## **How Is Cancer Studied?**

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# Why do we need cancer models? What is a cancer model system?

Cancer is a major health concern and a leading cause of death around the world. Fortunately, due to intensive research, long-term survival has improved dramatically for most cancer types. 1, 2 Cancer research and treatment development depends heavily on the use of laboratory systems designed to mimic at least some aspects of human cancer. These models can be used to study how cancer develops, grows and spreads, examine the body's response to cancer and test drugs to predict how cancer patients may react to them. 2 Cancer is a very complex disease and it is difficult to develop model systems that can be trusted to give accurate and reproducible results. 2 It is also challenging to create models for specific tumor and cancer types. Different cancer models have different costs, are more or less reproducible and vary in their ability to predict what is seen in humans patients. Common cancer models (described below) include cell lines, animals, organoids, patient-derived xenografts (PDX) and computer-based models.

## Cell Lines

A cell line is a group of cells that are able to reproduce indefinitely – they are immortal. Normal human cells can reproduce only about 50 times before stopping. 4 Cell lines can (as far as we know) reproduce forever. There are two common types of human cell lines: immortalized cell lines and cancer cell lines. 5 Immortalized cell lines are created in a lab. They have genes altered to allow them to reproduce indefinitely. Cancer cell lines, on the other hand, are cells taken from a tumor and grown in a laboratory. Both of these cell lines allow researchers to study cancer biology and test cancer treatments. 5 Cell lines can also be used to identify the types and numbers of genetic changes found in a tumor. 6 Much of our knowledge about cancer biology comes from the study of cell lines created from tumor samples removed from patients or animals. 6

### **Human Cell Lines**

Human cell lines are a valuable cancer model because they are consistent, convenient, easy to use and publicly availability. Human cell lines can be used to predict responses to anticancer drugs and help researchers understand drug mechanisms in preclinical studies. Importantly, once cells are removed from the body, they are in a different environment and may act differently. It is important to verify all results obtained with cell lines in other model systems before using any treatments in patients. Recent research indicates that the growth of cancer may be dependent on a small subset of cells called 'cancer stem cells'. Researchers believe that using patient-specific stem cells will improve the accuracy of cell lines in predicting treatment responses.

### Learn more about cancer stem cells.

### **Animal Cell Lines**

Researchers also use animal cancer cell lines to better understand the difference between cancer cells and normal cells. Animal cancer cells are developed from a range of animal species including mouse, rat, canine, and others .7 These models are an important tool to help humans overcome cancer and other debilitating diseases. Like human cell lines, researchers can test the effects of cancer drugs on the cells and use the results to improve drug development.8

See some examples of animal cell lines used in cancer research.

## **Animal Models**

Cancer research is dependent on the use of animal models. The goal is to simulate disease within animals and transfer the knowledge gained to human cancer. The two primary types of cancer animal models involve the use of rodents - primarily rats and mice - and the use of domesticated companion animals (dogs/cats).

### **Rodent Models**

Mouse models are the best-studied and commonly used animal model, but rats are also used. There are many advantages to rodent models, including short generation times and easy manipulation of genes. 9, 10, 11 Disadvantages include that the findings using rodent models may not translate well to clinical trials in humans. 9, 10 One reason for this is that mice can tolerate higher drug doses than humans can. 9, 10 The models do allow for the study of genes, proteins, and biological pathways in living animals. The animals can be followed to study the course of the disease. There are three main types of rodent models that researchers use: chemically induced models, genetically engineered models and xenograft models.

## **Chemically Induced Rodent Models**

Chemically induced rodent models are developed by exposing mice and rats to carcinogens. Carcinogens are substances that cause cancer. For example, many of the chemicals in cigarette smoke are classified as carcinogens and can cause lung cancer. Chemically induced rodent models allow researchers to study the complex process of cancer development but can be time-consuming.9, 10 Some of the very insights into how cancer develops came from mice studied by Isaac Berenblum. Berenblum was able to cause mice to develop skin cancer by placing chemicals on their skin.12, 13 Because it is readily viewed by researchers, skin cancer is a good model to learn how cancer begins.14

### **Genetically Engineered Rodent Models**

Genetically engineered models (GEM) are created by injecting DNA into the zygotes (fertilized egg cells) of mice and rats.9, 10 The introduced DNA often contains cancer-causing changes (mutations) in genes frequently found to cause cancer in patients. The cancers that form as the animals age mimic those that arise in people with those same genetic mutations. One advantage of genetically engineered rodent models is that the findings from testing anticancer therapies can often be translated and applied in human cancers [9].9, 10 Inserting genes into animals can lead to unexpected results that can make the results unreliable.9, 10 GEM exist for many different kinds of cancer, including liver cancer15, colorectal cancer16, melanoma17 and glioma18.

