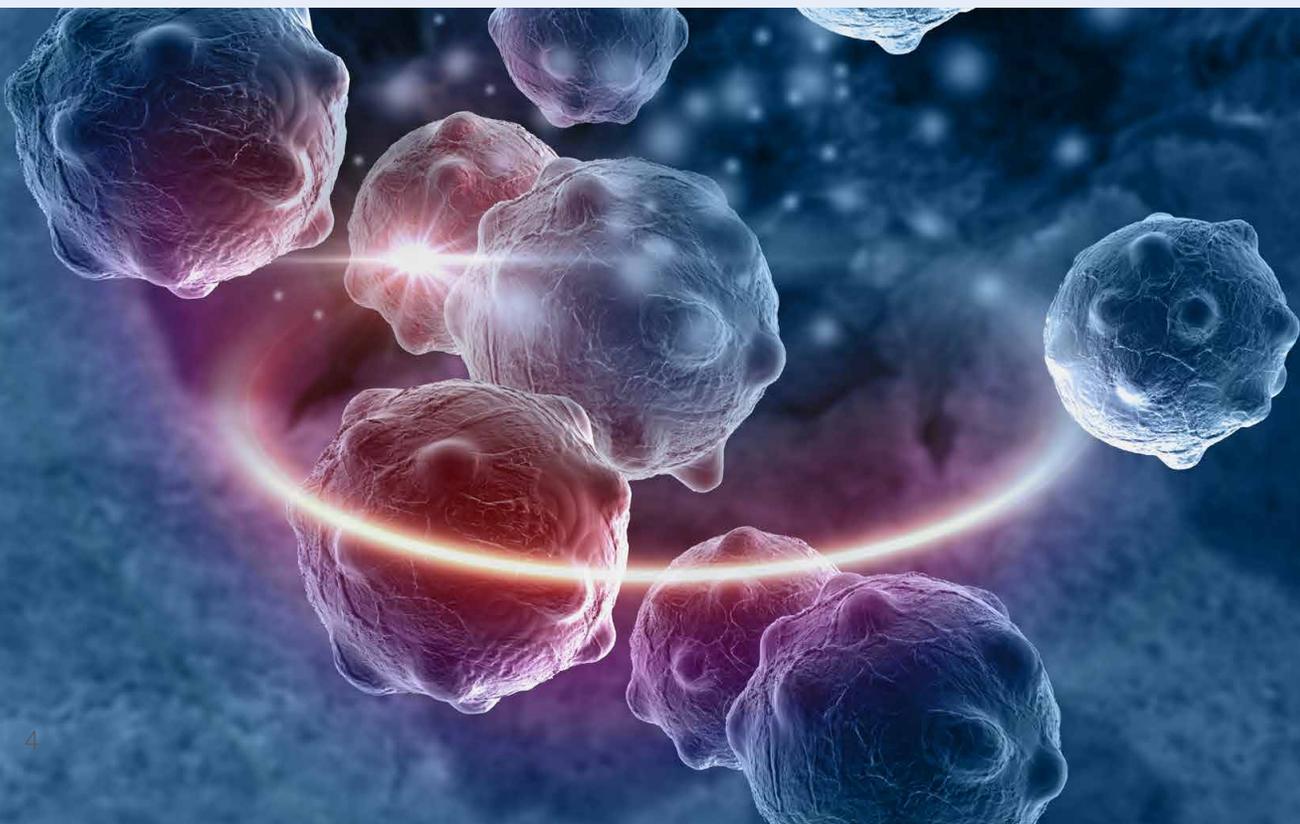


Figure 1. Hallmarks of cancer. Schematic representation of key features describing the transformation from a normal cell to a tumor cell. Adapted from Hanahan and Weinberg, 2000 [2], Hanahan and Weinberg, 2011 [3].



Replicative immortality

The hallmarks of cancer are described in more detail in Figures 2–11.

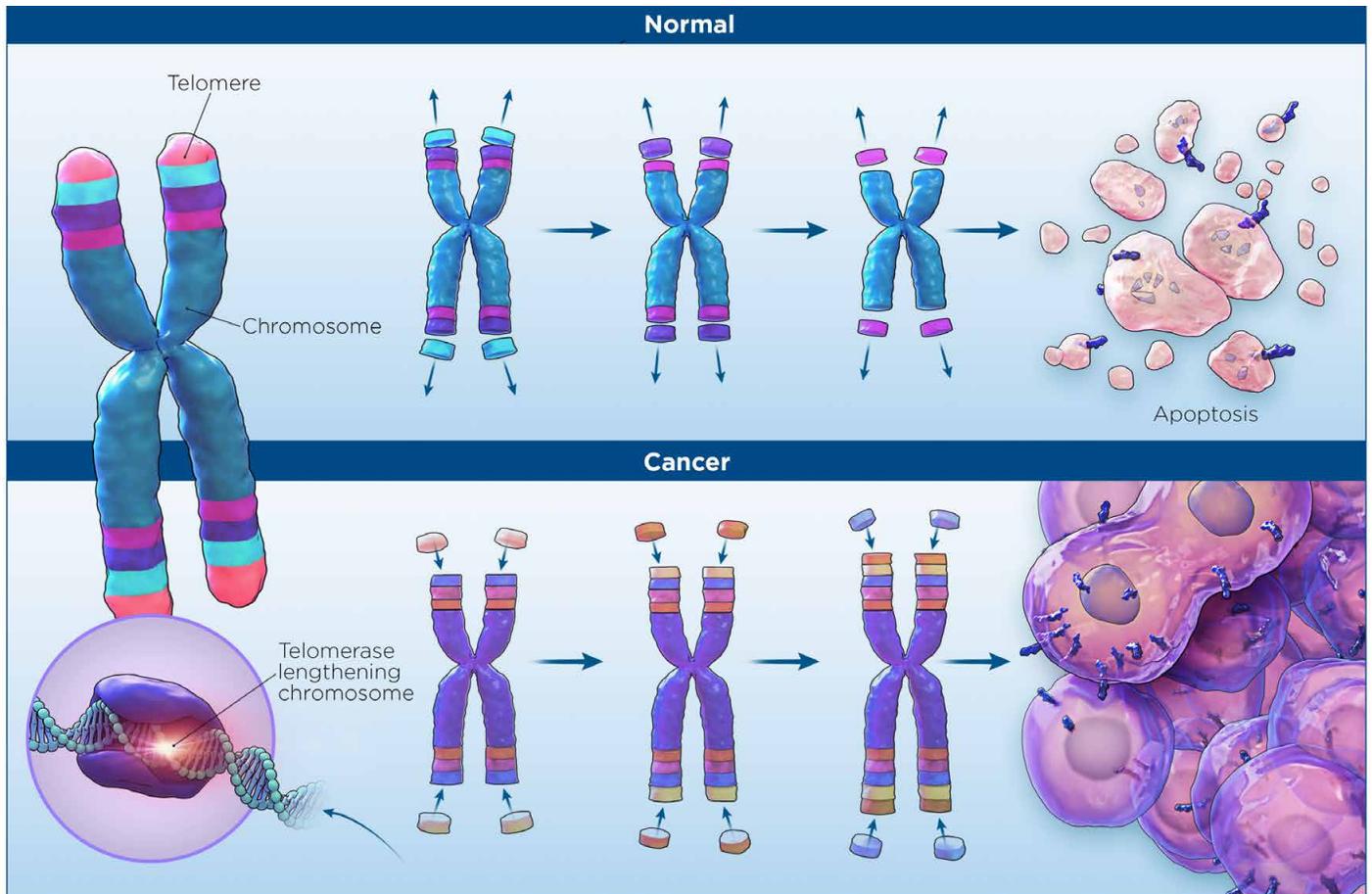


Figure 2. Replicative immortality. Cancer cells typically exhibit increased telomerase activity, which helps them to become immortal; that is, to divide indefinitely as long as proper nutrient and O₂ requirements have been met. Normal cells have normal telomere lengths and divide for finite numbers of replications.

Inducing angiogenesis

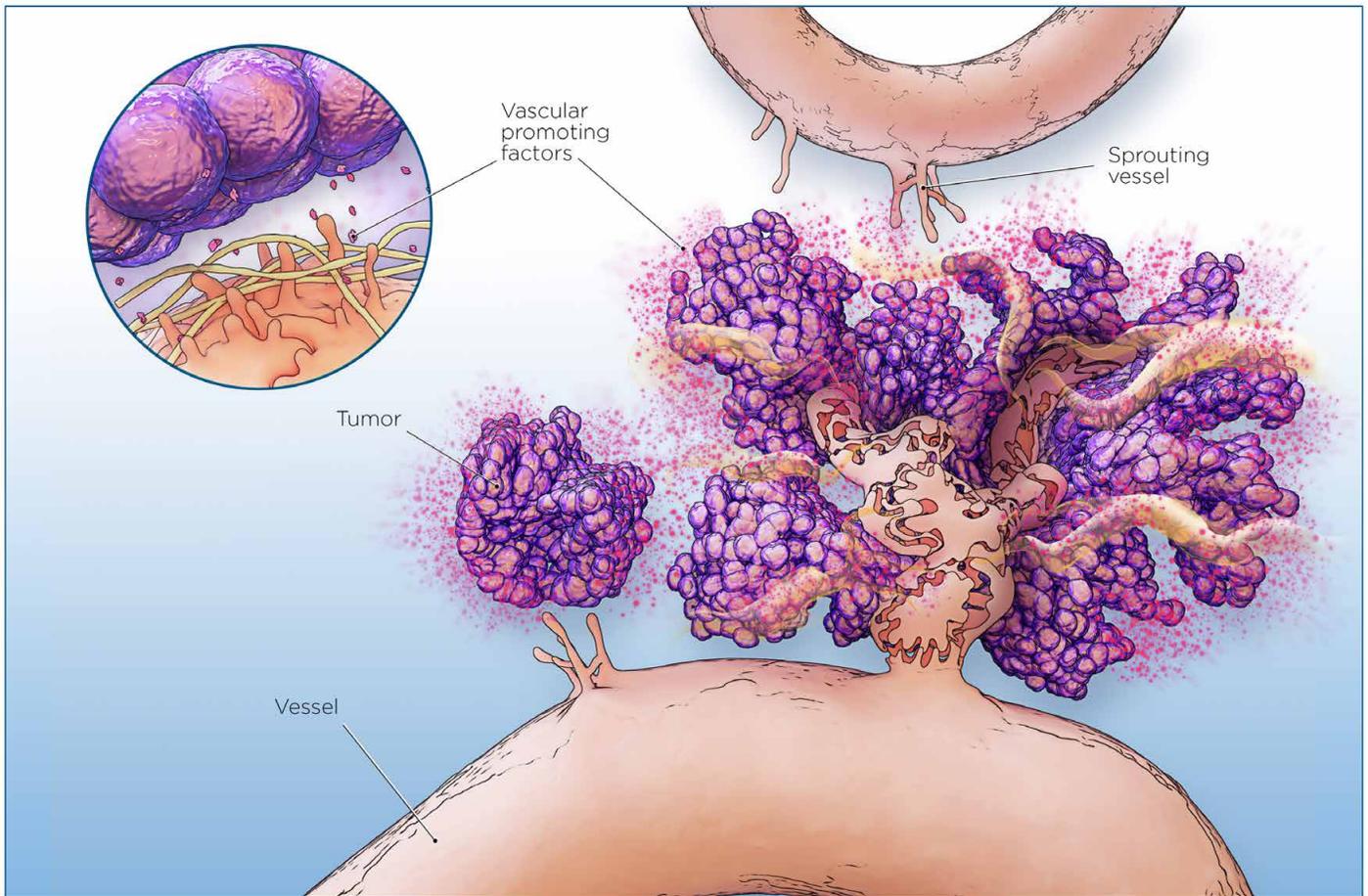


Figure 3. Inducing angiogenesis. Some tumor cells overexpress vascular endothelial growth factor (VEGF), which is a major angiogenic factor. Secretion of angiogenic factors such as VEGF by tumor cells creates blood vessels, which provide nutrients to the interior of tumors. These blood vessels are architecturally different from normal blood vessels, being less organized. In order to grow, tumors need to create a blood supply to their interior, allowing nutrients and O_2 to be delivered. The process of blood vessel growth is called angiogenesis. Most solid tumors secrete angiogenic factors.

Resisting cell death

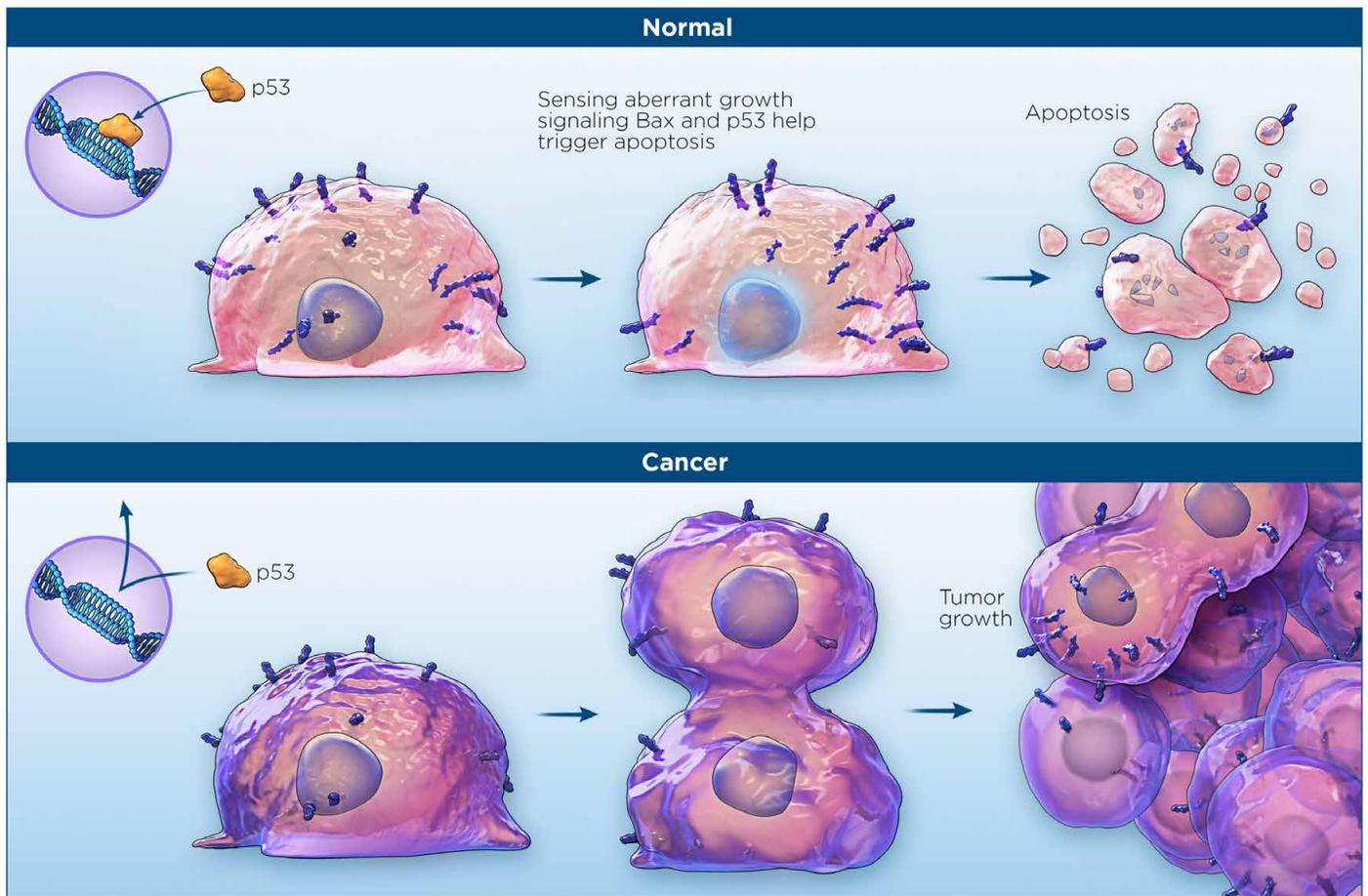


Figure 4. Resisting cell death. The p53 tumor suppressor protein is encoded by the *TP53* gene in humans. In normal cells, p53 suppresses abnormal growth in part through interactions with the proapoptotic protein Bax, whereas in tumor cells, mutations in the *TP53* gene lead to loss of tumor suppression, loss of interaction with Bax, and inhibition of apoptosis. Apoptosis, also known as programmed cell death, serves as a mechanism to prevent the accumulation of mutations or damage within normal cells.

