Cancer Biology

Bruce Woda, MD
James Liebmann, MD
Elizabeth Kurian, MD

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Summary and Key Points

1. Cancer is caused by mutations in the DNA of normal cells. Most cancers arise from cells that acquire somatic mutations over the course of years. Less commonly, some inherited germline mutations, present in all cells, can predispose cells to develop a cancer phenotype.
2. Some DNA mutations that contribute to the formation of cancer arise in normal genes known as proto-oncogenes. Proto-oncogenes usually play key roles in the control of normal cell growth and division.
3. Mutated proto-oncogenes that cause cancer are known as oncogenes.
4. Tumor suppressor genes also normally function to control growth. When a cell's tumor suppressor gene is inactivated or lost, there is an increased probability of the cell proliferating and developing into a cancer.
5. Division of cells with DNA mutations results in identical cancer cells (or a clone). Cancers can acquire additional mutations over time to develop multiple subclones which contribute to variability in a cancer's response to drugs and behavior (i.e. ability to metastasize).
6. Metastasis is a multi-step process requiring cell separation from neighboring cells, infiltration through the basement membrane, entrance into blood vessels or lymphatics and tumor angiogenesis.

Introduction

Cancer cells arise from normal cells. Given that all our cells have the same genotype; it is reasonable to wonder what changes are needed to make normal cells become cancer cells. The answer appears to be that most cancers arise as the result of multiple somatic mutations to DNA. This chapter will focus on DNA mutations that cause cancer, abnormal regulation of cell growth and death, and metastasis.

Carcinogenesis

Most cancers occur only after a cell has acquired many mutations to create chromosomal instability. As a cell acquires mutations, it undergoes a variety of changes. There is a continuum from a perfectly normal cell to a cancer cell. Along this continuum, cells often change their appearance and behavior, a phenomenon known as dysplasia, before they become malignant. Although dysplastic cells appear quite different from normal cells, they are NOT cancer cells, and will not invade tissue or metastasize to other organs. Dysplastic cells may never progress to become cancer at all. However, if dysplastic cells acquire additional mutations, they may become frankly malignant.

While mutations in DNA are a fundamental cause of cancer, other causes exist as well. Epigenetic changes affect both DNA and associated chromatin, without affecting the nucleotide sequence, and contribute to carcinogenesis by influencing gene expression. The classic example of an epigenetic change is methylation or demethylation of DNA to control cell division. Methylation of promoter regions of genes blocks transcription and silences the gene. If a gene promoter region is demethylated, the gene may be inappropriately transcribed.

In general, the transformation of a normal cell into a cancer cell requires disruption of a variety of genes. However, there may instances where abnormal expression or function of a single gene may be sufficient to give rise to a malignancy. The translocation between chromosomes 9 and 22 (t(9;22)(q34;q11.2)), also called the “Philadelphia chromosome”, results in a bcr-abl fusion gene which is diagnostic of and seems to be sufficient for the development of chronic myeloid leukemia (CML). However, additional mutations can and do occur in CML cells and these mutations can contribute to progression of disease to the rapidly lethal

"accelerated phase" and "blast phase" forms of the disease. The additional mutations can also cause resistance to the drug imatinib, which targets the tyrosine kinase function of the inappropriately expressed ABL. While the Philadelphia chromosome can also been seen in acute lymphocytic leukemia (ALL), the clinical course of ALL is different from CML. Ultimately; the form of a malignancy depends on all of the specific DNA mutations found in the cancer cell. Increasingly, clinicians utilize molecular studies to screen cancers for the presence of specific mutations. In many instances mutation analysis of cancers can lead to selection of specific treatments and provide information regarding prognosis of a cancer.

Genes Involved in Cancer

Oncogenes

It is not surprising that many mutations found in cancer occur in genes responsible for cell division and growth. One such class of genes is termed proto-oncogenes. Proto-oncogenes are essential to growth and mitosis in a normal cell. However, mutations in proto-oncogenes give rise to oncogenes, which contribute to the formation of cancer cells.

It is useful to keep in mind the following four functional categories of proto-oncogenes. These include:

1. Growth factors- protein hormones secreted by cells to stimulate growth of other cells
2. Growth factor receptors
3. Transcription factors- proteins that bind to gene promoter regions and control gene transcription
4. Genes that regulate the synthesis of a number of proteins involved in intracellular signaling

A partial list of proto-oncogenes and their normal cell function is provided in Table 1.