### Rodent Xenograft Models

A xenograft is when cells of one kind of organism (i.e. human) are placed into a different organism (i.e. mouse). The term 'xeno' is derived from the Greek xénos meaning stranger or guest. Patient derived xenografts (PDX) are small tumor fragments removed from patients and transplanted into mice or other animals. 19 Xenografts evolved from animal models used to test drug development in the 1950s. 19 Although these models were the standard at the time, they often displayed traits different from the original tumor. Modern PDX more accurately reflect the cellular and tissue structure of the patient tumors and can be used to study responses to anti-cancer therapies [6] and can be used to develop personalized treatment plans. 20

The transplanted cancer cells are often inserted under the skin of the animal, but they can also be placed into the organ where the cancer formed in the patient (i.e. human lung cancer samples can be put into the lungs of an animal). The rodents used have weakened immune systems, allowing the human cells to survive in their bodies.

Like all cancer models, there are drawbacks and limitations for PDX. PDX models take 4-8 months to develop, which is longer than some patients can wait for treatment. 19 Rat and mouse xenograft models can't fully represent cancer in humans due to the weakened immune systems of the rodents. 9, 10 Also, the optimal implantation site varies with the type of tumor being studied. 19 PDX can also be very expensive. 21

### **Companion Animals**

Companion animals include dogs and cats. These models are not used nearly as much as those described above, but they are still valuable to cancer research. Companion animal models often use dogs and cats with naturally occurring cancers. The animals can be studied to test anticancer drugs that have shown promise in rodent models and to understand the biology of human cancer. 22, 23, 24 These models also have more clinical and biological similarities to humans than other animal models and respond similarly to treatments and therapies. 22, 23, 24 Additionally, the shorter lifespan and reproductive cycles of dogs and cats provide an advantage because researchers can get results quickly. 10

# **Organoid Cancer Models**

Organs are highly organized structures that perform specific tasks. Examples include the stomach, lungs and heart. Organoids are tiny, three-dimensional structures grown from cells in a lab. The idea is to create a miniature version of the organ that can be easily studied. Ideally, these tiny models have similar cellular arrangements and some functions of the organ they are created to mimic. As an example - A colon organoid can't absorb nutrients and water like the real thing, but it can form tube-like structures and produce many of the same products as a colon inside the body.

Organoids have several advantages over cell lines. Unlike cells growing on a dish, organoids are three dimensional. They are often composed of several different kinds of cells, like a real organ. This helps preserve some of the traits of the original tissue. 25, 26 Organoids are less expensive than some other models, easy to use, and can be developed within 4 weeks. 25, 26 Like cells, cancer organoids can be frozen and stored in "biobanks" for later use. Through the use of gene-editing (changing DNA to cause changes in physical traits), organoids from normal tissues can be changed into tumor-like organoids. Tumor organoids allow researchers to study specific genetic changes that lead to cancer initiation and progression. A major advantage of organoids over cell lines is that organoids contain different kinds of cells. They are more complex and more accurately represent what happens in the body.

Organoid models exist for many different kinds of cancer, including lung cancer27, pancreatic cancer28, breast cancer29 and colon cancer30. Organoids can be used to study the formation of cancer (called carcinogenesis) - the process in which normal cells are changed into cancer cells.31 By studying organoids created with mutated cells, researchers have identified specific mutations that lead to tumor formation for several types of cancers. Organoids allow researchers to study the way cancer cells interact with neighboring cells and how changes in the environment around them influences cancer cells.25, 26, 26 Even the way cancer cells interact with the immune system can be studied with organoids.32 This is something that would be extremely difficult in a two-dimensional system like a petri dish.

### Learn more about carcinogenesis.

Metastasis is the spread of cancer cells from the original site to other areas of the body. Perhaps surprisingly, cancer organoids, and the related 'spheroid' modles can be used to study metastasis. Even though the organoids outside a body, the movement of cancer cells can be tracked and specific mutations that lead to metastasis can be identified. 25, 26 Spheroid models have been used to identify 'leader' cells that appear to guide metastasis, and 'follower' cells that move in response to signals from leaders. 33

Learn more about metastasis.

