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Cancer Genetics Fundamentals

KEY WORDS

Carcinogenesis

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Angiogenesis

Genetic counseling

It is often said that cancer is genetic. What exactly does that mean? This article is our answer to that question at the turn of the millennium. We present models of carcinogenesis, review basic cancer genetics terminology, and explain some of the fundamental genetic changes common to all types of cancer. These are organized into 6 sections of (1) self-sufficiency in growth signals, (2) insensitivity to growth-inhibitory signals, (3) evasion of programmed cell death, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastases. Underlying all of these changes are the even more fundamental enabling factors of genetic instability on both the chromosomal and the gene level. Finally, we look toward the future in a field where the future is now!

■ Introduction

Cancer is a set of diseases in which the regulation of growth and maturity of normal cells is disturbed. There are numerous types of cancers, and even cancers of the same type can behave very differently from each other. Despite this diversity, there are fundamental changes common to all types of cancer. As Nobel Laureate and former National Institutes of Health (NIH) director Harold Varmus explains, "Cancer cells divide without restraint, cross boundaries they were meant to respect, and fail to display the characteristics of the cell lineage from which they were derived."^{1(p1)} These changes in growth and development are under genetic control.

Much has been learned about the fundamentals of cancer genetics throughout the last few decades. This article covers some of the more basic issues as described in our earlier publi-

cation² as well as recent updates that offer expanded understanding. There are a number of publications that summarize cancer genetics at the introductory level.³⁻⁹

Models of Carcinogenesis

Research indicates that carcinogenesis in humans is a multistep process (Figure 1) and that these steps reflect genetic alterations that drive the progressive transformation of normal tissue through steps of initiation, promotion, and progression into malignant states.¹⁰⁻¹² Initiation generally involves deoxyribonucleic acid (DNA) damage from chemical carcinogens, reactive oxygen species, ultraviolet or other radiation, as well as inherited and spontaneous mutations. Promotion from the initiated cell to a preneoplastic one involves additional genetic steps of oncogene activation, tumor suppressor gene (TSG) inactivation, proliferation, and possible inflammation

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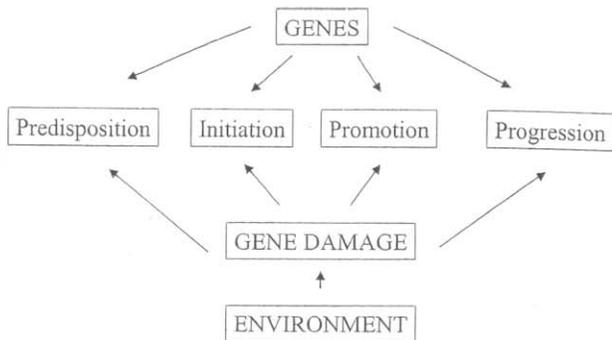


Figure 1 ■ General carcinogenesis model.

to produce clonal selection of a group of cells with growth advantage over neighboring cells. These premalignant cells may then progress to true neoplasias if they have further genetic alterations leading to uncontrolled proliferation, loss of normal cell functioning and morphology, and the ability to invade and possibly metastasize.

The evidence available a decade ago for the common cancers of adulthood indicated that between 4 and 7 genetic steps are necessary for malignant transformation.¹³ This was in keeping with the predominant model from the 1990s for understanding the interplay of gene mutations, and the elucidation of the evolution from a normal cell to a cancerous cell in colorectal carcinoma.¹⁴⁻¹⁷ The Fearon-Vogelstein model illustrates the progression from a normal epithelial cell to a benign adenoma to metastatic colorectal cancer (Figure 2). The accumulation of genetic alterations leads to gradual phenotypic changes in the colonic epithelium that can result in polyps or occasionally in carcinoma if the process progresses completely.

The Fearon-Vogelstein model used observations that at least 4 critical gene mutations not found in normal colonic epithelia have been noted in colorectal carcinomas: the activation of *K-Ras* oncogenes on chromosome 12p; the loss of TSGs and *p53* on chromosome 17p; the mutation or loss of the *APC* gene on chromosome 5q (Figure 2); and the loss of TSG DCC on chromosome 18q. Each of these genetic changes is associated with a phenotypic change that is subject to evolutionary forces. In other words, the processes governing the initiation and progression of cancers are ruled by natural selection, which acts upon the inherent or acquired differences of various

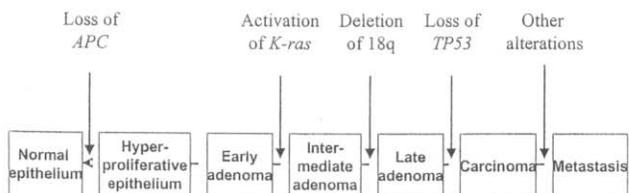


Figure 2 ■ Fearon-Vogelstein model of colorectal carcinogenesis. Adapted from Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990;61:759-767. Reprinted with permission from ASCO, the American Society of Clinical Oncology, Alexandria, Va.

clones of cells causing those with the most growth advantage to outperform and overcome their neighbors.