Table 1. Proto-Oncogenes

Proto-Oncogene Type	Comment
Growth Factor	
Sis	Platelet-Derived Growth Factor
Growth Factor Receptor	
erbB	Epidermal Growth Factor Receptor (EGFR)
neu (erb-2/her-2)	Member of EGFR Family
Intracellular Signaling	
Src	Tyrosine Kinase: Animal Homolog is Rous Avian Sarcoma
Raf	Serine/Threonine Kinase
K-ras	GTPase Activity
Nuclear Transcription Factors	
Jun	
Myc	

The epidermal growth factor receptor (EGFR) gene family is an example of proto-oncogenes. These genes code for membrane receptors and also have tyrosine kinases which normally regulate signal transduction for cell proliferation, survival, adhesion, migration, and differentiation. The family includes four transmembrane receptors which include EGFR (erb-1), Her2 (also known as neu, erb-2, Her2/neu), HER3 (erb-3), and Her4 (erbB-4). While each receptor can be activated by a ligand, Her2 is activated through heterodimerization with the other erb tyrosine kinases. Mutations can lead to constitutive (or constant) activation and promote carcinogenesis. The presence of these mutations can be tested. For example, Her2 testing is commonly performed in breast and gastric cancers. The presence of Her2 mutations allows for treatment with drugs such as trastuzumab, a monoclonal antibody directed against the Her2 receptor.

Another classic proto-oncogene is B-raf (or BRAF). BRAF is involved in the intracellular signal transduction of the MAPK pathway, which is a key pathway that can lead to mitosis. Mutations within this pathway lead to a number of cancers including melanoma, thyroid cancer, and colorectal cancers. For example, in melanoma the most common BRAF mutation is

a nucleotide substitution, leading to expression of a different amino acid at codon 600 (V600E). This mutation site is present in 50% of melanomas. Testing of melanoma for the presence of BRAF mutations permits the use of drugs such as vemurafinib or dabrafenib, which inhibit BRAF which possess the V600E mutation.

Tumor Suppressor Genes

Tumor suppressor genes represent another common class of genes frequently altered in cancers. Tumor suppressor genes encode proteins which downregulate cell proliferation and control cell cycle checkpoints necessary for cell growth. Tumor cells commonly exhibit mutations in tumor suppressor genes such as Rb and p53.

Rb is a nuclear phosphoprotein which regulates the cell cycle at the G1/S checkpoint. When hypophosphorylated, Rb binds E2F, blocking entry into S phase. In its hyperphosphorylated state, Rb releases E2F permitting entry into S phase. A germline mutation in Rb is associated with retinoblastoma.

P53, on chromosomal locus 17p13.1, is known as the gate keeper of the cell cycle. P53 is a transcription factor which regulates hundreds of genes and prevents proliferation of cells with DNA damage or shortened telomeres. For example, activated p53 upregulates the mir34 family of microRNAs which inhibits translation of growth promoting genes (such as CDK4, MyC and anti-apoptosis genes such as BCL-2) to act as a pro-apoptotic protein. P53 acts to produce a temporary or permanent cell cycle arrest or to direct cells into the apoptotic pathway.

Mutations in P53 are some of the most common genetic alterations in cancer. The function of p53 is abrogated by an acquired somatic mutation of both alleles. Mutations of p53 are usually present in its DNA binding domain. More than 50% of malignancies have a p53 mutation, including many of the most common tumors (i.e. breast, colon and lung cancer).

In fact, most cancers show a defect in one or more of the crucial proteins involved in control of the cell cycle. Some of the proteins commonly affected include Rb, cyclin D1, CDK4, and p16INK.

Abnormalities in the apoptotic pathways allow a cancer cell to circumvent cell death. Indeed, most cancer cells are potentially immortal – if provided with constant nutrition and removal of waste products. The immortality of cancer cells was first exploited to develop tissue culture cell lines in which multiple generations or passages of cells can be maintained for decades, whereas normal cell enter apoptosis after 60-70 doublings.

This potential immortality of cancer cells is in part due to preservation of telomeres. Telomeres are a series of short tandem nucleotide repeats that are present at chromosomal ends. As normal cells replicate, their telomeres shorten. Eventually cells with short telomeres undergo cell cycle arrest mediated by the Rb and p53 pathways. The enzyme telomerase maintains telomere length, and is active in stem cells and cancer cells. This confers unlimited growth potential.