# Cancer 'Omics'

Cancer develops when at least one gene becomes broken (mutated) or lost. This change throws off the balance in the cell. Most of the time there are several defects in cancer cells. To understand cancer and develop cancer drugs, most research focuses on the critical genes in any particular cancer type.

There is another approach that is actively being pursued. Instead of studying a single gene or biological pathway, it is now possible to look at large numbers of biological molecules or pathways. The term 'omics' is used informally to describe research areas that work to understand how living things work by looking at <u>collections</u> of biological molecules instead of individual molecules. As an example, to understand differences between a normal liver cell and a cancerous liver cell, researchers can now compare ALL of the proteins made in both kinds of cells to look for changes that may be important. Some proteins may be lost in a cancer cell and others may be more common. By looking at many proteins at once the 'big picture' can become clearer. This kind of work is only possible with computers and high-tech devices.

There are many different kinds of biological molecules that are now studied in this way. It is also possible to study small changes that are made to biomolecules (things like the addition of a methyl group to DNA or a phosphate group to a protein). Some of the 'omics' being used to study cancer include:

- 1. Genomics This is the study of changes in the genes of cancer cells. The changes can be very small at the single nucleotide level, or large whole chromosomes being changed, altered or lost/gained. Genomics is used to study the basic biology of cancer, but also to help develop drugs and to choose the best drugs for a given cancer patient. 34, 35, 36
- 2. Transcriptomics <u>Transcription</u> is the process by which the information in genes (DNA) is read and an RNA copy is created. The RNA can then be used to make protein, or can have a function as an RNA. Cancer cells change the kinds and amounts of RNA are made from DNA. Comparisons between normal cells and cancer cells from the same tissue can give important information about how the behavior of cancer cells is changed. Advances in this area are rapid. It is now possible to study the transcriptome from a single cell and determine which cells in a tissue sample contain RNAs of interest. 37, 38 Transcriptomics is also being used to personalize cancer treatments. 39
- 3. Proteomics This is the study of large numbers of proteins. 40 The proteins that are gained/lost/altered in cancer can be studied in large numbers. The work can help us understand the development and progression of at least some kinds of cancer. 41, 42 Proteomics is also being used to guide personalized cancer treatment decisions. 43
- 4. Lipidomics Lipids are important in cell structure and can also change the cell activity. By studying many of the lipids in a cell/tissue at the same time, a bigger picture can be formed about the changes that are caused by (or may help cause) cancer.44, 45
- 5. Interactomics Perhaps the most challenging of the omics, interactomes are an attempt to learn how the biomolecules in cells/organisms work together to get things done. Interactomes are used to find out how changes in one or a few biomolecules affect many other parts of the cell and organism. 46, 47 Because cancer cells often have many changes, the interactomes can become VERY complex. Not surprisingly, this research relies heavily on computers and machine learning to identify patterns and connections.

# Mathematical and Computer-based Models

For many decades, scientists and researchers have used mathematics and physics to better understand biology. Cancer is complex, and mathematical models are often created to identify connections between multiple parts of complex systems. 48 Different computer-based models are used to study the many aspects of cancer. For example, in the clinic, models analyzing tumor growth are used to develop personalized cancer treatment plans. Oncologists play an important role in this research. They provide the patient information and tissue samples used to make computer-based model accurate and effective. 48 There are currently two main types of computer-based models.

The first type of computer model is designed to help researchers analyze large amounts of data. In fact, understanding of cancer and other complex biological systems is frequently gained from information that has been collected in large electronic databases. 49 The information is often openly shared. For example, the Cancer Genome Atlas Program collected genetic information on over 20,000 cancer samples from 33 different kinds of cancer. In order for scientists and researchers to share data and computational models, a common software language has to be used so that both parties can communicate effectively. 48 Data analysis models are used in drug development, to study gene interactions, in image analysis, and more. 48

The second computer-based model uses mathematical models to understand the physical nature of tumors. 48 This model analyzes factors like tumor size and growth to determine how they influence overall tumor behavior. 48 These models help

define factors like the metastatic potential (chance the tumor will spread) of the cancer and other traits, They provide researchers with guidance for treatments, and prevention. 50

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