There have been changes to the conceptualization of the carcinogenic process in the decade since this model was first presented.¹⁸ The early events include changes not only in the *APC* gene, but also *bcl-2*, *c-myc*, and *cox-2*, as well as changes in methylation status. *K-ras* changes are involved in promotion from mild dysplasia to polyp formation. Then, mutations in *SMAD2* and *SMAD4* as well as *DCC* cause the polyp to become a seriously abnormal adenoma. *p53* remains key in the progression of adenomatous polyps to adenocarcinomas.

We now know that most cancers involve many more genetic alterations than were heretofore recognized. This and other recent advances in cancer genetics understanding are discussed below. Before we proceed, we will use the first half of this article to provide some introductory material for the novice reader in cancer genetics. This should prepare the reader for the discussion of essential carcinogenesis processes in the second half of this article.

■ Clarification of Terminology and Concepts

Genes, Proteins, Pathways, and Networks

Genes are sections of DNA that are transcribed into ribonucleic acid (RNA), which is then modified and translated to produce protein products. It is the protein products that can interact with other pieces of DNA, RNA, or other proteins to cause specific changes within cells. This simple linear model is the basis for much biomedical research that involves determining specific effects of one gene or one protein on another gene or protein.

However, this era of linear modeling is rapidly passing and is being replaced by an era of greater complexity. One gene product may act in many different ways. For example, depending on intracellular and intercellular circumstances, alteration of one gene, such as *myc*, can have a variety of different effects on diverse processes, such as differentiation, proliferation, angiogenesis, invasion, apoptosis, and senescence.¹⁹ Often alternate forms of the same or related proteins can be substituted for each other. To complicate matters further, proteins are assembled into pathways, and those pathways can be associated into larger networks of interacting pathways with all types of positive and negative feedback loops, redundancies, and counterbalancing forces.²⁰

Generally, damage to any one protein in a pathway obviates the need for other mutations in genes in the same pathway. For example, the genes *MSH2* and *MLH1* and others are associated in what is known as the mismatch repair pathway, which is described in more detail below. Disabling this pathway does not require multiple mutations in the individual pathway components because when any one of the genes in this pathway is disrupted, the repair process pathway as a whole is disrupted.

Genetic, Congenital, Inherited, Germline, and Somatic

Although all cancer has a genetic origin at the cellular level, this does not mean all cancer is inherited. There is often confusion regarding whether a disease is *genetic*, *congenital*, *familial*, or *inherited*. There is further confusion in cancer about genetic changes that are *somatic* vs those affecting the *germline* cells.

Genetic

Genetic is a general term that is used when discussing genes or chromosomes. These genetic changes may be somatic or germline. Genetic is also sometimes used interchangeably with the terms *familial* or *hereditary*.

Congenital Conditions

A condition is *congenital* when it is present at birth. For example, sacral teratomas may be present at birth. Retinoblastoma (RB) or Wilms' tumor may be congenital and yet may be diagnosed in infancy or early childhood. The fact that a condition is present at birth does not imply that it is necessarily inherited. For example, we know that about 40% of RB is due to a mutation inherited from a parent and 60% is sporadic.

Familial

Conditions that occur in at least several members in a family are called *familial*. Familial clustering of a condition is often assumed to be inherited, but this is not always the case. In addition to their genes, family members may also share ethnicity and culture, childhood environment, geographic location, diet, lifestyle, and exposures to environmental carcinogens in the air, water, or ground. Conversely, there may be a mutation in a cancer susceptibility gene in the family, but a familial clustering may not be obvious. Possible explanations include small family size, low gene penetrance, presence of risk modifiers, or other factors. With all of these caveats recognized, familial clustering of a given type of cancer is still one of the characteristics of hereditary cancer susceptibility.

Inherited Conditions

A condition is *inherited* when it is due to a genetic alteration derived from one or both of the parents. There are more than 4,000 conditions that are caused by an alteration of a single gene, thought to be inherited from one or both parents in inheritance patterns known as autosomal dominant, recessive, or x-linked. Conditions that are believed to be *inherited* are cataloged in a printed compendium called *Mendelian Inheritance in Man*.²¹ The Web-based version is known as Online Mendelian Inheritance in Man, OMIMTM.²² Neoplasia may occur as a primary or secondary feature in hundreds of these known inherited conditions.^{6,7,23,24} The hereditary cancers are not discussed in detail in this article. This topic is ade-

quately addressed in the publications previously cited and in subsequent contributions about genetic testing in this series.²⁵

Multifactorial or Complex Disease Genetics

Many common conditions of adulthood such as heart disease, mental illness, diabetes, and cancer have a complex and heterogeneous etiology. In these conditions, the inherited contribution becomes relevant only in combination with lifestyle and other environmental causes such as carcinogen or viral exposure.