Sustained proliferative signaling

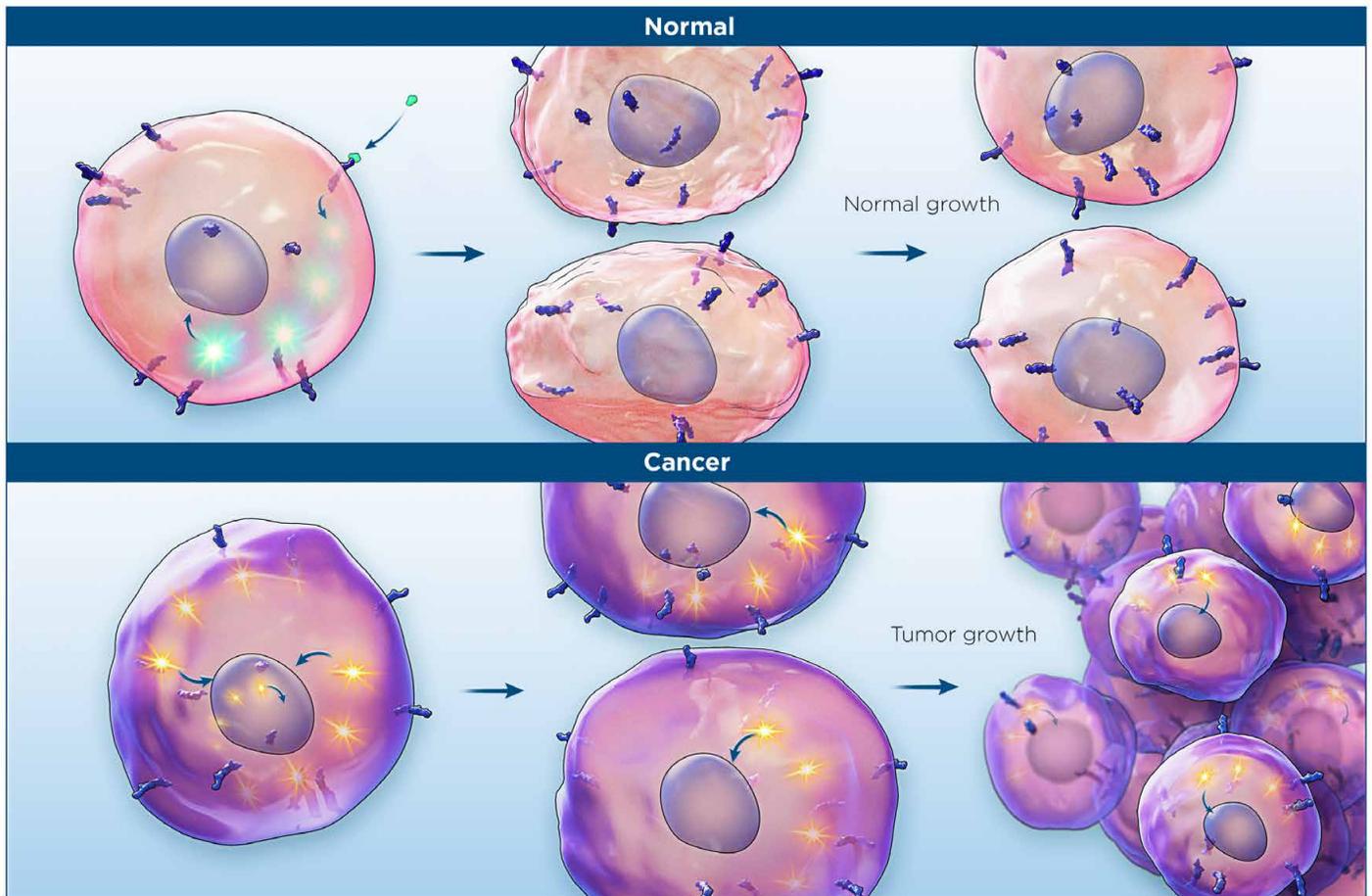


Figure 5. Sustained proliferative signaling. Expression of proliferation and survival signals by tumor cells allows the cells to grow continually as immortalized cells. To achieve growth independent of external growth factors, some tumor cells express activating mutations in proteins involved in cell growth. For example, ~50% of melanomas bear mutations in the gene coding for the serine-threonine kinase BRAF, and of these mutations, about 90% are V600E point mutations [4].

Invasion and metastasis

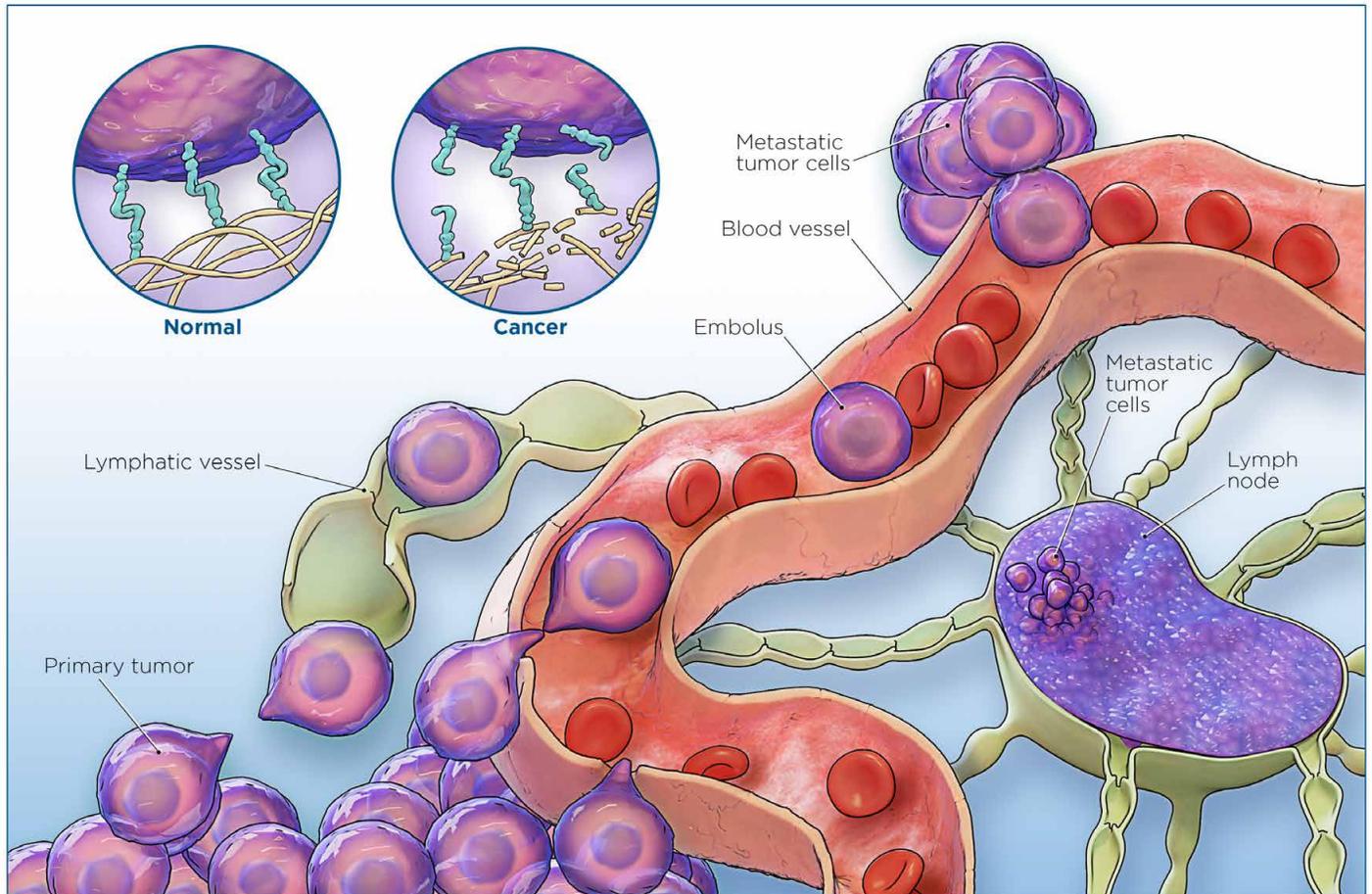


Figure 6. Invasion and metastasis. Tumor cells spread from the original site of growth to distal sites through the circulatory system. The movement of cancer cells from the original site is termed metastasis. Tumor cells are transported via blood or the lymphatic vessel to colonize distant organs. In some cases, metastasis occurs when tumor cells spread in body cavities, such as the peritoneal cavity, or when tumor cells are inadvertently seeded during surgical removal of a tumor. Metastasis is aided by deregulation of epithelial adhesion molecules such as E-cadherin.

Evading growth suppressors

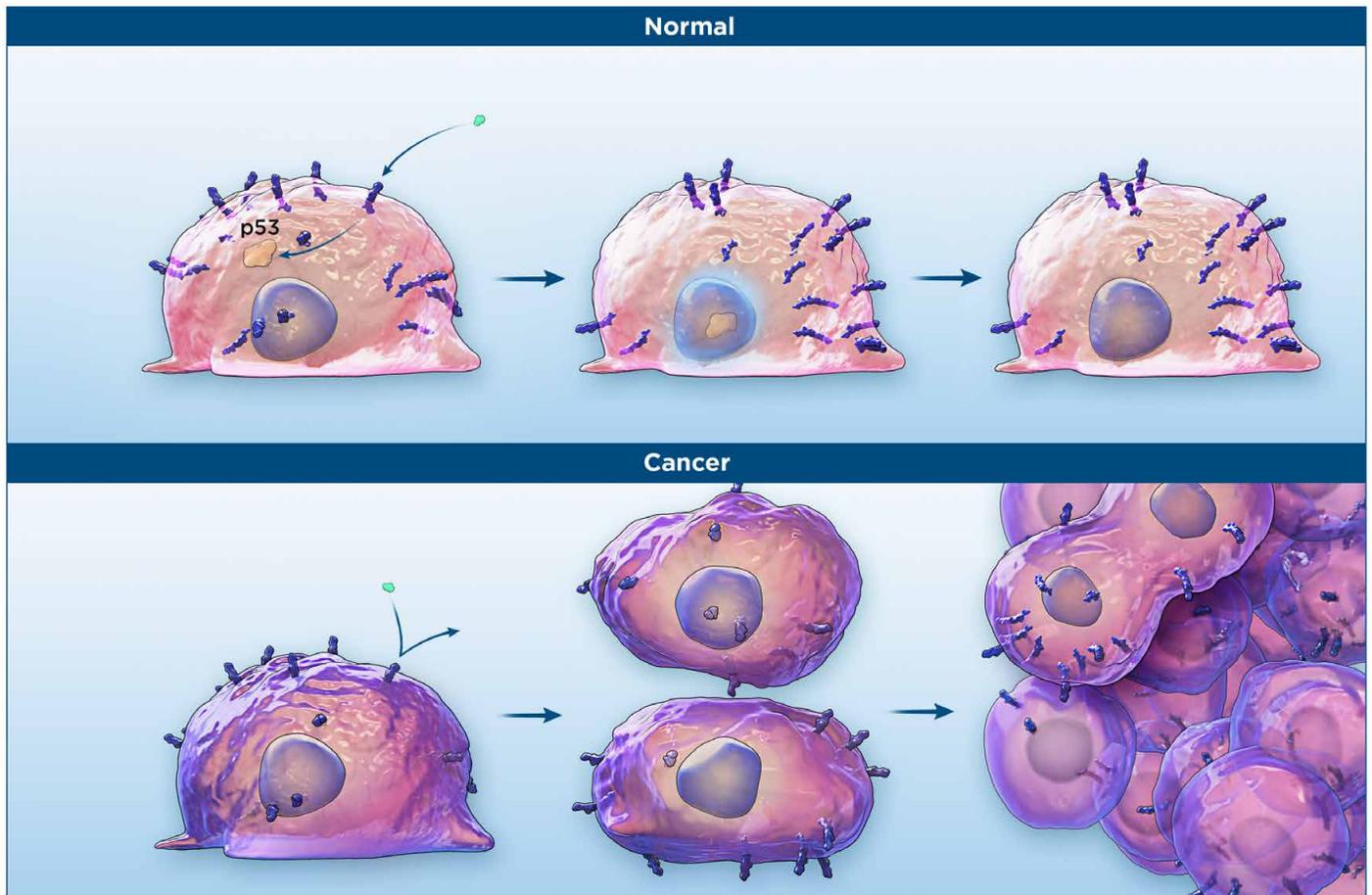


Figure 7. Evading growth suppressors. Alteration in tumor-suppressing signaling proteins, such as p53, drives sustained proliferation of tumor cells. Escaping from growth-suppressing signaling is another mechanism by which proliferation of tumor cells is driven. Mutations in tumor suppressor genes are frequently found in cancer cells; for example, p53 is often mutated and alters the cell cycle in tumor cells.

Genome instability and mutation

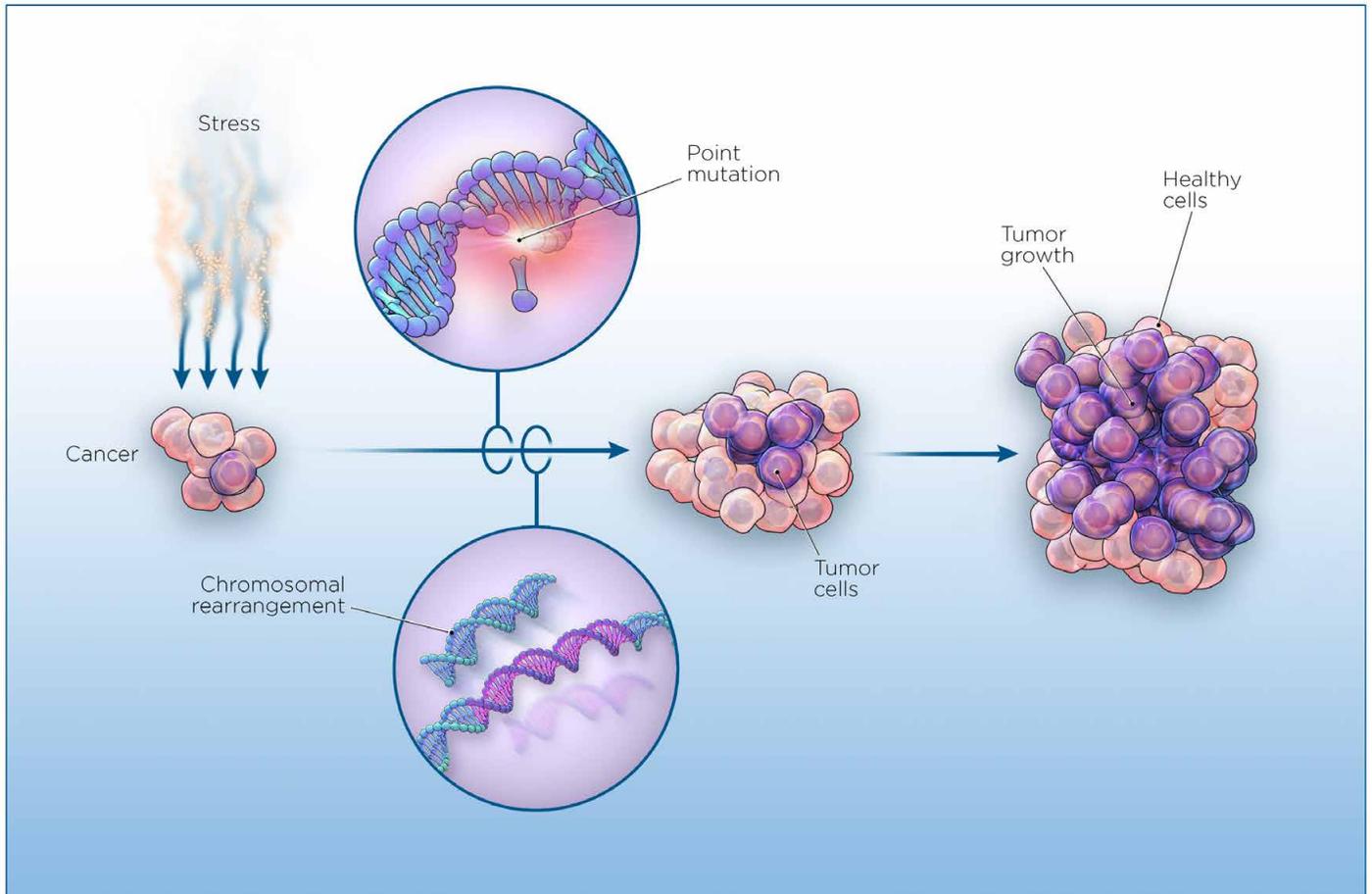


Figure 8. Genome instability and mutation. Genetic instability—consisting of loss of heterozygosity (LOH), chromosomal rearrangements, and more—favors continual growth of tumor cells. For example, mutations in genes (e.g., *TP53*) are recessive, thus LOH leads to expression of mutated p53 protein sufficiently to sustain growth.