DNA Repair Genes

DNA mutations can occur with remarkable frequency in the life of a cell through errors introduced during replication. A variety of DNA repair enzymes exist to remove and fix these mutations. Loss of DNA repair enzymes leads to an increased risk of permanent DNA damage with consequences including progression to cancer. A number of inherited syndromes that lead to an increased risk of cancer are due to loss of DNA repair enzymes. Examples include deleterious mutations in BRCA1 or BRCA2 and in any of the genes responsible for the hereditary non-polyposis colon cancer syndrome (HNPCC, also known as Lynch Syndrome). In all of these examples, the affected gene encodes a DNA repair enzyme. Loss of function of that enzyme increases genetic instability in affected cells, leading to carcinogenesis.

microRNA

MicroRNA is single stranded RNA of 18-25 nucleotides in length. First identified in the early 1990's, it has become increasingly clear in the last two decades that microRNAs control a variety of critical cellular functions. Over 2000 microRNAs have been identified in human cells. They are synthesized and processed in the nucleus and then transit to the cytoplasm where they bind specific mRNAs, leading to interruption of translation and destruction of mRNA.

The expression and function of miRNAs is cell specific and miRNAs can act as both "oncogenes" and "tumor suppressor genes". For example, miR-21 is overexpressed in a variety of malignancies and this leads to inhibition of negative regulators of the RAS/MEK/ERK pathway, resulting in cell proliferation and inhibition of apoptosis. By contrast, miR-34

expression induces apoptosis and downregulation of miR-34 in cancer cell lines leads to cancer cell proliferation.

MicroRNAs can be found circulating in blood or shed into sputum or feces. Measurement of miRNAs has been proposed for screening for cancers and as a way to gain prognostic information in patients diagnosed with cancer.

Cancer Stem Cells

It is thought that cancers possess a population of stem cells sufficient to propagate a tumor. These cancer stem cells need not arise from a pool of normal stem cells. Rather, somatic mutations may result in a differentiated cell reverting to a stem cell phenotype. If this hypothesis is correct, true eradication of cancers would require therapy targeting the stem cell population. Cancer stem cells likely comprise less than 1%-25% of a tumor population but may be resistant to conventional chemotherapy.

Cancer Genetics

Cancer cells are prone to acquire additional mutations. It is therefore common for cancers to have multiple sub-clones of the original malignant cell. These new clones possess different metastatic abilities or develop resistance to different chemotherapeutic drugs. The presence of clonal variation within cancers explains the frequently observed phenomenon of cancers initially shrinking in response to chemotherapy, but subsequently growing back. Presumably, the recurrent cancer cells are derived from clones selected out from the bulk of tumor by their resistance to chemotherapy. This phenomenon has also been referred to as tumor cell heterogeneity, a term that stresses the variability that can exist between cancer cells within the same tumor.

Inherited Cancer Syndromes

This discussion of carcinogenesis has emphasized that cancers arise through acquired mutations. However, rare inherited cancer syndromes do exist and have informed our understanding of carcinogenesis. The syndromes listed below (Table 2) are inherited in autosomal dominant fashion.

Table 2. Inherited Cancer Syndromes

Mutation	Syndrome
RB	Retinoblastoma
P53	Li-Fraumeni Syndrome (multiple cancers)
APC	Familial adenomatous polyposis (colon)
MLH1, MSH2, MSH6, PMS2	Lynch Syndrome (colon, ovary)
BRCA1, BRCA2	Breast, Ovary

These syndromes involve an inherited mutation in a single allele of a tumor suppressor gene. Loss or inactivation of a single allele is clinically silent. The acquisition of a mutation in the second normal allele results in complete loss of function of the gene and markedly increases the probability of cancer development. This phenomenon has been referred to as “loss of heterozygosity” because individuals with these syndromes are genotypically heterozygous for the normal gene and its mutated counterpart.

Cancer Cell Kinetics

Cancer growth rates vary greatly. Highly aggressive lymphomas or leukemias may double every 24 hours. Many cancers that arise from solid organs, however, may have doubling times of many months. Within the same cancer, growth rates may change as the cancer expands, possibly through the emergence of clones with varying rates of growth. Doubling times of cancer have important clinical implications. A tumor with a diameter of about a centimeter has roughly one billion (10^9) cells. Assuming constant doubling of the original cancer cell with no loss of cells during tumor growth, this represents roughly 30 cell doublings ($2^{30} \approx 10^9$). As noted above, many cancers may have doubling times of several months or more. This implies that formation of a tumor with a 1 cm diameter – probably the smallest size that a nodule can be reliably found on chest x-ray – may take three to ten years from the initiation of the original cancer cell in the tumor. This also means that growth of many cancers will proceed for years before the cancer reaches a size that is clinically detectable.