A commonly cited prototype for multifactorial etiology of malignancy is lung cancer. The incidence of lung cancer varies significantly by chronic exposure to tobacco smoke but also by a variety of other factors, including genetic variations contributing to addiction to tobacco as well as the variable rates and efficiency of one's metabolism of specific carcinogenic components of smoke.²⁶

Somatic or Acquired Mutations

Somatic cells are all body cells that are not reproductive cells, eg, skin, liver, breast, colon, and every organ except the gonads. A *somatic* mutation is a gene alteration in an individual cell in one of these organs. These are also known as *acquired* mutations, meaning that the cells acquire the changes postconception. A somatic mutation that is not repaired may be replicated at mitosis and thus be incorporated into all future descendants of that altered cell in that particular organ. The group of altered descendent cells is said to be a genetic clone, and the organ exists in a mosaic state of having normal and altered cells. These clonal changes are unique to the cells in that particular organ cell line and are not passed on to one's offspring because the somatic mutations are not present in the egg or sperm cells. In general, for a cell to become cancerous, it must develop more than one somatic mutation. This is especially true for the common epithelial cancers of adulthood, as exhibited in the carcinogenesis section. The need for a cell to experience multiple changes before malignant transformation provides protection to the organism.

Germline Mutations

Germ cells are the reproductive cells of the body, ie, the sperm and egg cells. Germline mutations are those present in a parent that can be passed on to offspring through the egg or sperm cell. Because the mutation is present at conception in the original fertilized egg, when that cell copies and divides in cell, the mutation is copied in all future cells. Thus, an inherited mutation is present in every cell of the body. A person who inherits a mutation in a specific cancer susceptibility gene can develop cancer more easily, have bilateral or multiple cancers, and at a younger age than a person born without such a mutation. Why? Because every cell already carries an inherited mutation. Therefore, every cell is already one step closer to malignant transformation (Figure 3).

Oncogenes

Oncogenes are a class of genes that play a role in the growth of cells but when overexpressed by mutation, amplification, translocation, or other means, they can foster the growth of cancer, uncontrolled by the normal signaling pathways of the cell.^{27,28} Oncogenes typically encode for proteins that stimulate mitosis or inhibit apoptosis. Some are now known to increase both mitosis and apoptosis.

Initial knowledge of oncogenes came from early studies (before 1970) that focused on cancer-causing viruses as the culprits in initiating transformation from normal cell to tumor at the cellular level. The classic definition of an oncogene is a cancer-causing gene carried by an acute transforming retrovirus that has a normal counterpart (homologue) referred to as a proto-oncogene.²⁷ Cancer-causing oncogenes were identified and generally named based on the virus in which they were originally carried. For example, *SRC* is an oncogene from the Rous sarcoma virus, *SIS* from the Simian sarcoma virus, *RAS* from the rat sarcoma virus, and so forth.¹

In the 1970s it was discovered that oncogenes and proto-oncogenes encode proteins involved in signal transduction, the orderly and specific transmission of growth-regulatory messages from outside the cell to the machinery controlling replication inside the cell's nucleus.²⁹

When signaling growth in a normal orderly fashion, oncogenes are termed *proto-oncogenes*. Mutations and other alterations of the proto-oncogenes turn them into oncogenes, which are unresponsive to outside influences, leading to a permanent activation of the pathway promoting cellular division and growth, thus leading to cancer. More of this is addressed below in the section on insensitivity to growth signals.

A simple analogy is often made of oncogenes being similar to the accelerator in a car, allowing the driver to go faster or slower, depending on the pressure applied. However, one might imagine a defect in which the accelerator suddenly becomes stuck in the down position, creating runaway acceleration. Thus it is with oncogenes, creating an unnatural buildup of cells.

Oncogene mutations are dominant at the cellular level. In other words, they produce an effect when only one allele is altered, regardless of the presence of the corresponding wild-type protein. Oncogenes may increase their function through a variety of mechanisms described below in the genomic instability section.