Tumor-promoting inflammation

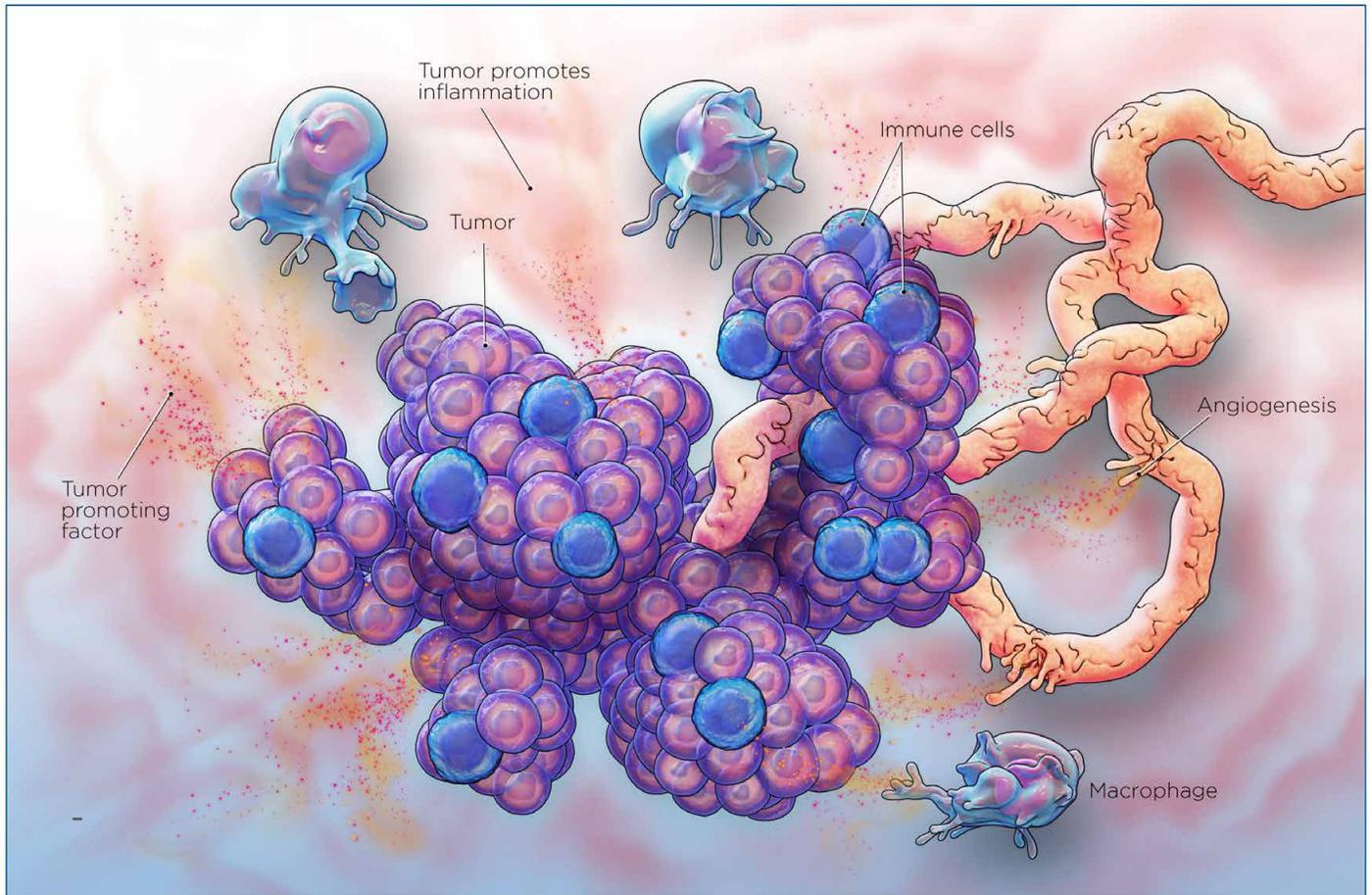


Figure 9. Tumor-promoting inflammation. Infiltration of tumors by cells of the immune system helps drive tumor growth by providing growth, survival, and angiogenic factors. On the other hand, in some tumor types, infiltrating immune cells mediate responses against the tumor, resulting in tumor shrinkage.

Deregulating cellular energetics

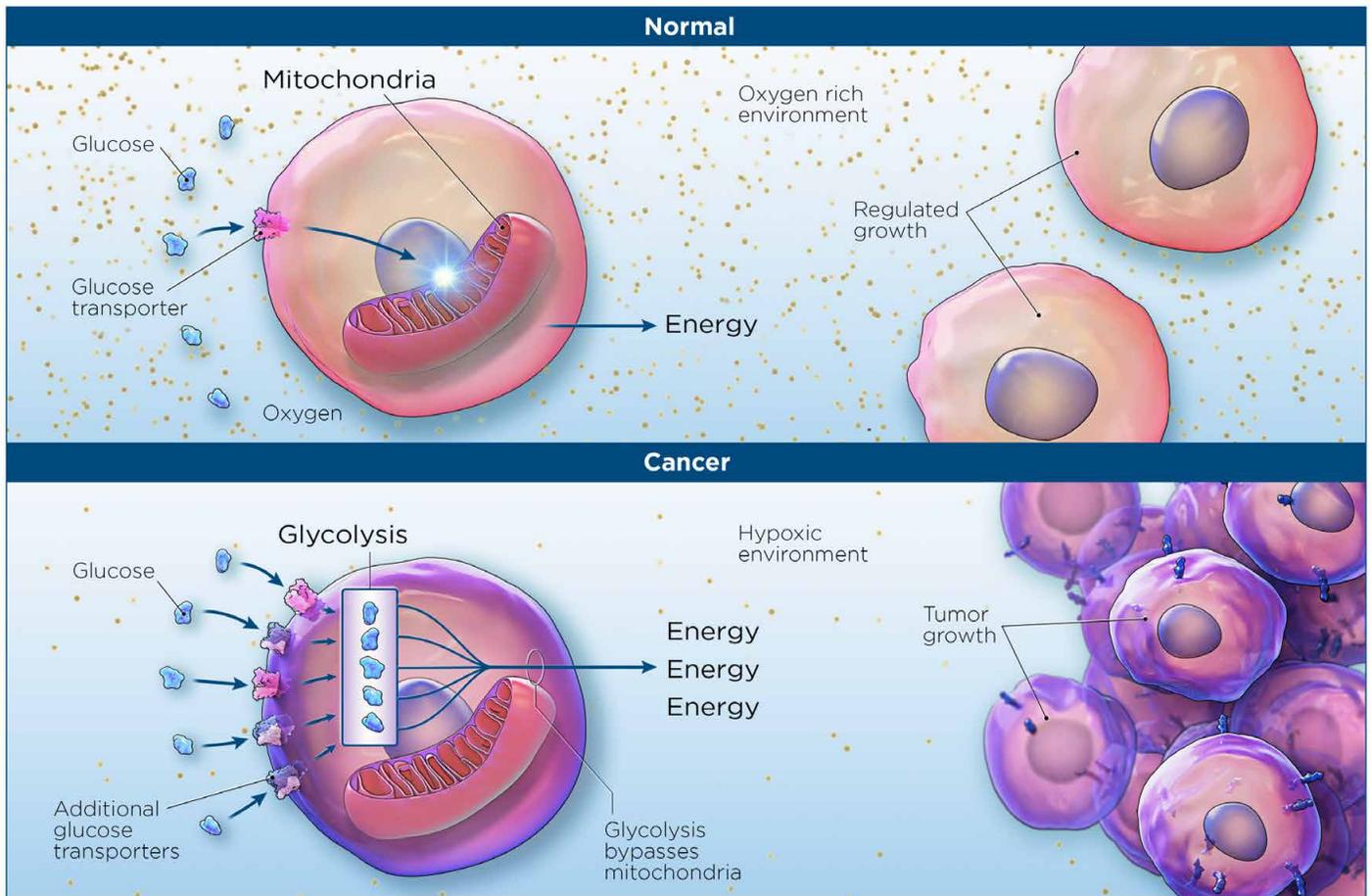


Figure 10. Deregulating cellular energetics. Cancer cells require increased energy consumption to fuel their growth [5]. Tumor cells mostly use glycolysis instead of mitochondrial oxidative phosphorylation, thus they need to increase their uptake of glucose relative to normal cells. Moreover, hypoxic conditions and Ras expression increase the expression of HIF1 α and HIF2 α , which upregulate glycolysis. In addition, HIF1 α and HIF2 α upregulate growth and angiogenic factors [5].

Evading immune surveillance and destruction

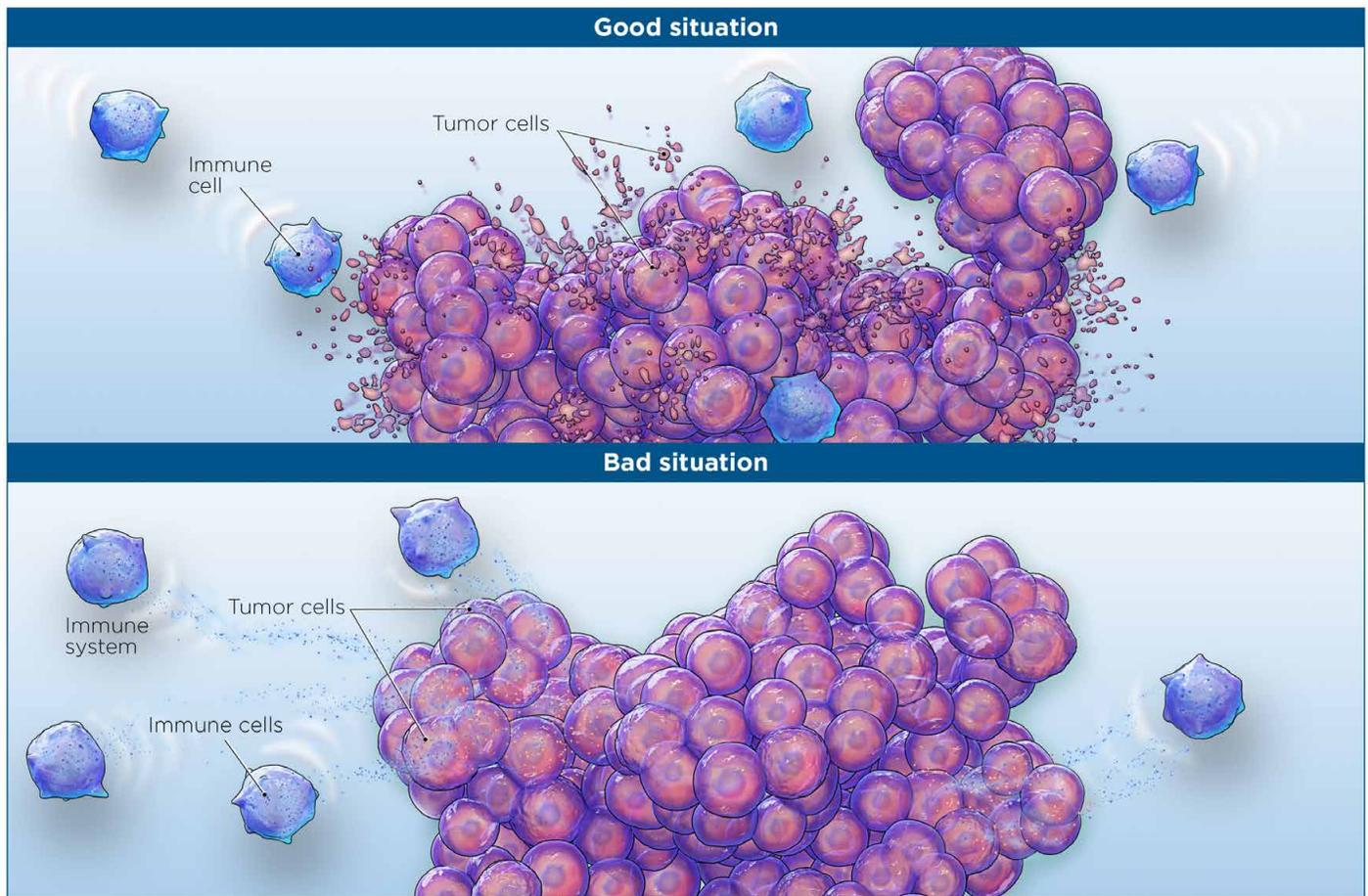


Figure 11. Evading immune surveillance and destruction. Infiltration of tumors by T cells often accompanies a better prognosis than tumors not infiltrated by T cells. Thus, many tumor types have evolved mechanisms to escape surveillance or avoid destruction by the immune system.

An additional hallmark of cancer cell lines is loss of contact inhibition. Untransformed (normal) cells display contact inhibition when grown on a solid substrate, such as a culture dish or flask; they form a monolayer. Cancer cell lines, however, continually grow. Contact inhibition is an anticancer mechanism that stops cell division when cells reach a high density. Interestingly, cancer cells and, more generally, cancer cell lines display no contact inhibition. Indeed, when confluence is reached, the cells continue to divide and pile up on top of each other.

The processes described previously may ultimately result in tumor formation, with concomitant invasion of surrounding tissues and emergence of metastases. Throughout this text, tumor and cancer will be used interchangeably, although the terms are not identical. For example, tumors can be benign or malignant, and leukemias do not form tumors.