Metastases

Metastases are the hallmark of a malignant tumor. They represent spread of tumor cells away from the primary tumor. Fortunately, the development of metastases presents a hurdle for many cancers. It is thought that while millions of cancer cells are shed into the circulation daily, few (<0.01%) are able to initiate tumor growth at a distant site. For most malignancies, metastases are responsible for death.

Normal cells typically adhere to their neighboring cells and the surrounding extra-cellular matrix. A variety of protein families, including integrins, cadhedrins, selectins, and other cell adhesion molecules (CAMs) anchor cells to one another. Mutations in CAMs are frequently present in cancer cells. These mutations make it easier for cancer cells to disaggregate from one another in order to spread out beyond the normal cell's usual anatomic boundary.

In order to metastasize, a cell or group of cells must detach from the primary tumor, digest and move through the intercellular matrix and penetrate the vascular basement membrane. Enzymes, like matrix metalloproteinases are upregulated in cancer cells to facilitate this process.

Once in the circulatory system, the cells must escape the immune and coagulation systems. Cancers must then must lodge in a distant capillary or venule and again travel through the vascular wall, lodge in the extracellular matrix and grow.

As soon as cancer cells have moved to a new location, their growth will be limited unless they can establish a blood supply. Diffusion of oxygen and nutrients into a collection of cells only permits growth of spherical colonies smaller than a few hundred microns. Larger growth requires the formation of new tumoral blood vessels. Successful cancers can elaborate growth factors such as vascular endothelial growth factor to stimulate angiogenesis (new blood vessel growth). Few cells succeed in this series of steps required for metastasis, but as so many cells attempt this journey, metastasis is common for many tumors.

Tumor metastasis is not a random process. Certain tumors have a propensity to metastasize to specific sites. Some tumor spread is related to normal anatomy. For example, the liver is the most common site of metastatic spread for colorectal cancers. Invasion into the portal vasculature results in the liver being the first organ that metastasizing colorectal cancer cells enter. However, many common sites of metastases are not so easily explained. For example, prostate and breast cancers tend to spread to bones while lung cancers preferentially spread to the adrenal glands. In these instances, it is likely that local growth factors within tissues provide a receptive "soil" for the metastatic "seed" to grow.

Conclusion

Cancers arise from normal cells as the result of mutations in critical genes. These mutations are usually acquired during life but, less commonly, can also be inherited. Cancers are characterized by poorly regulated cell growth and the ability to metastasize. The mutations that give rise to cancers are passed down to successive generations of cancer cells. However, individual cells within a cancer can acquire their own new mutations that may result in subclones with different properties than the rest of the tumor.

Understanding the biology underlying the development, growth, and spread of cancers is critical if one hopes to develop treatments that target cancer cells. Once cancer cells have moved to a new location, their growth is limited unless they can establish a blood supply. Diffusion of oxygen and nutrients into a collection of cells permit growth of spherical colonies to diameters of a few hundred micrometers. Larger growth requires only blood vessels. Tumors are capable of elaborating growth factors such as vascular endothelial growth factor to stimulate new blood vessel growth.

Thought Questions

1. With increasing exposure to carcinogens on a daily basis and longer survival rates, what prevents everyone from getting cancer?

   Your answer

   Expert Answer

2. If an invasive tumor is capable of generating many circulating tumor cells, why might only a few succeed in becoming metastatic cancers? Why do cancers have preferred metastatic sites?

   Your answer

   Expert Answer

Glossary

Apoptosis- Programmed cell death; a normal process of cell death that is triggered by a variety of factors, including sometimes as a part of normal cell development.

Dysplasia- Abnormal cell appearance and behavior

Matrix metalloproteinases- Enzymes that catalyze the hydrolysis of extracellular matrix proteins. These enzymes typically contain a zinc atom in their catalytic domain.

Oncogene- Product of a mutation in an oncogene. This leads to their constitutive activation which allows cells to grow without normal control mechanisms.

Proto-oncogene- Normal cellular counterpart of an oncogene

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Suggested Reading

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