Most oncogenes are altered in somatic and not germline cells. The notable exception is the *RET* gene. Mutations in this oncogene can be inherited and can cause multiple endocrine neoplasia type 2, medullary thyroid cancer, and/or Hirschsprung disease.^{30,31}

The "Two-hit" Theory of Tumor Development

The concept of inherited vs acquired cancer susceptibility is well illustrated in Dr Knudson's "Two-hit" theory. Three decades ago, Dr Alfred Knudson proposed a model to explain

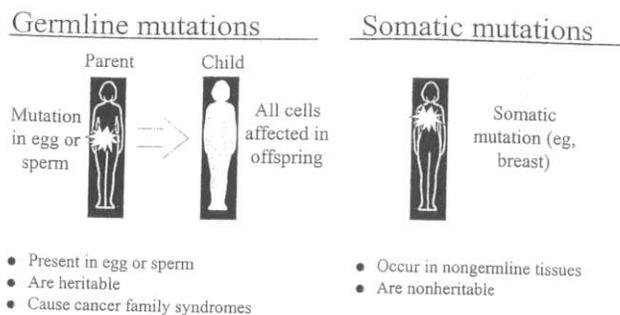


Figure 3 ■ Cancer arises from gene mutations. Reprinted with permission from ASCO, the American Society of Clinical Oncology, Alexandria, Va.

the epidemiology of RB, a childhood cancer distinguished by retinal tumors.³² He suggested that more than one genetic mutation ("hit") was necessary for either inherited (positive family history) or sporadic (no family history) RB to occur. People born with a germline Rb mutation have a DNA mutation in every cell of their bodies present at birth (the first "hit"). Any retinal cell that acquires a second "hit" in the *RB* gene can develop into an RB tumor. Therefore, people bearing a germline mutation are more likely to develop cancer than those born without this first mutation. The tumors usually occur earlier in life (eg, infancy vs toddler), and more often present as multiple tumors versus one (because more than one cell involved in malignant transformation may have experienced the second "hit"). In addition, persons with inherited RB are more prone to specific second primary malignancies (eg, osteosarcomas, hepatoblastomas, melanoma, lung).

In contrast, people born without an *RB* mutation in their germline cells must acquire 2 mutations ("hits") in the same retinal cell to develop cancer. This noninherited form of RB manifests as later onset disease (pediatric vs newborn), is more often unilateral, and is not as frequently associated with other cancer development (Figure 3).

Knudson's "Two-hit" theory of tumor development may not apply to all cancers, but the basic concept was validated with the cloning of the *Rb* TSG.^{33,34} It was demonstrated that in RB tumors, both copies of a pair of *RB* genes are inactivated, thus the "hits" necessary to transform a cell toward malignancy can be both inherited and acquired.

Tumor Suppressor Genes and the "Two-hit" Theory

TSGs are genes that are present in all normal cells and function to restrain cell growth, but when missing or inactivated by a mutation or other means, allow cells to grow in an uncontrolled manner.^{27,35} Continuing our auto analogy above, TSGs are analogous to the brakes (growth inhibitors). Generally, we have 2 functional TSG alleles, similar to 2 functioning sets of brakes, front and back. Having one functioning copy of a TSG pair is sufficient for slowing growth in the same way as having one set of functioning brakes is usually sufficient to slow and stop one's car. If the second gene copy is lost, the cell is left

with no copy of that gene working to control growth. This is the cellular equivalent of a runaway car that has lost all its braking capacity. The “Two-hit” theory previously described applies to the TSGs. The 2 hits are shown in Figure 4.

Tumor suppressor mutations are also known as loss of function mutations, because they inactivate the protein and act in a recessive manner, ie, are silent in the presence of the corresponding wild-type protein. When the first allele in a given gene is lost, that cell is said to be *heterozygous*, ie, having 2 different forms or alleles. When the second copy is lost through mutation, deletion, or other mechanism, this is referred to as “loss of heterozygosity,” (commonly abbreviated as LOH). Many cancer genetic studies report LOH in tumor tissue when compared to normal tissue, ie, places where the second copy of a relevant cancer susceptibility gene has been lost. The significance is that sites of LOH indicate potential TSG locations. The patterns of LOH often differ in different tumors as well as include some common sites of loss. These results are interpreted to mean that there are both some common etiologic pathways to cancer as well as organ-to-organ and person-to-person differences, with various cancer types caused by specific combinations of tumor suppressor losses. Tumor suppressors may be either gatekeepers or caretakers.

GATEKEEPER GENES

Vogelstein and Kinzler¹⁷ introduced the terminology *gatekeeper*. They refer to gatekeepers as those genes that produce proteins that directly regulate the growth of tumors by inhibiting mitosis or promoting apoptosis. The function of these genes is rate limiting for tumor growth, hence they are the gatekeepers. Examples include *APC*, *VHL*, *p53*, *NF1*, and *PTEN*.