Introduction to specific cancer types

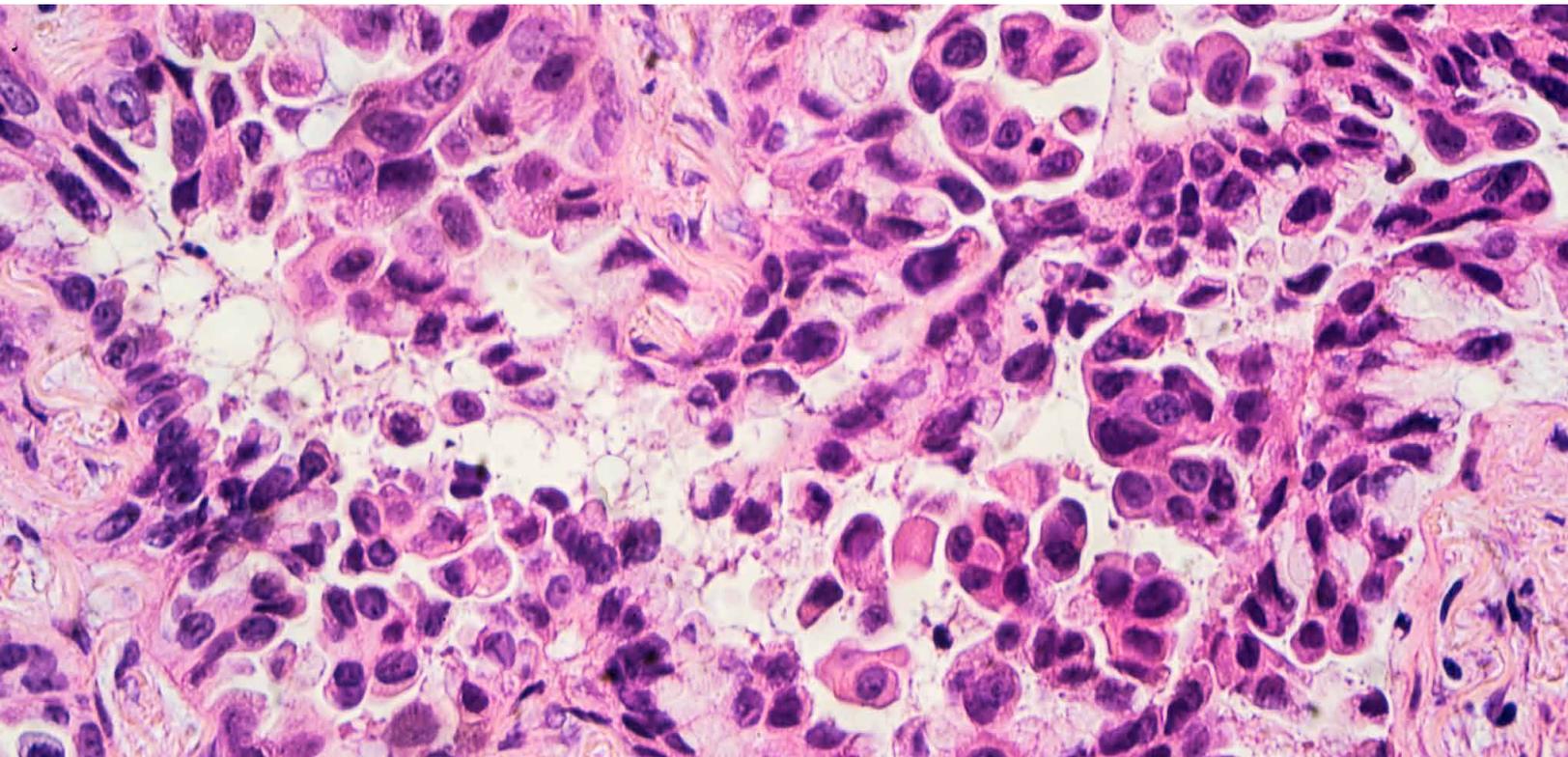
In this handbook, four cancer types will be discussed in detail: lung, breast, liver, and colorectal. These four types were chosen in part for their global prevalence and incidence. Lung cancer is the leading cause of cancer death worldwide, and has a high overall mortality. Breast cancer is the second most common noncutaneous cancer among women and the second leading cause of cancer death for women. Liver cancer is the sixth most prevalent cancer globally, and the third leading cause of death due to cancer. Prostate cancer is the most common noncutaneous cancer among men. Although mortality from prostate cancer has decreased, its incidence is rising.

Lung cancer

Lung cancer is the leading cause of cancer-related death worldwide. Despite recent advances in lung cancer treatments, mortality remains high (overall five-year survival: 5–10%), partly due to the advanced stage of the disease at diagnosis. There are two major forms of lung cancer, non-small cell lung cancer (NSCLC, about 85% of all cases), and small cell lung cancer (SCLC, about 15% of cases) [6]. NSCLC occurs in three main histological subtypes: adenocarcinoma (40%), squamous cell carcinoma (25%), and large cell carcinoma (10%) [7].

Smoking is the principal cause of lung cancer and is associated with SCLC and squamous cell carcinoma. Adenocarcinoma is the most frequent lung cancer in nonsmoker patients. According to recent epidemiological studies, the prevalence of smoking is decreasing, and the incidence of adenocarcinoma is increasing, particularly in women.

Advances in genetic research have allowed the identification of genomic alterations in NSCLC, which have contributed to both development of new therapies and increased patient survival. About 15% of adenocarcinomas harbor mutations in the epidermal growth factor receptor (*EGFR*) gene, and thus respond well to EGFR inhibitors. Currently, EGFR inhibitors are used in a first-line setting with excellent efficacy and tolerance profiles. About 5% of adenocarcinomas harbor a translocation mutation in the anaplastic lymphoma kinase (*ALK*) gene, which causes the production of an abnormal fusion protein EML4–ALK that promotes cell proliferation. Inhibitors targeting this abnormal protein have been approved, and patients on these inhibitors have excellent responses. Inhibitors for *BRAF* mutations, *MET* mutations, *NTRK* fusion, *ROS1* translocation, or *HER2* insertion are currently under clinical investigation. The emergence of onco-immunological agents has further revolutionized treatment of NSCLC, and represents a great hope for patients.



Breast cancer

Breast cancer is the second most common noncutaneous cancer after lung carcinoma and the second leading cause of cancer-related death in women.

Immunochemical markers, such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and proliferation marker Ki-67, have been used to classify breast carcinoma for predicting the prognosis and guiding treatment. Based in part on these markers, breast cancer is categorized into three main subtypes, basal-like (HER2-, ER-, PR-; also known as triple-negative breast cancer or TNBC), HER2+/ER-, and luminal, with the luminal subtypes subdivided into luminal A (ER+ and/or PR+, HER2-, low Ki-67) and luminal B (ER+ and/or PR+, HER2+ or HER2-, high Ki-67) [8,9]. Luminal A subtype cancer has the best prognosis; that of luminal B is slightly worse. HER2+ is amenable to therapy targeting HER2, while basal-like is the most difficult to treat, owing to its lack of specific targetable markers. Some add an additional subtype, normal-like, which resembles luminal A in its pattern of marker expression, but has a slightly worse prognosis than luminal A [9].

About 5 to 10% of breast cancers are associated with gene mutations, the most common inherited cause being mutations in either *BRCA1* or *BRCA2*, which increase the risk of developing breast cancer [10]. *BRCA1* and *BRCA2* are tumor suppressor genes. Mutations in *BRCA1* are slightly more common than in *BRCA2*. The prevalence of TNBC with *BRCA1* mutations ranges from 57% to 88%, and clusters of *BRCA1* mutations are seen in certain ethnic groups [11]. *BRCA2* mutations are also seen with TNBC, albeit at lower frequencies [12]. *BRCA1* and *BRCA2* proteins play important roles in DNA repair, such as homologous recombination (HR). Defects in HR enhance carcinogenesis. Recent studies showed that targeting poly-ADP-ribose polymerases in HR-deficient cells leads to cell death. This therapeutic strategy may yield future therapies for patients with TNBC.

Liver cancer

Liver cancer, also known as hepatic cancer, is the sixth most prevalent cancer and the third leading cause of cancer-related death worldwide [13]. Liver cancer includes two main subtypes, hepatocellular carcinoma (HCC) and intrahepatic bile duct cancer [14]. Of these, the predominant subtype seen is HCC and its incidence is rising as a result of infection with hepatitis B or C virus (HBV or HCV) [15].

Hepatocellular carcinoma (HCC) is the most common form of adult liver cancer, accounting for ~90% of all cases, and is frequently seen in people with cirrhosis. Intrahepatic bile duct cancer (cholangiocarcinoma) is the second most common form [14]. Pediatric liver cancer occurs in two main forms, hepatoblastoma and HCC [16].

Currently, there are no specific targeted therapies for liver cancer. However, multiple treatment options are available, such as surgical resection, liver transplantation, radiation therapy, and chemotherapy. Moreover, vaccination against HBV and HCV may reduce the future incidence of HCC. Data from a 20-year study of HBV childhood vaccinees showed that protection against HCC extended from childhood into early adulthood [17]. There is no vaccine approved for HCV as of this writing; however, vaccines against HCV in clinical trials show efficacy and tolerability [18], and at least 10 different vaccine versions are in current trials [19].

Prostate cancer

Prostate cancer is the most common noncutaneous cancer in men. Most prostate cancers are adenocarcinomas, characterized by slow growth. Screening for prostate-specific antigen (PSA) is the most commonly used test for prostate cancer, as the risk of prostate cancer increases with the level of PSA. However, clinical guidelines recommend using parallel methods of screening, as PSA levels alone do not indicate the presence or absence of prostate cancer.

Some inherited genetic alterations increase the risk of developing prostate cancer:

- Inherited mutation in the *BRCA1* or *BRCA2* gene, especially in the *BRCA2* gene, increase the prostate cancer risk
- Rare inherited mutation in the *HOXB13* gene has been associated with early prostate cancer
- Inherited mutation in the *RNASEL* gene increases the risk of developing prostate cancer

Male hormones, called androgens, are directly involved in prostate cancer cell proliferation. Hormone therapy disrupts androgen activity and impairs tumor cell proliferation. Recent research has identified molecular alterations in prostate cancer cells (GSPT1, PTEN, p27, NKX3.1), which are under investigation for targeted therapy.

Cancer cell lines as model systems for cancer study

Cancer cell lines are widely used in biomedical research; they provide excellent model systems to study mechanisms associated with cancer development, thereby improving the understanding of cancer cell biology. Furthermore, these cell lines are used for the development and screening of novel anticancer drugs.

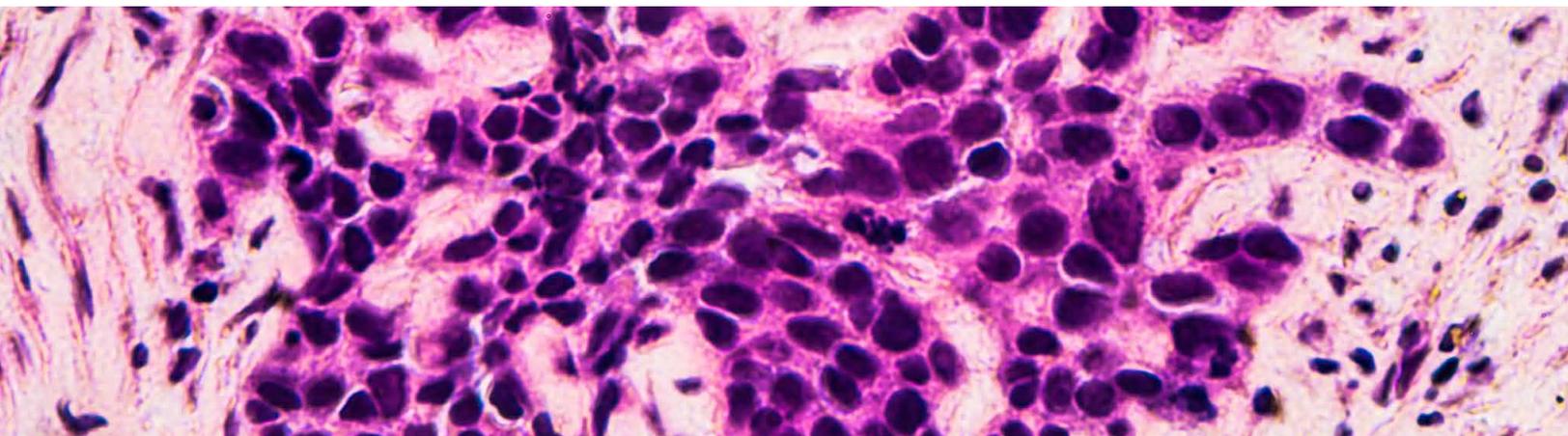
Cells can be obtained from tumors and subsequently cultured. Such primary cells can be acquired from solid tumors or cell suspensions (pleural effusions, blood, or ascites). Two main methods are used for cell extraction: mechanical or enzymatic dissociation (e.g., trypsin, Gibco™ TrypLE™ Express Enzyme, papain, dispase II, collagenase). Primary cells represent the initial clonal tumor's heterogeneity. However, heterogeneity itself is a disadvantage, because studying a mixture of cell phenotypes makes it more difficult to draw incontrovertible conclusions regarding the cells. To obtain a homogenous population of tumor-derived cells, it is possible to select specific cell types in the dissociated cell population through various methods: a specific antibody coupled to magnetic beads, density centrifugation, or selective culture medium. Currently in biomedical research, most experiments are carried out in cancer cell lines as they are easier to obtain, manipulate, and maintain *in vitro*.

One question to consider when using cancer cell lines is how accurately the cell line captures or mimics the cancer *in vivo*. Obviously, metastatic potential cannot be examined at all, nor angiogenesis per se, but surrogate assays for both aspects of cancer growth can be used, such as testing for clonal growth in soft agar, production of known angiogenic factors, or formation of endothelial cell tubes.

Another feature cancer cell lines should preserve is the genomic profile of the original tumor type. How well cell lines retain the genomic profiles of parent tumors has been examined. The Cancer Cell Line Encyclopedia (CCLE) is one of several genomic datasets [20]. The CCLE represents roughly 1,000 cell lines from 36 cancer types. A strong, but not perfect, correlation was noted regarding genomic data between tumor and the derived cell line [21]. It is interesting to note that DNA methylation profiles correlated strongly between tumors and cell lines, and in at least some cases, differences in DNA methylation might be attributed to the process of cell culture [21].

Despite the differences, there are many similarities between original tumors and the cell lines derived from them. This is the reason why cancer cell lines are considered representative of specific tumors and therefore valid experimental models. Indeed, tumors and cell lines share certain genotypic and phenotypic characteristics. This is demonstrated by the preservation of tumor histopathology in immunodeficient mice subcutaneously transplanted with cancer cell lines. There are several advantages to the use of cancer cell lines:

- Cell lines can be modified genetically using siRNA or shRNA expression vectors or other gene-modifying techniques, making them easy to maintain; they provide a renewable source of cells for reproducibility of experiments
- They share a genotypic and phenotypic similarity with the original tumor



- Cancer cell lines are a fairly homogenous population of tumor cells. However, this can be seen as a disadvantage, as the cell lines are no longer representative of the primary tumor's heterogeneity. Using a large enough panel of cell lines will help recreate the clinical picture more accurately [22]
- Cancer cell lines for most tumor types are commercially available (see cancer cell line collections, Table 3)

There are also some disadvantages to their use:

- Long-term culture can lead to genetic alterations that may affect the genotypes and phenotypes of cancer cells; this can be solved in part by limiting the number of passages and replenishing the culture from early-passage frozen stocks for use in experiments
- It is important to work with validated cell lines, since it has been shown that some cancer cell lines have been contaminated with HeLa cells, are not from the purported organ source, or are contaminated with mycoplasma

Commonly used lung, breast, liver, and prostate cancer cell lines

It is important for the investigator to be familiar with the phenotypes of cancer cell lines available from various commercial and noncommercial sources. For example, among prostate cancer cell lines there are those that are sensitive to the effects of androgens, and those that do not respond to androgens. Furthermore, it is important to recheck the desired phenotypes over time. Freezing quantities of early-passage cells of any line is highly recommended.

Investigators may find cell line databases of pharmacogenomics useful when planning experiments. These databases link cancer cell line genomics with pharmacologic data [21]. Besides the CCLE, other databases include the Cancer Therapeutics Response Portal (CTRP), the Genomics of Drug Sensitivity in Cancer (GDSC), and the National Cancer Institute collection of 60 cell lines (NCI-60). Some of the tumor types represented in these databases are listed in Table 1.

Table 1. Representative cancer cell lines among pharmacogenomic databases.*

Tumor type	Number of cell lines			
	CCLE	GDSC	CTRP	NCI-60
Bladder	28	18	5	0
Breast	60	43	1	5
Colon	63	35	37	7
Hematopoietic and lymphoid	181	113	24	6
Liver	36	14	4	0
Lung	187	141	91	9
Ovary	52	20	26	7
Prostate	8	5	1	2
Skin	62	45	9	10

* Adapted from Goodspeed et al. 2016 [21]. CCLE, Cancer Cell Line Encyclopedia; GDSC, Genomics of Drug Sensitivity in Cancer; CTRP, Cancer Therapeutics Response Portal; NCI-60, a set of 60 cell lines collected by the National Cancer Institute.

Some of the commonly used cell lines for each cancer type are discussed in the following sections. Unless otherwise indicated, the cell lines grow in complete medium with serum and no other supplements. The investigator should refer to specific protocols or reference articles to decide on the best culture medium to use for each cell line.

Lung cancer cell lines

NSCLC cell lines

Several human NSCLC cancer cell lines are available for research and drug testing, all of which are grown as adherent layers. Their characteristic doubling times, and EGFR and KRAS mutations, are listed in Table 2. Of these, the A549 adenocarcinoma cell line is most often used (Figure 12). Besides adenocarcinoma histology, these NSCLC cell lines also have squamous cell and large cell carcinoma histologies.