CARETAKER GENES

This term was also introduced by Vogelstein and Kinzler¹⁷ to describe genes that encode proteins that maintain the integrity of the genome. Their inactivation contributes to genomic

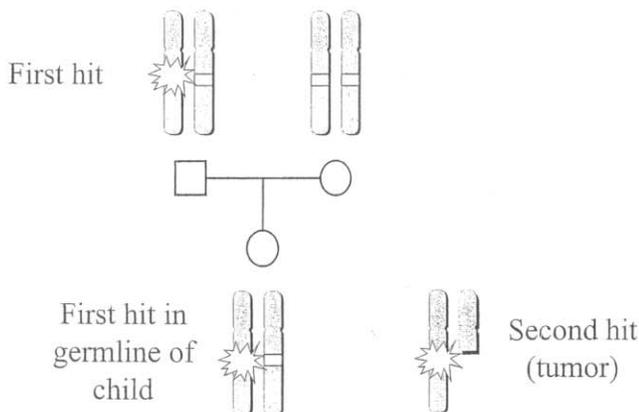


Figure 4 ■ The two-hit hypothesis. Reprinted with permission from ASCO, the American Society of Clinical Oncology, Alexandria, Va.

instability. Examples include the mismatch repair (MMR) genes (eg, *MSH2*, *MLH1*, *PMS2*, and *MSH6*) associated with hereditary nonpolyposis colorectal cancer, *ATM* associated with ataxia telangiectasia, and *BRCA1* associated with hereditary breast and ovarian cancers. It is interesting to note that some genes, such as *p53*, can be both a gatekeeper and a caretaker because *p53* affects cell growth through its role in a key cell cycle checkpoint and also is known as the “guardian of the genome” because of its role in detecting and either repairing DNA damage or sending the cell toward apoptosis.

Inactivation of caretaker genes leads to a genetic instability that indirectly promotes tumor growth by causing an increased mutation rate. The targets of these further mutations are TSGs and oncogenes involved in a given carcinogenic pathway. Because numerous mutations are required for the full development of a cancer, inactivation of caretakers can greatly accelerate the development of cancers. These genes are related to genetic instability.

■ Essential Features of Cancer

In their review of the hallmarks of cancer, Hanahan and Weinberg¹² suggest that there are 6 essential alterations in cell physiology that collectively dictate malignant growth. Changes in these processes are made possible by an underlying genetic instability. These essential carcinogenic processes are enumerated in Figure 5.

Although each cancer is different in a variety of ways, all cancers probably share certain features, such as the phenomena of genomic instability affecting specific pathways involving oncogenes and TSGs. However, the genomic instability is not sufficient to cause cancer. Rather, this allows other changes to occur at ever-increasing rates within the cell in certain systems essential to all cells. For example, normal cells respond appropriately to growth, antigrowth, apoptosis, senescence, angiogenesis, and contact inhibition signals. In contrast, cancer cells acquire deficits in each these capacities in some way for malignant transformation to be complete. The following

Enabling factor

- Genomic instability

Six essential systems that are altered in malignancy

- Self sufficiency in growth signals
- Insensitivity to growth inhibitory (antigrowth) signals
- Evasion of programmed cell death (apoptosis)
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastases

Figure 5 ■ Biological processes associated with malignancy. Adapted from Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100:57-70.

section follows the conceptualization of Hanahan and Weinberg¹² in describing various acquired characteristics of cancer cells.

1. Self-sufficiency in Growth Signals, Aberrant Signaling Pathways, and the Cell Cycle

Cells are generally produced only when a new one is needed, as in normal growth and development or replacement of aging or damaged tissue. In contrast, tumor cells proliferate when normal cells would not. Normal cells require mitogenic growth signals before they move from a quiescent state into an active proliferative state. These signals are generally external factors, such as diffusible growth factors, extracellular matrix components, and cell–cell adhesion or interaction molecules. These growth signals then are transmitted into the cell by transmembrane receptors that bind particular growth signal molecules. Many oncogenes operate by mimicking normal growth signaling.

THE CELL CYCLE

Normally the process of cell reproduction takes place through an ordered process, generally known as the cell cycle as shown in Figure 6.³⁶ For one cell to reproduce into two, several things are necessary: replication of the genome, doubling of cell mass, and precise segregation of the chromosomes and other cell components.³⁷ The coordination of these is divided into 4 phases of S, G₂, M, and G₁. During the synthesis (S) phase, the chromosomes containing all the genetic material are replicated. Then there is a gap (G₂) in DNA activity. Next, the chromosomes and other cell constituents segregate to the 2 daughter cells during mitosis (M). Finally, there is another gap (G₁) in the replication sequence before the cycle starts again. Sometimes at this point, cells enter a temporary resting phase known as G₀ or permanently leave the cell cycle to differentiate. Although the DNA synthesis, chromosome replication, and segregation occur only during S and M phases, cell growth

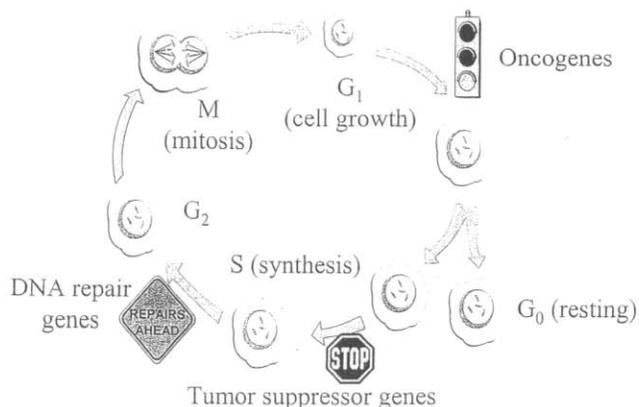


Figure 6 ■ The cell cycle. Reprinted with permission from ASCO, the American Society of Clinical Oncology, Alexandria, Va.

occurs continuously throughout the cell cycle, usually in response to growth signals. The S and M phases are the bases in pathology reports that refer to the S-phase fraction and the Mitotic Index when describing the growth characteristics of the tumor. It is typically during G₁ and G₂ when the cells are typically responding to the proliferative and antiproliferative signals that determine whether the cell cycle proceeds or comes to a halt, either momentarily or permanently. This means that the faithful reproduction of the cell requires that multiple events are carefully orchestrated via molecules linked together into pathways that promote or inhibit cell cycle progression. In other words, the orderly execution of cell cycle events results from a series of dependent relationships in which the completion of one event is required for the beginning of the next.

Some of the many factors that regulate the cell cycle include specific proteins, growth factors, and environmental signals, such as nutrients and temperature. Events that disrupt or damage the cell's internal regulation system, trapping it in unceasing cycles of growth, are key to development of cancer. Understanding these disruptions in the cell cycle is important in understanding specific genes involved in cancer causation, prevention, and treatment presented in upcoming modules.

Many, and possibly all, cancer cells contain mutations in cell-cycle regulatory proteins.^{38,39} Disruptions in cell-cycle proteins can promote cell proliferation directly by allowing the cell to override or bypass the controls that would ordinarily regulate growth. In particular, one or another element in the “RB regulatory pathway” (Figure 7), including *p16*, *cyclin D*, *cdk4*, *E2F*, or *RB* itself, may be mutated.⁴⁰ They can also cause the cell to ignore internal alarms that signal the presence of errors.

Cell-cycle checkpoints refer to the phenomenon of genetic damage causing the cell to pause in G₁ or G₂, allowing time for repair enzymes to correct the lesions, thereby preventing the cell from passing along damaged genetic material. Inactivation of checkpoint pathways is believed to underlie the genetic instability seen in tumor cells. Thus, many TSGs are part of checkpoint pathways and inactivation of these genes con-

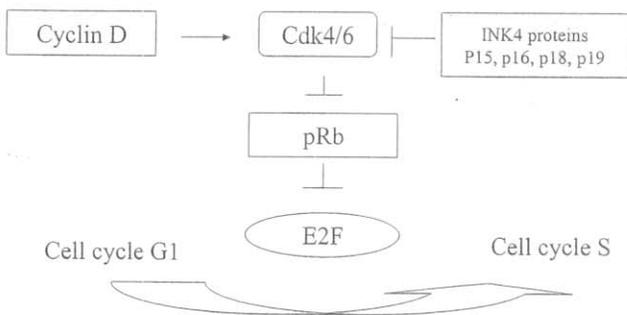


Figure 7 ■ Simplified Rb pathway intersecting with the cell cycle. Adapted from Kaelin W Jr. *Cancer genetics*. In: *American Society of Clinical Oncology ASCO Comprehensive Review of Clinical Cancer Genetics*. San Francisco: ASCO; 2001; and Clurman BE, Roberts JM. Cell cycle control: an overview. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill; 2001.

tributes to the clonal evolution of cancer cells by allowing the accumulation of genomic errors that normally would have resulted in either cell cycle arrest or cell death (apoptosis). The protein product of the *p53* TSG is important in regulating cell-cycle checkpoints. The *p53* pathway is involved in both cell-cycle inhibition and apoptosis as shown in Figure 8. TP53 is a nuclear protein that binds to sections of DNA in many different genes to regulate their transcription. Either the *p53* gene itself or part of the *p53* pathway is altered in many cancers. The antiproliferative effect of wild-type *p53* is exerted at the G_1/S checkpoint through *p21* and has a role in control of the mitotic spindle and cell diploidy at the G_2/M checkpoint.