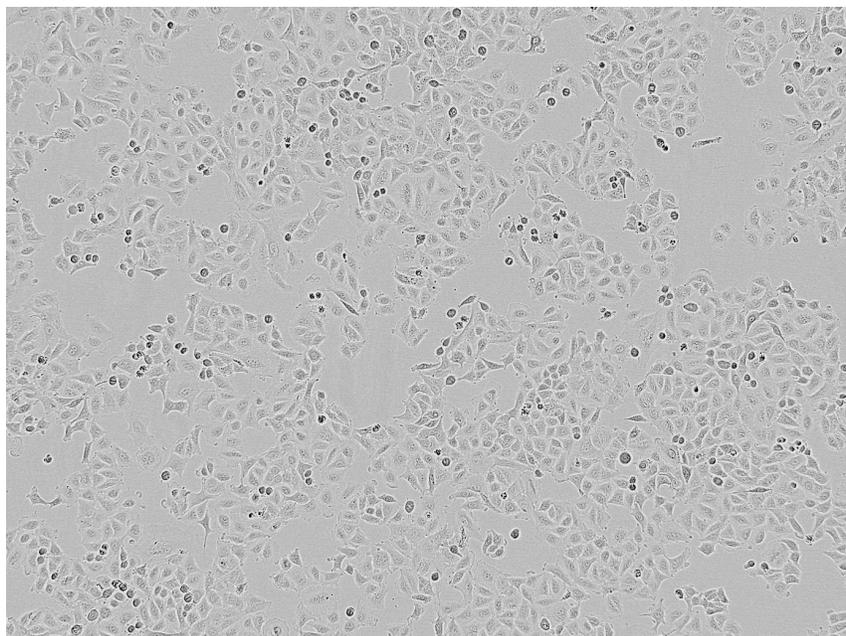


Figure 12. Brightfield image of A549 cells in culture. Cells were grown in DMEM supplemented with FBS, GlutaMAX™ Supplement, and sodium pyruvate (all Gibco™ cell culture reagents).

Table 2. Commonly used NSCLC cell lines.

Histology subtype	Cell line	EGFR mutation	KRAS mutation	Doubling time
Adenocarcinoma	A549	Wild type	G12S	24 hr
Adenocarcinoma	NCI-H1975	L858R and T790M	Wild type	30 hr
Adenocarcinoma	HCC4006	L747_E749del	Wild type	40 hr
Squamous cell carcinoma	NCI-H226	Wild type	Wild type	61 hr
Large cell carcinoma	NCI-H460	Wild type	Codon V61	20 hr

SCLC cell lines

The SCLC cell line most often used is NCI-H69, which grows as packed floating aggregates. They also form colonies of adherent cells spontaneously on flasks coated with poly-L-lysine. DNA analysis shows that adherent and suspension sublines of NCI-H69 are genetically identical [23]. The doubling time for the NCI-H69 cell line is about 30 hours. Another SCLC cell line, NCI-196, grows as an adherent layer of cells with a doubling time of approximately 64 hours.

The T47D cell line, another commonly used breast cancer cell line, was derived from a pleural effusion of a breast ductal carcinoma, and also bears the luminal subtype A (ER+, PR+) and exhibits low proliferative activity (low expression of Ki-67) [25]. The T47D cells grow as an adherent layer, with a doubling time of 43 hours.

Commonly used HER2-overexpressing cell lines

The SKBR3 and AU565 cell lines were derived from a breast adenocarcinoma and a pleural effusion, respectively; these cell lines are characterized by overexpression of the HER2 gene product (HER2+) and the absence of ER and PR [24]. Cell lines display epithelial morphology and grow as adherent layers with a doubling time of 70 hours for SKBR3 and 38 hours for AU565.

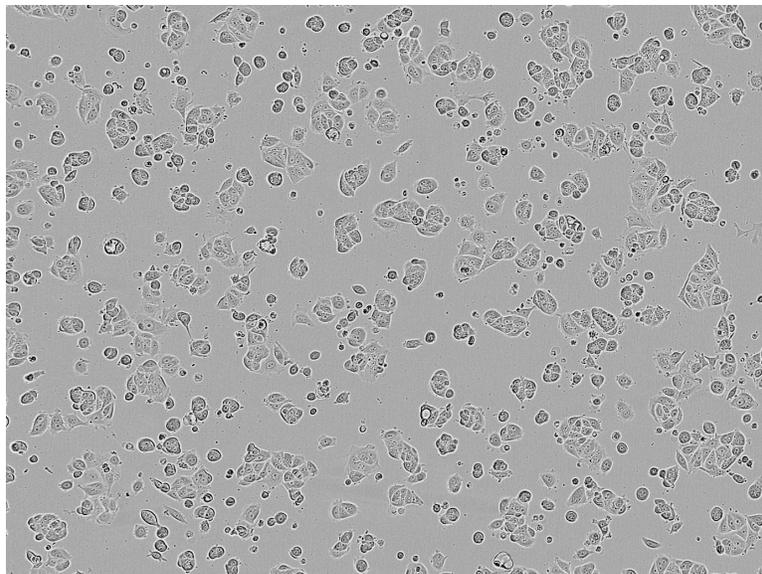


Figure 13. Brightfield image of MCF7 cells in culture. Cells were grown in DMEM supplemented with FBS, GlutaMAX Supplement, and sodium pyruvate (all Gibco cell culture reagents).

Breast cancer cell lines

Breast cancer is a heterogeneous disease with a high molecular complexity. Cell lines bearing phenotypes of the main breast cancer subtypes are available for use in research. All breast cancer cell lines described below were derived from pleural effusions.

Commonly used luminal subtype cell lines

MCF7 is perhaps the most commonly used breast cancer cell line. MCF7 cells have a luminal A subtype. They express ER and PR, are functionally hormone sensitive, may express low levels of HER2, and are somewhat positive for Ki-67 [24]. MCF7 cells grow as an adherent layer with a doubling time of 29 hours (Figure 13).

Commonly used basal subtype cell lines

The MDA-MB-231 cell line was derived from a breast adenocarcinoma. These cells have the triple-negative subtype characterized by the absence of ER, PR, and HER2 [24]. Moreover, MDA-MB-231 is a claudin-low cell line (low expression of claudins 3, 4, and 7) [25]. MDA-MB-231 cells display epithelial morphology and grow as an adherent layer with a doubling time of 35 hours.

The MDA-MB-468 cell line is derived from a breast adenocarcinoma; like MDA-MB-231, this cell line belongs to the basal triple-negative subtype of breast cancer cell lines. MDA-MB-468 cells express cytokeratins 5 and 6 [25]. These cells display epithelial morphology and grow as an adherent layer with a doubling time of 28 hours.

Liver cancer cell lines

Hepatocellular carcinoma cell lines

The Hep3B cell line was derived from a patient with HCC. It displays an epithelial morphology and grows as an adherent layer with a doubling time of 24 hours. Hep3B cells have HBx DNA from the HBV virus [26], and a deletion of the *TP53* gene, resulting in a lack of p53 protein expression.

The HuH6 hepatoblastoma line and the Huh-7 hepatoma cell line are permissive to infection by HCV; in fact, Huh-7 is the cell line most often used to study HCV infection [27]. These two cell lines display differential sensitivity to interferon- α , in that infected Huh-7 cells respond, whereas infected HuH6 cells are resistant [27]. Both cell lines grow as adherent layers with doubling times of 99 hours for HuH6 and 36 hours for Huh-7.

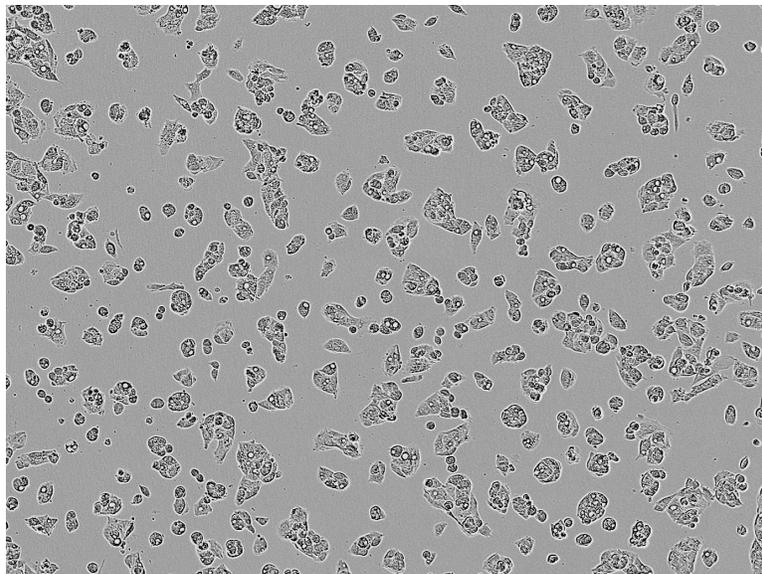


Figure 14. Brightfield image of HepG2 cells in culture. Cells were grown in DMEM supplemented with FBS and GlutaMAX Supplement (all Gibco cell culture reagents).

The HLE cell line was established from a patient with HCC; the cells have an epithelial morphology with some cytoplasmic granules and grow as an adherent layer with a doubling time of 40 hours.

Hepatoblastoma cell lines

The most widely used liver cancer cell line is the HepG2 line, which was derived from a patient with hepatoblastoma [26]. Unlike most other commonly used liver cancer cell lines, HepG2 is not tumorigenic in nude mice [26]. It does not contain HBx DNA [26]. It has an epithelial morphology, grows as an adherent monolayer, and has a doubling time of 24 hours (Figure 14).

Cholangiocarcinoma cell lines

The HuCCA-1 and RMCCA-1 cell lines were derived from cholangiocarcinomas; these cell lines have epithelial morphologies and grow as adherent layers. Doubling time for the HuCCA-1 cell line is 55 hours, and doubling time for the RMCCA-1 cell line is 48 hours [28]. The HuCCA-1 cell line secretes mucin and is of epithelial origin [29]. The RMCCA-1 cell line shows a high degree of motility *in vitro* [28], which makes it valuable for studying cell functions related to metastasis.

Prostate cancer cell lines

In prostate cancer research, the PC3, DU145, and LNCaP cell lines are most widely used, although as technology advances, patient-derived xenografts may be used as adjunct systems or instead of cell culture. Especially for studying the transition from androgen dependence to castrate resistance, patient-derived xenografts may prove more useful than a series of cell lines [30]. Still, for rapid screening of protein expression, genomics, or candidate drugs, prostate cancer cell lines will continue to be used in the foreseeable future.

The PC3 cell line was isolated from a bone metastasis. This cell line has the characteristics of a poorly differentiated adenocarcinoma and grows as an adherent layer, with

a doubling time of 33 hours. PC3 cells are androgen insensitive, expressing no androgen receptor. In addition, they do not respond to glucocorticoids, EGF, or FGF [31]. Moreover, they don't secrete PSA, although mRNA can be detected, and are PTEN deficient. In xenografts, PC3 cells have a high potential to metastasize [31].

The DU145 cell line was isolated from a brain metastasis of a patient with prostate cancer. Like PC3 cells, they don't secrete PSA and are androgen insensitive. DU145 cells have an epithelial morphology and grow as an adherent layer with a doubling time of 34 hours (Figure 15). In xenografts, DU145 cells have a moderate potential to metastasize, especially to bone [32,33].

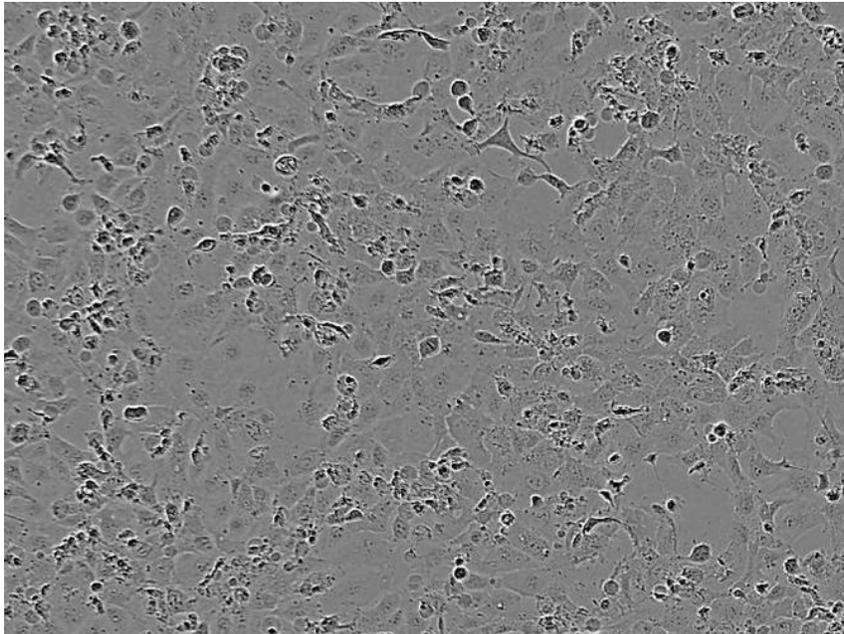


Figure 15. Brightfield image of DU145 cells in culture. Cells were grown in MEM supplemented with FBS, MEM Non-Essential Amino Acids Solution, HEPES, and sodium pyruvate (all Gibco cell culture reagents).



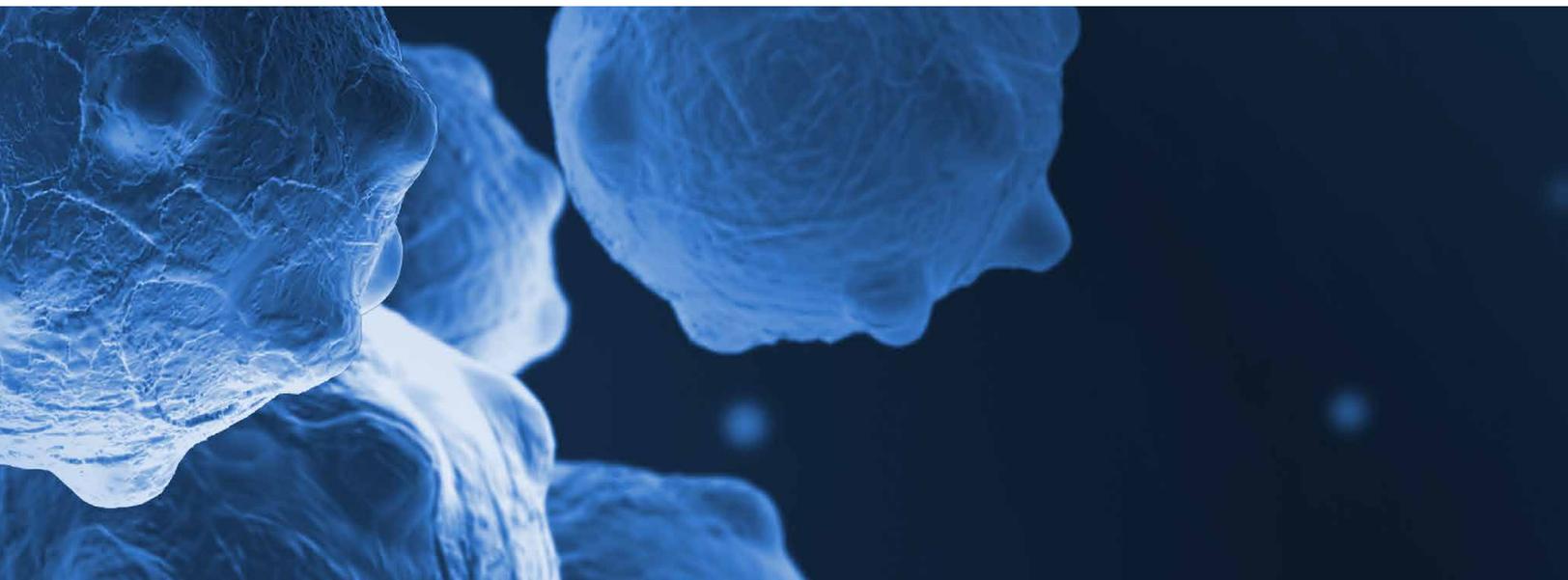
The LNCaP cell line was isolated from a lymph node metastasis of a patient with prostatic adenocarcinoma. LNCaP cells are androgen sensitive, express the androgen receptor, and secrete PSA. LNCaP cells grow slowly as aggregates and as single cells, with a doubling time of 65 hours [34]. They tend to metastasize to lymph nodes when used in xenografts. When using LNCaP cells to study androgen response, it is important to remove androgens from the serum for several days prior. Androgens can be removed by treating with charcoal, or special serum supplements can be purchased for this purpose (e.g., Gibco™ dialyzed FBS, Cat. No. A3382001).