The RB and the *p53* pathways interact in a *p53*-pRB network as seen in a simplified version in Figure 9. This is part of larger networks of growth signaling and inhibiting pathways at one or more sites in complex interacting signaling pathways in the cell as illustrated in Figure 10. What is most noticeable in addition to the complexity of this model is the redundancy of most sensing, regulatory, and effector components. This undoubtedly plays a key role in cell survival, but also may complicate cancer treatment in situations where drug mechanisms that block one path to cell proliferation may be eventually overridden by other cell pathways. This is addressed in a future publication in this series on applications of cancer genetics.

2. Insensitivity to Antigrowth Signals

Normal cells exist in a state of homeostasis between cell growth and factors that oppose growth. Antigrowth signals can block proliferation by 2 mechanisms: (1) causing cells to move out of the cell cycle into a temporary G_0 quiescent state or 2) causing cells to move toward a more permanent postmitotic state, often associated with cell differentiation or apoptosis.

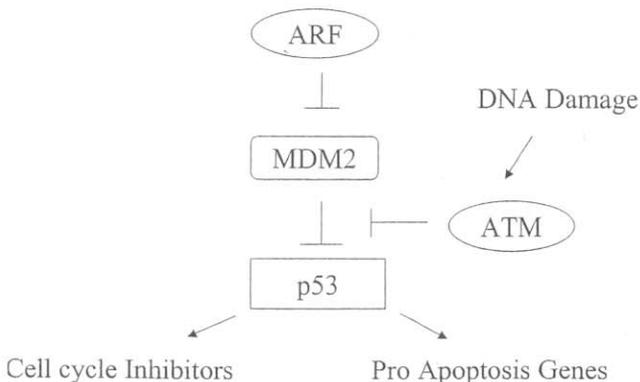


Figure 8 ■ A simple *p53* pathway. Cancer Genetics. In: *American Society of Clinical Oncology ASCO Comprehensive Review of Clinical Cancer Genetics*. San Francisco: ASCO; 2001; and Clurman BE, Roberts JM. Cell cycle control: an overview. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill; 2001.

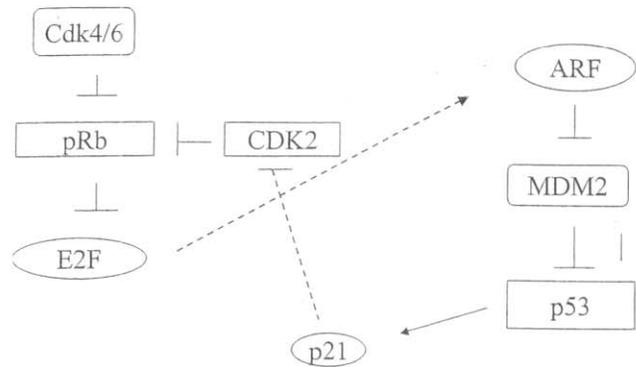


Figure 9 ■ Network integrating pRB-*p53* paths. Adapted from Kaelin W Jr. Cancer Genetics. In: *American Society of Clinical Oncology ASCO Comprehensive Review of Clinical Cancer Genetics*. San Francisco: ASCO; 2001; and Clurman BE, Roberts JM. Cell cycle control: an overview. In: Scriver CR, Beaudet AL, Sly WS, et al, eds. *The Metabolic and Molecular Bases of Inherited Disease*. 8th ed. New York: McGraw-Hill; 2001.

There are specific antigrowth molecules just as there are specific growth-signaling molecules.⁴¹ As with the paths described above, these involve cooperative interaction of a number of gene products. For example, *TGF-Beta* blocks advance through G_1 by suppressing expression of the *c-myc* oncogene, which helps to regulate the G_1 cell-cycle machinery. *TGF-Beta* also causes synthesis of the *p15* and *p21* proteins that block the *cyclin-CDK* complexes. Mutation or disabling of *TGF-Beta* thus removes barriers to the cell proceeding through the G_1 cell-cycle checkpoint to proliferation.

Other factors cause cells to enter irreversibly into postmitotic differentiated states. Once a cell is differentiated, eg, into a mature breast ductal cell, or a CD4 lymphocyte, it generally has a finite lifespan to perform its designated cell function within the organ. Finding extensive numbers of immature cell types, eg, blast cells in the peripheral blood, is a sign that cells have overridden the antigrowth signals that would have ordinarily allowed differentiation in favor of uncontrolled proliferation.

The bottom line is that proliferation and antigrowth circuits exist and form interlocking circuits within cells. Normally these are balanced; in malignancy, they are not.