Cancer cell line collections

Thousands of cancer cell lines are available through online cell banks (Table 3). The most reliable source is one that provides a guarantee of authentication and no contamination. It is always a best practice to isolate a newly acquired cell line and check it for mycoplasma or other contaminants before allowing it to be cultured with the remaining laboratory stock. Often, this can be done by using a dedicated incubator. The new cell line should be cultured last in the day to minimize the risk of spread in the cell culture hood.

Table 3. Cell culture collections.

Collection	URL
American Type Culture Collection (ATCC)	atcc.org
European Collection of Animal Cell Culture (ECACC)	phe-culturecollections.org.uk
German Collection of Microorganisms and Cell Cultures (DSMZ)	dsmz.de
Cell Bank Australia	cellbankaustralia.com
Coriell Cell Repository	catalog.coriell.org
Japanese Collection of Research Bioresources (JCRB)	cellbank.nibiohn.go.jp/english/
RIKEN Bioresources Center	cell.brc.riken.jp/en/



Characterization of cancer cell lines

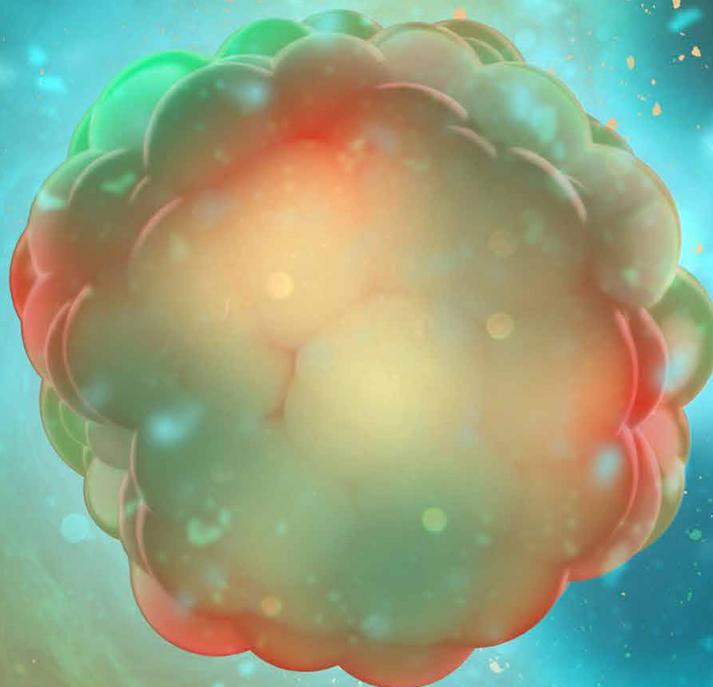
For cancer cell lines to be credible models for cancer research, they need to be validated by molecular characterization. Many researchers rely on the guarantees provided by cell repositories like ATCC or ECACC, but it is known that cross-contamination by HeLa cells or cell line misidentification happens. Recently, the lack of characterization was discussed by Allen and colleagues [35]. One conclusion the authors drew was that a glioblastoma cell line U87MG, available from the ATCC, is not the same as the original cell line [35].

In response to the issue raised by this article, several scientific journals now require evidence of cell line authentication. There are several methods used for authentication. To determine if previous investigators identified potential cross-contamination, use PubMed by cross-referencing the name of the cell line with the term “cross-contamination”. To carry out the authentications journals often require, it is important to characterize cancer cell lines as soon as possible after receiving them, documenting the details of the authentication.

Short tandem repeat (STR) profiling is the technique most often used [36]. With this method, laboratories can

authenticate their cancer cell lines at minimal cost. The technique involves the amplification of polymorphic STR loci with commercial primers. The resulting PCR product is compared to the donor tissue or validated samples. However, there are limitations to the use of STR profiling in cancer cell lines. Indeed, cancer cell lines are highly heterogeneous and contain genetic alterations. Moreover, genetic drift can occur during long-term culture. Thus, the STR profiling analysis parameters need to be redefined. The search for a close rather than a perfect match will be a necessity, and most studies suggest that a match equal to or greater than 80% is sufficient. Currently, there are some online resources available on the ATCC and DSMZ cell bank websites for comparing STR profiles. Another database, the Cell Line Integrated Molecular Authentication (CLIMA), is available [37], and there are also publications of STR profiles [36,38]. Finally, the ATCC has a testing service for cell line authentication by STR analysis.

Scientific journals will continue to ask for cell line authentication. The materials and methods section should clearly specify the cell type used and its origin, as well as the method used for authentication. One can expect more methods for authentication to become available.



Cancer spheroid culture

Researchers have cultured cells in aggregates since the 1950s, but it wasn't until 1971 when the term "spheroid" was coined in work using Chinese hamster V79 lung cells as a model for nodular carcinomas, which formed perfect spheres. Robert Sutherland's early research not only provided some of the first glimpses into the effects of nutrition and oxygenation on cell growth, but also allowed for the determination of growth fraction following treatment with drugs or radiation [39].

By the 1980s, Mina Bissell and her team at Lawrence Berkeley National Laboratory pioneered the use of three-dimensional (3D) techniques for more accurate *in vivo* cell models [40]. This shift from traditional 2D culture systems was first published in a paper highlighting the importance of the extracellular matrix (ECM) along with the crucial role of the microenvironment. This was critical for driving the uptake of spheroid culture as a widespread and biologically relevant system with obvious advantages over the widely used monolayer culture methods.

3D or spheroid cancer cell culture is believed to be more representative of *in vivo* tumors than monolayer cell culture. Spheroid culture represents the complexity of solid tumors better than flat cultures can. Three-dimensional culture is defined by the aggregate of cells that grow in suspension or in a 3D matrix (Figure 16). Cancer cell spheroids, sometimes called multicellular tumor spheroids (MCTS), have the characteristics of avascular tumor nodules or micrometastases. This type of cell culture allows researchers to characterize tumor invasion and perform drug screening. Spheroids with a size of 200 to 500 μm display oxygen and nutrient gradients resulting in the formation of a necrotic core, which is like that found in *in vivo* avascular tumors. *In vivo*, tumors are more resistant to drug therapy than cancer cells cultured as adherent layers. This resistance is due to the complex architecture of the tumors: cell-cell contact, 3D shape of the tumors, cell-matrix contact, and the distance to a blood vessel. These features limit the diffusion of cancer treatment. Spheroid cultures exhibit these features and are thus more accurate models for drug screening.

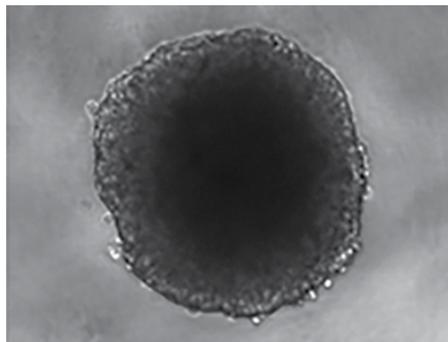


Figure 16. Single cancer spheroid grown using HCT116 human colon carcinoma cells in complete DMEM containing 3% methylcellulose in Thermo Scientific™ Nunclon™ Sphera™ plates. Image courtesy of Professor Dolznig from the Institute of Medical Genetics at the Medical University of Vienna.

Spheroid formation

There are different types of culture methods for spheroid formation:

- Suspension culture: This method promotes spheroid formation in suspension through agitation, which permits mass production if needed. However, suspension culture does not allow uniform generation of spheroids, and therefore, size control is lost.
- Hanging drop method: Drops of medium containing cells are suspended on the inner surface of a specialized plate lid. Gravity allows aggregation of cells in the bottom of the drop, enhancing spheroid formation. This technique has a low throughput, but spheroid size is generally uniform [41] (Figure 17).
- Culture on nonadherent surface: Cells in suspension are cultured on nonadherent surfaces; this promotes spheroid formation. Another name for this culture method is the liquid overlay technique. Nonadherent surfaces can be agar, agarose, poly-hydroxyethyl methacrylate, or Nunclon Sphera surface technology. This technique is also used for mass production, but does not allow for the control of spheroid size. To increase the throughput, an agarose microwell array (with agarose in each well) can be used (Figure 18).

- Microfluidic device: This method requires a specialized laboratory device. Microfluidic devices allow precise regulation of spheroid size and continuous production [42].

Applications of spheroid cell cultures

Spheroid cultures are used by researchers for various applications, some of which are described below:

- Drug screening: Spheroid culture permits a better estimation of drug efficacy than 2D culture. Moreover, since the need to generate uniform spheroids with the same size is a prerequisite for drug testing, it is critical to have highly reproducible spheroid cultures. Small spheroid cultures (with a maximum diameter of 200 μm) are widely used by researchers to carry out drug screening.
- Tumor cell biology: The gene expression of spheroid cell cultures reflect *in vivo* tumors more closely than 2D cell cultures, as previously mentioned. Thus, 3D cell culture is an excellent model to study tumor cell biology by analyzing protein and gene expression, proliferation, cell death, and differentiation in 3D space.

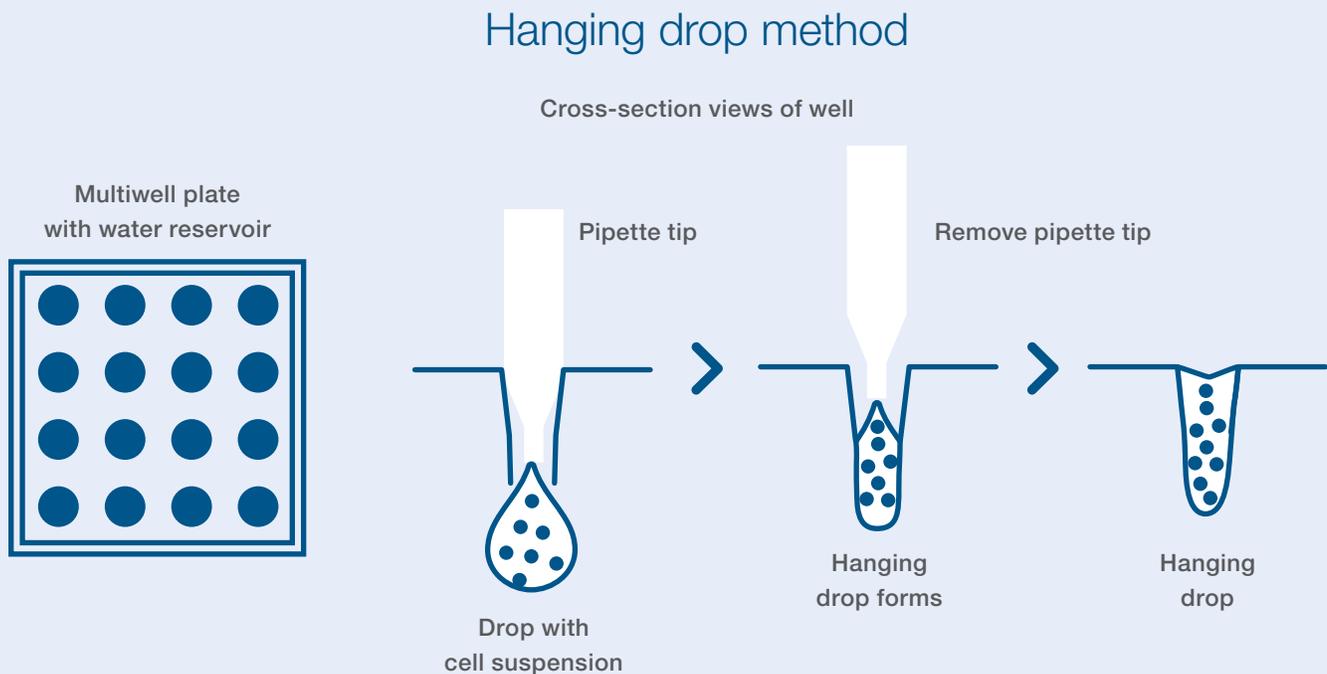


Figure 17. Hanging drop method. Cells in medium are suspended from the inner surface of a culture plate lid. Cells aggregate due to gravity, which enhances spheroid formation.

- Invasion potential: Spheroids are used to test the invasive potential and migration of tumor cells. The invasive potential of spheroids is measured by placing them on coated surfaces or embedding them in gels and tracking their movement using various techniques.
- Immune cell responses: Multicellular tumor spheroids can be cocultured with immune cells such as natural killer, monocyte, macrophage, or T cells to assess the type and magnitude of the immune response to the tumor. Responses can be monitored and quantified by analyzing infiltration and migration of immune cells into spheroids. Moreover, spheroids can be used to develop therapies to stimulate immune cell infiltration and antitumor cytotoxic responses.
- Cancer stem cells: In recent years, studies have elucidated the presence of cancer stem cells (CSCs) involved in tumorigenesis. CSCs are a known subpopulation of cancer cells with unlimited self-renewal and the ability to promote tumor growth, and are involved in resistance to therapy. The isolation of CSCs can be done after enzymatic dissociation, or they can be obtained by using cancer cell lines. Some detailed protocols using different methods adapted for various tumor types can be found in Palmini et al. 2016 [43]. Stem cell media do not contain serum and are supplemented instead with growth factors (e.g., EGF, FGF). The exact growth factors added depend on the type of cancer cells being grown. In addition, other factors known to be involved in stem cell proliferation can be added to the medium, such as hydrocortisone, progesterone, or insulin.

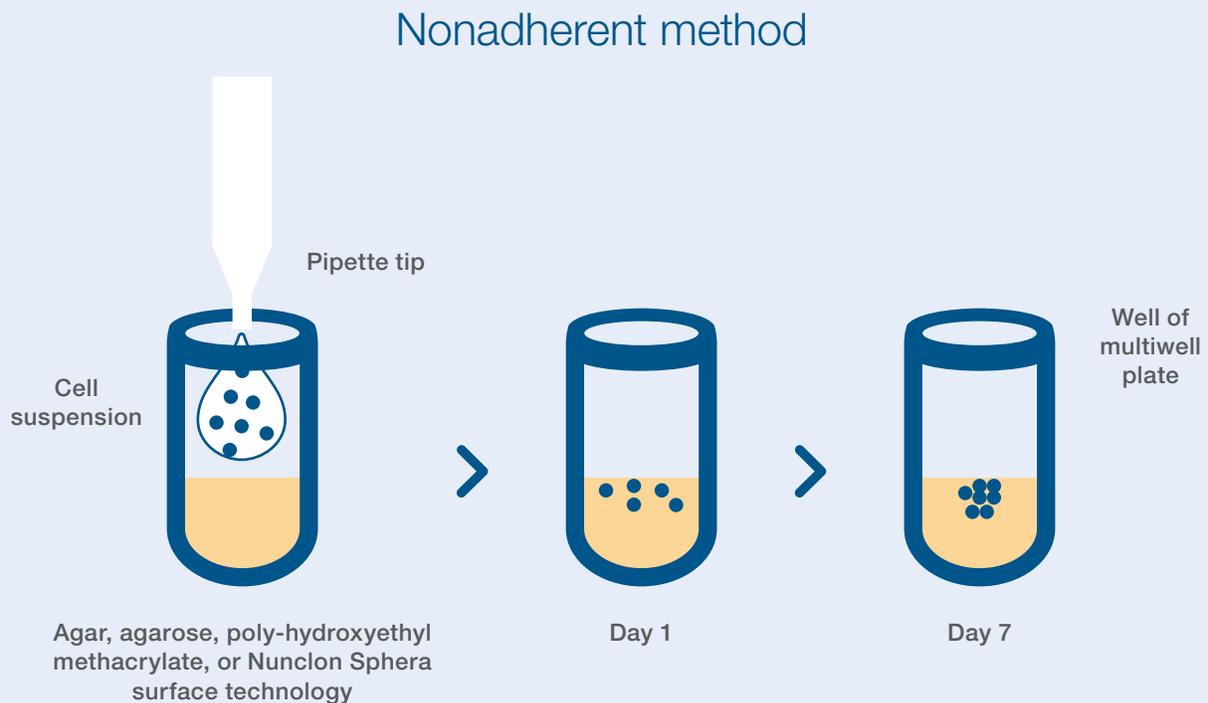


Figure 18. Nonadherent method to create spheroid cultures. Spheroid formation is promoted by culturing cells suspended in medium on nonadherent surfaces.

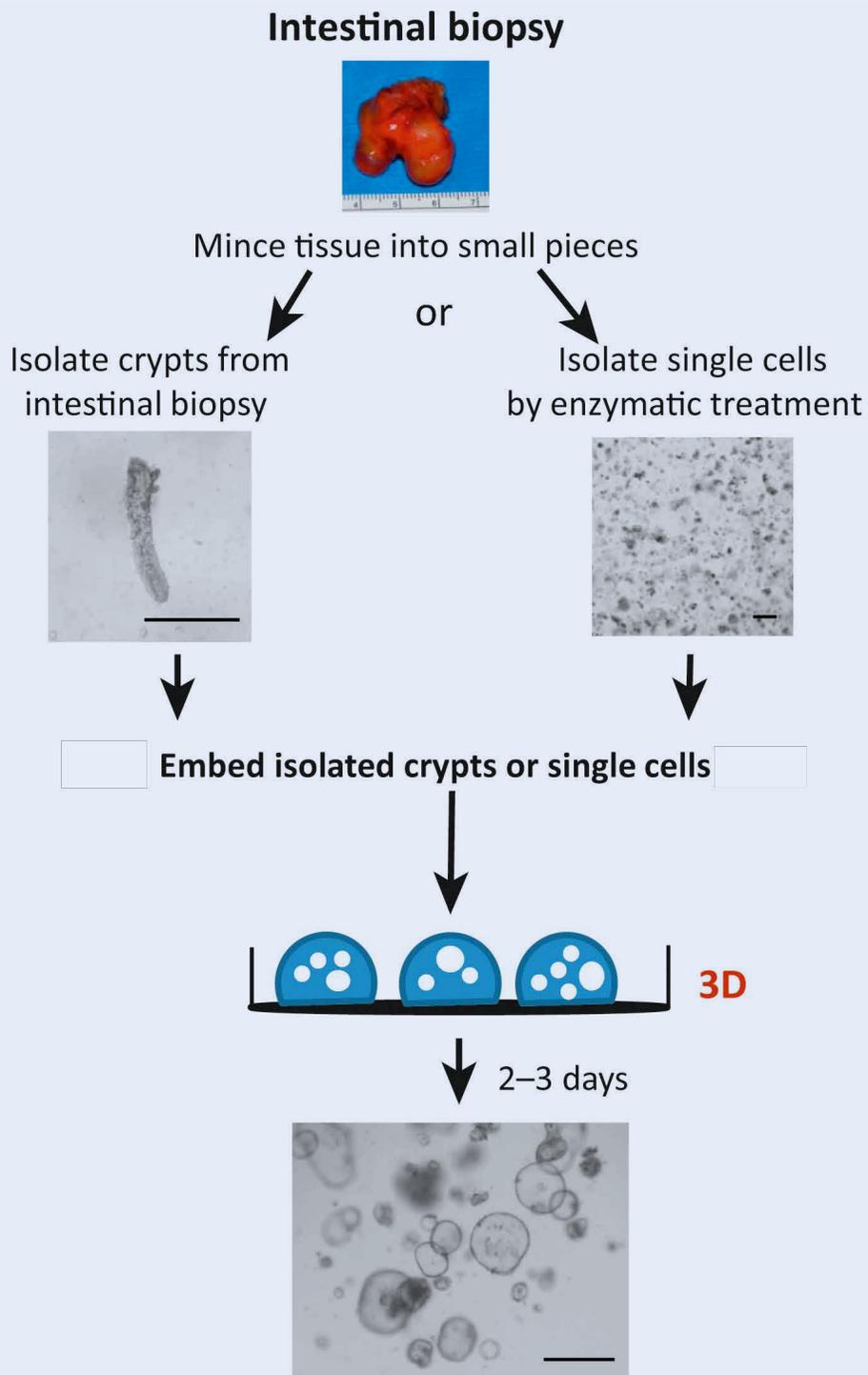


Figure 19. Derivation of organoid culture from organ specimens, in this case, from intestinal biopsies. Dissociation of the specimens and subsequent culturing on an appropriate matrix, with complete medium and growth factors, will cause the dissociated cells to form organoids within days. Adapted from Dutta et al. 2017 [44].

Cancer organoid culture

Organoid cultures are a way of creating organs in a dish. Instead of a layer of homogenous cells, as in 2D cell culture, organoid culture creates organized tissue that mimics organs in a body. In some cultures, organoids are derived from embryonic stem cells, induced pluripotent stem cells, or adult stem cells [44] (Figure 19).

Organoid cancer cultures are a further refinement of spheroid cultures. They are 3D, not 2D adherent layers, and have features that more closely resemble the clinical picture of tumors. Scaffolds (extracellular matrices) are used, and the cancer cells for seeding the culture can be obtained from resected patient tumors. It is possible to create organoids from tumor types not amenable to culture by traditional 2D methods [45].

Several types of acellular matrices are available for organoid cultures. These include collagen, polysaccharide scaffolds, basement membrane extract, polyethylene glycol (PEG)–fibrin hydrogel, and synthetic hydrogels [46]. It may be necessary to try different materials to find the best one. Moreover, in at least one study, the matrix had a significant effect on the morphology and composition of the resulting organoid [47].

Although organoid cultures have been described for >40 years, their usefulness had been limited by available technology and knowledge regarding organogenesis, as well as the requirement for large numbers of cells to seed the cultures [48]. With the development of more precise media formulations, increased knowledge of growth factor requirements, and growing awareness of the key role of stem cells, organoid cultures have been developed to reproduce specific features of the desired organ (e.g., crypts of intestines) [49].

Table 4. Comparison of preclinical cancer models using cell culture methods.*

Desired feature	2D cell culture	Organoid culture	Xenograft
Ease of establishment	+	+++	++
Ease of maintenance	+++	+	+/-
Expansion	+++	+++	+
Biologic stability	+	++	++
3D growth	-	++	+++
Tumor–stroma interaction	-	-	+++
High-throughput screening	+++	++	-
Representative of cancer	+	++	++
Immune cell infiltration	-	-	+++

* Adapted from Sachs and Clevers 2014 [53].

Organoid cultures from adult somatic tissue can also be used to create organoids. Liver and pancreas organoid cultures derived from adult human and mouse organs could be expanded into self-renewing organoids using dissociated cells, defined medium, and an extracellular membrane for support. Liver organoid cultures could differentiate *in vitro* into hepatocytes, while passage *in vivo* could induce liver and pancreas organoid cultures to differentiate into ductal cells [50]. Furthermore, genome editing methods such as CRISPR-Cas9 make it possible to alter genomic expression of organoids for specific experimental objectives [51].

There are limitations to organoid cultures as model systems. They lack interactions with microenvironments, and cannot interact with or be infiltrated by cells of the immune system. Similarly, they cannot be used to study inflammatory pathways or effects of inflammation. Some growth factor signaling pathways cannot be mimicked *in vitro*, nor can biomechanical forces be studied [52].

Organoid cancer cultures help fill the gap between 2D culture and physiologically relevant xenografts (Table 4). It should be noted that cancer stage may play a role in the success of establishing organoid cultures; specimens from advanced-stage cancer may be more difficult for establishment of organoid cultures than those from earlier-stage cancers [53]. Organoid cancer cultures have been developed for several types of human cancer. The most important cancer types are discussed below.

Lung cancer organoid culture

Because lung cancer is so prevalent and has such high mortality worldwide, studying it in an organoid model might be useful for the development of new drug treatments and for identifying biomarkers. The interactions of stromal and cancer cells in lung cancer can be studied more accurately in organoid than in 2D cultures. Such interactions may affect drug distribution and metabolism, and ultimately, the final therapeutic effect of any new agent. In particular, lung cancer organoid cultures were used to study oncolytic virus as a possible therapeutic agent. Using a PEG-fibrin hydrogel matrix, investigators observed that the effects of the oncolytic viruses on organoid lung cancer cultures closely resembled what was observed *in vivo* [54].

Breast cancer organoid culture

Growing mammary tumor cells with ductal architecture has been a challenge, one that was not met by using human primary mammary cells alone. For more accurate organoid cultures, researchers used explants from patients with breast cancer. The explants were seeded onto hydrogel scaffolds, containing collagen, laminin, and fibronectin, as well as hyaluronan. Explants thus seeded, self-organized, expanded, and differentiated to form mature breast tissue [55].

Breast cancer organoids were generated from frozen tumor specimens. Investigators found little difference in organoid cultures between those derived from fresh explants and those derived from frozen ones. Explants could generate organoid cultures whether they had been flash-frozen or frozen more slowly in DMSO; however, explants frozen using DMSO had more consistent responses to drugs (paclitaxel or trastuzumab) than did flash-frozen explants [56].

Colorectal cancer organoid culture

Patient-derived explants from colorectal specimens have been used to form organoid cultures [57]. For this cancer type, one group of investigators passed patient explants through mice as xenografts prior to creating the organoid cultures. Results showed that passing the explants created organoid cultures that grew well and had the expected drug sensitivity features of colorectal cancer cells to adriamycin, paclitaxel, mitomycin C, and [s]-10-hydroxycamptothecin [58].

Another group of researchers established parallel organoid cultures from patients with colorectal cancer, using both malignant tissue as well as adjacent benign tissue. By creating these malignant and benign organoid cultures in parallel, the investigators had accurate control tissue to study the effects of novel therapeutic agents, to sequence DNA, and to study RNA expression [57].

CRISPR-Cas9 genome editing was used in another series of experiments to introduce four of the most frequent mutations found in human colorectal cancer into human small intestine organoid cultures [59]. By introducing mutations in *APC*, *TP53*, *KRAS*, and *SMAD4*, investigators examined when growth independence arose, as well as when other changes consistent with the transformed phenotype occurred. The investigators noted extensive aneuploidy when *APC* and p53 functions were lost due to mutations [59]. This series of experiments demonstrates the power of organoid cultures in studying the development of cancer from normal cells.

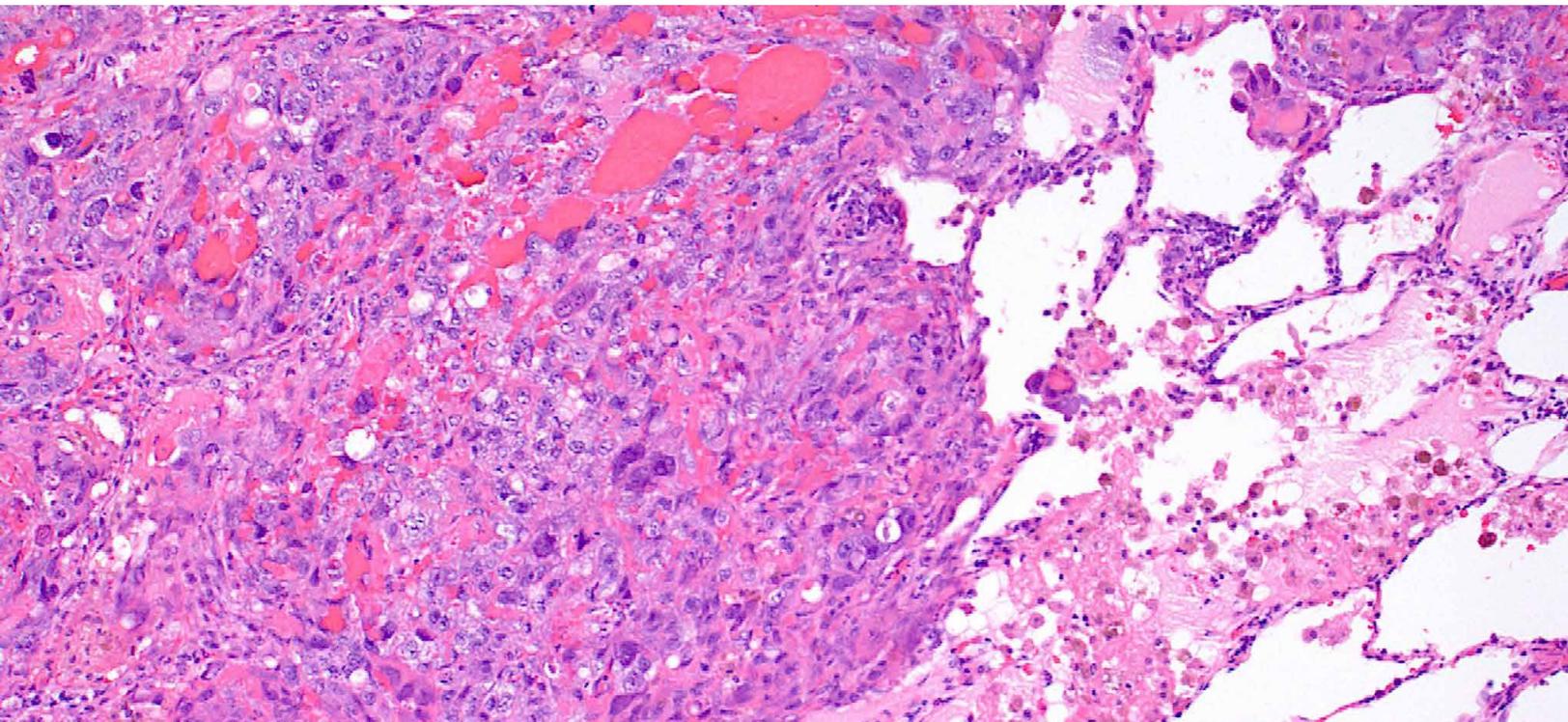
Prostate cancer organoid culture

As prostate cancer is the most common noncutaneous cancer among men, finding suitable model systems to advance treatment for it has been the objective across many laboratories. Organoid cultures represent an advancement relative to 2D cultures, which have limited similarity to prostate cancer cells *in situ*. To this end, researchers created prostate cancer organoid cultures from primary tumors and from metastatic tissues. The organoids retained key genetic signatures, such as *PTEN* loss, *TMPRSS2-ERG* interstitial deletion, *SPOP* mutations, and alterations in *TP53*, *PIK3R1*, and *FOXA1*. In addition, the organoid lines retained characteristics of their specific patient-derived origin. For example, a bone metastasis-derived organoid culture stained negative for androgen receptor, while an organoid culture derived from a primary tumor showed an intraductal growth pattern. Overall, prostate cancer organoid cultures recapitulated the histology found *in situ* [60].

Other organoid cancer cultures

Renal cell carcinoma (RCC) is particularly challenging to study *in vitro*, due in part to the complex architecture of the kidney [47]. Using a scaffold created from decellularized kidney, Batchelder and colleagues created a 3D extracellular matrix (ECM) for growing RCC organoid cultures from tumor explants [47]. They noted that signature gene expression was maintained by RCC organoid cultures for up to 21 days, and that the ECM they prepared was superior to a commercially available polysaccharide scaffold for the formation of RCC organoids [47]. These experiments highlight the importance of checking all aspects of the culture conditions when creating organoid cultures.

It is possible to create organoids from brain tissue. For studying glioblastoma, Hubert and colleagues created glioblastoma organoid cultures from primary tumors as well as metastases, which are not otherwise amenable to culture *in vitro* [45]. The glioblastoma organoid cultures grew for months, displayed rapidly dividing outer regions, and a hypoxic core containing more senescent cells and quiescent cancer stem cells [45]. Orthotopic xenotransplantation of glioblastoma organoids retained tumor invasiveness and the expected histology [45]. The hypoxic gradients within the organoids induced differential gene expression and differential radioresistance [45]. Thus, organoid cultures showed themselves to be valuable for studying cancer arising from brain, and in the future, for more tissue types as well.



Appendix

Most commonly used cell lines in recent publications

Lung:

A549
GCT
NCI-H1299
NCI-H460
U937
EML4-ALK Fusion-A549 Isogenic Cell Line Human (ATCC)
MOR
Calu-3
NCI-H23
V79-HG04
NCI-H292
NCI-H358
NCI-H1975
T84
NCI-H441
HCC827
NCI-H69
NCI-H1650

Breast:

MCF7
MDA-MB-231
JC
T-47D
MCF10A
SK-BR-3
MDA-MB-468
BT-474

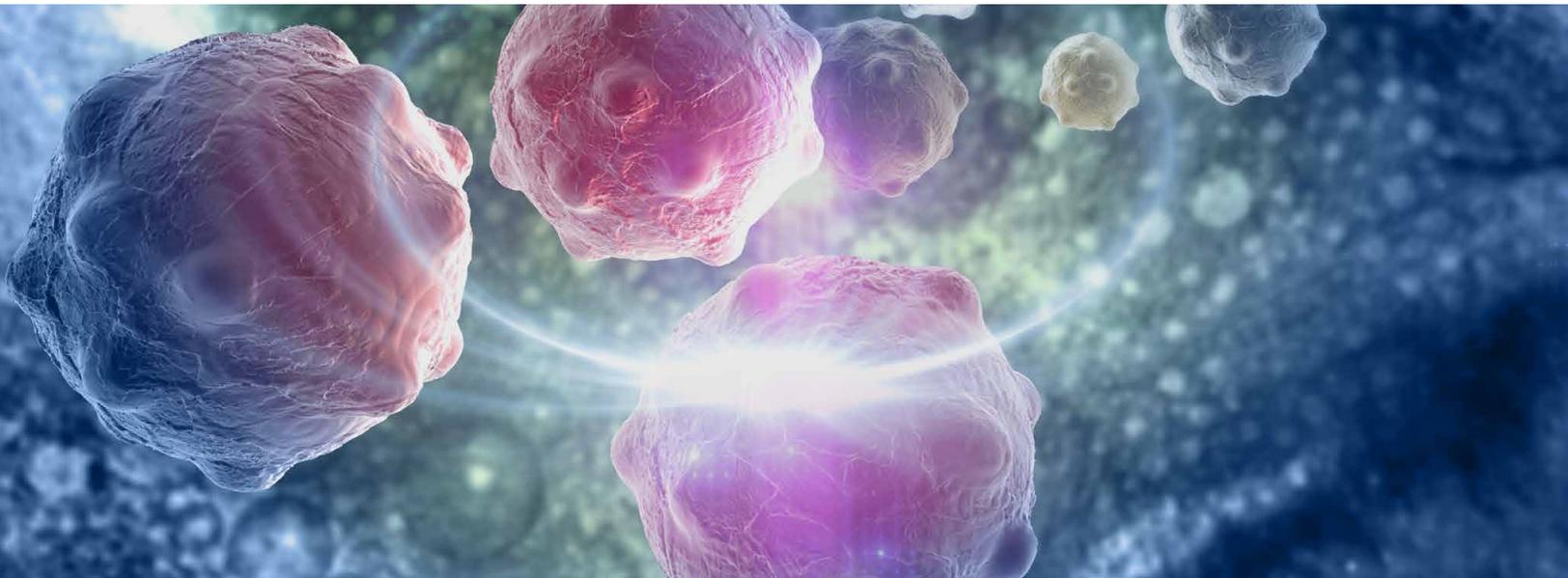
Liver:

HepG2
Hep 3B2.1-7
c4 (B13NBii1)
Fao
HepG2/C3A
H5

Prostate:

PC-3
LNCaP
DU145

View protocols for these cell lines at
[thermofisher.com/cancer/protocols](https://www.thermofisher.com/cancer/protocols)



Troubleshooting

The following table lists some potential problems and possible solutions that may help you troubleshoot your cell culture experiments. Note that this table includes only the most commonly encountered problems in cell culture, and only provides guidelines for solutions. To help evaluate your results more successfully, we recommend that you consult the manuals and product information sheets provided with the products you are using as well as the published literature and books on the subject.

For these and more tips, visit thermofisher.com/cellculturebasics

Problem	Reason	Solution
No viable cells after thawing stock	Cells were stored incorrectly	Obtain new stock and store in liquid nitrogen. Keep the cells in liquid nitrogen until thawing.
	Homemade freezer stock is not viable	Freeze cells at a density recommended by the supplier.
		Use low-passage cells to make your own freezer stocks.
		Follow procedures for freezing cells exactly as recommended by the supplier. Note that the freezing procedure recommended in this handbook is a general procedure provided as a guideline only.
		Obtain new stock.
	Cells were thawed incorrectly	Follow procedures for thawing cells exactly as recommended by the supplier. Note that the thawing procedure recommended in this handbook is a general procedure provided as a guideline only.
		Make sure that you thaw the frozen cells quickly, but dilute them slowly using prewarmed growth medium before plating.
	Thawing medium is not correct	Use the medium recommended by the supplier. Make sure the medium is prewarmed.
Cells are too dilute	Plate thawed cells at high density as recommended by the supplier to optimize recovery.	
Cells not handled gently	Freezing and thawing procedures are stressful to most cells. Do not vortex, bang the flasks to dislodge the cells (except when culturing insect cells), or centrifuge the cells at high speeds.	
Glycerol used in the freezing medium was stored in light (if applicable)	If stored in light, glycerol gets converted to acrolein, which is toxic to cells. Obtain new stock.	
Cells grow slowly	Growth medium is not correct	Use prewarmed growth medium as recommended by the supplier.
	Serum in the growth medium is of poor quality	Use serum from a different lot.
	Cells have been passaged too many times	Use healthy, low passage-number cells.
	Cells were allowed to grow beyond confluency	Passage mammalian cells when they are in the log phase before they reach confluence.
	Culture is contaminated with mycoplasma	Discard cells, media, and reagents. Obtain new stock of cells, and use them with fresh media and reagents.

Cell culture products

We offer a variety of reagents, media, sera, and growth factors for your cell culture experiments. For more information on our products, please refer to these helpful links:

Culturing cells:

[thermofisher.com/cellculture](https://www.thermofisher.com/cellculture)

[thermofisher.com/media](https://www.thermofisher.com/media)

[thermofisher.com/3dculture](https://www.thermofisher.com/3dculture)

[thermofisher.com/fbs](https://www.thermofisher.com/fbs)

[thermofisher.com/cellcultureplastics](https://www.thermofisher.com/cellcultureplastics)

[thermofisher.com/growthfactors](https://www.thermofisher.com/growthfactors)

[thermofisher.com/transfection](https://www.thermofisher.com/transfection)

[thermofisher.com/heracell](https://www.thermofisher.com/heracell)

Imaging cells:

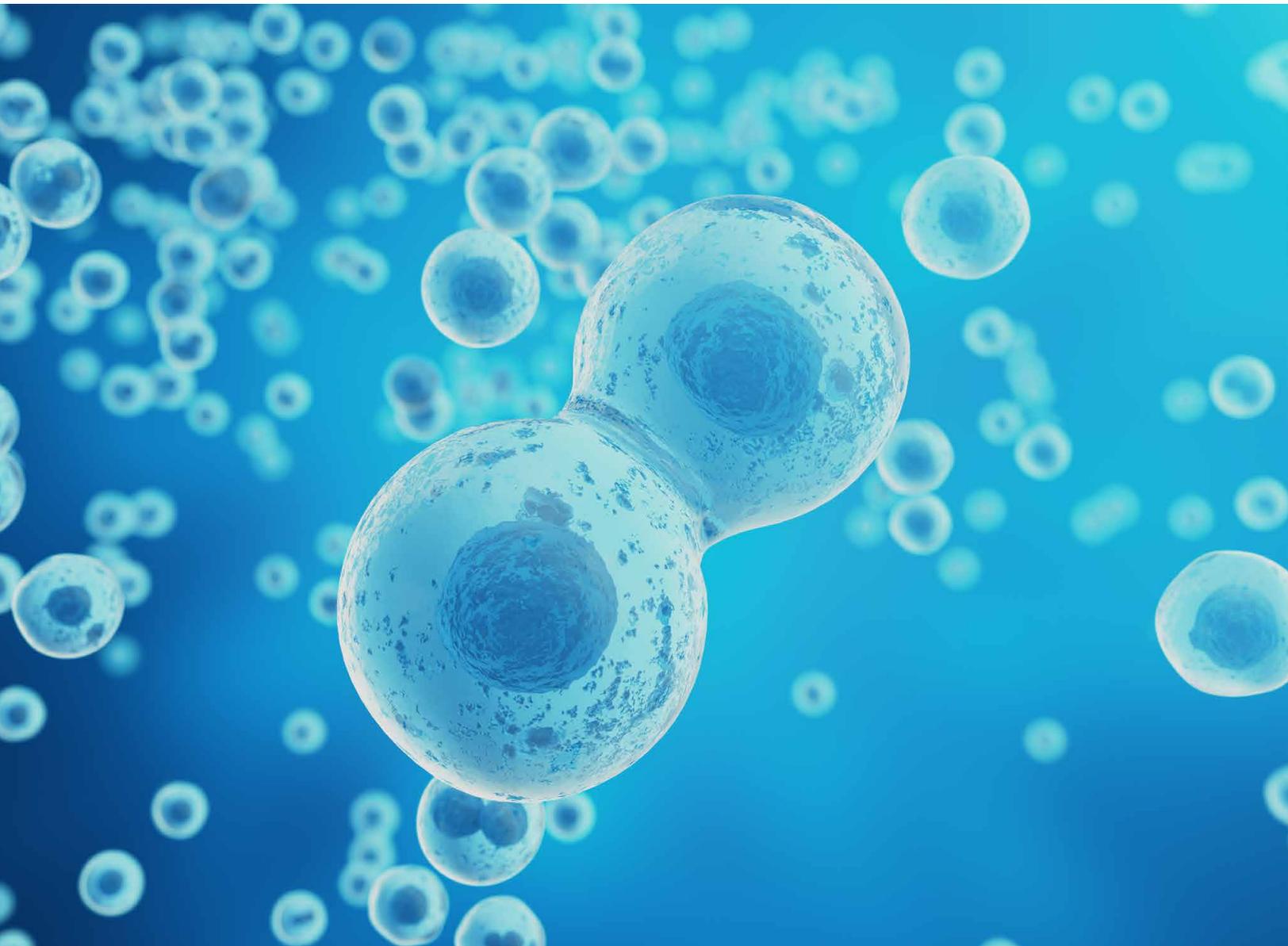
[thermofisher.com/countess](https://www.thermofisher.com/countess)

[thermofisher.com/evos](https://www.thermofisher.com/evos)

[thermofisher.com/antibodies](https://www.thermofisher.com/antibodies)

Genome editing:

[thermofisher.com/genomeediting](https://www.thermofisher.com/genomeediting)



Additional resources

Gibco cancer basics

Introduction to cancer cell culture, covering topics such as the biology of cancer, cell line culture, and the culture of cancer spheroids and cancer organoids.

For more information, go to

thermofisher.com/cancer/cellculturebasics

Gibco virtual training labs

Free, interactive laboratories where you can get complete Gibco™ cell culture training, get tips for optimizing *in vitro* research, see best practices for working with your cells, take quizzes to test your understanding, and receive a badge to verify your knowledge.

For more information, go to

thermofisher.com/cellculturebasics

Gibco cell culture heroes

Meet the researchers driving tomorrow's breakthroughs in the fight against cancer. Heroes don't seek recognition, but they deserve it. Boldly determined and deeply committed, our Cell Culture Heroes work tirelessly to lay the foundation for discoveries that may lead to cures. We want to show the world what they're doing.

See heroic work at thermofisher.com/cellcultureheroes

Safety Data Sheets

Safety Data Sheets (SDS) are available at

thermofisher.com/sds

Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to thermofisher.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Technical support

For more information or technical assistance, please contact us at thermofisher.com/support

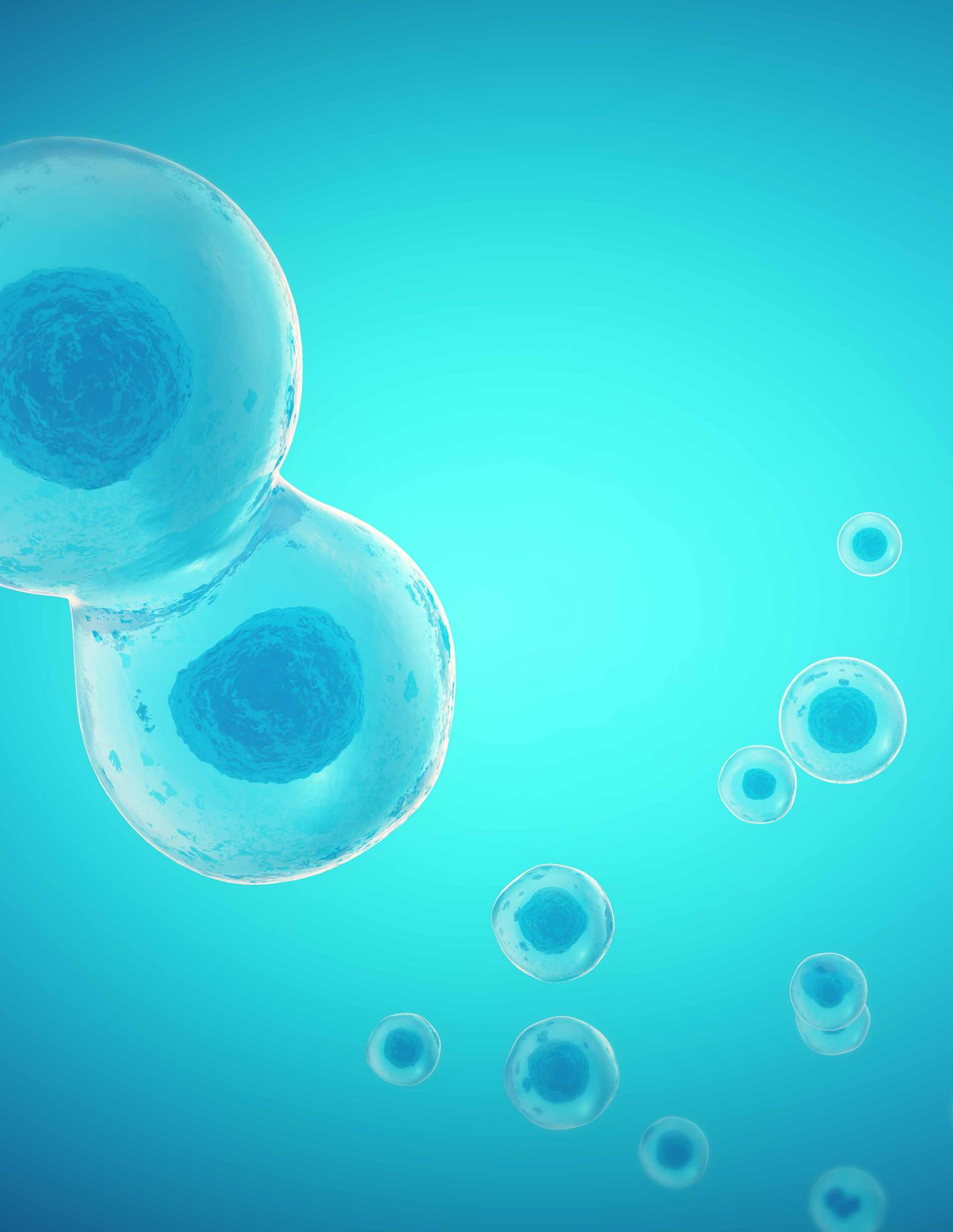
Limited product warranty

Thermo Fisher Scientific Corporation and/or its affiliate(s) warrant their products as set forth in the General Terms and Conditions of Sale found at thermofisher.com/termsandconditions

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Audrey Mouche, from the University of Rennes, Belgium, graduated with a bachelor's degree in molecular and cellular biology. She then received a master's degree in immunology from the University of Strasbourg, France. During her PhD studies at the University of Rennes (OSS Laboratory), Audrey investigated the role of the inhibitor of growth (*ING*) tumor suppressor gene in DNA damage response, under the supervision of Dr. Rémy Pedeux. Audrey is currently studying for her medical degree at the Medical University of Rennes 1, with the intention of doing clinical research in oncology.



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