3. Evading Apoptosis (Programmed Cell Death)

The term *programmed cell death* refers to the induction of cell death by a regulated pathway inherent to the cell by which it is programmed to die.⁴² This programmed cell death pathway is called *apoptosis* (from the Greek word “falling off” or “falling leaves”). Apoptosis involves a series of steps in which the cellular membranes are disrupted, the cytoplasmic and nuclear skeletons are broken down, the cytosol is extruded, the chromosomes are degraded, and the nucleus is fragmented.¹² This process contrasts necrotic cell death, which results from trauma or other factors external to the cell. Apoptosis plays a

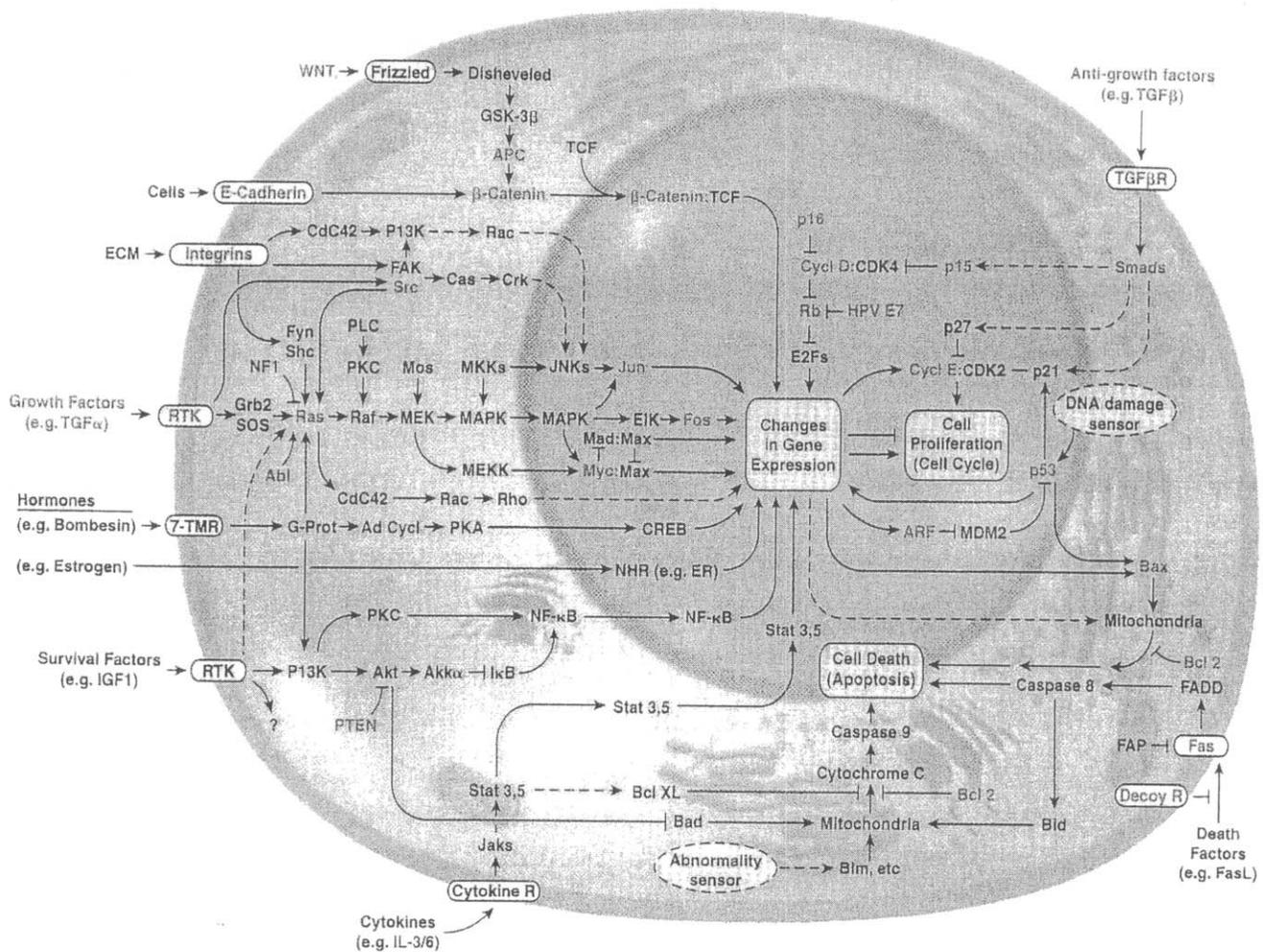


Figure 10 ■ Cell circuitry. Printed with permission from Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100:57-70.

critical role in normal development, permitting the necessary elimination of surplus cells in the formation of many complex organs, including fingers, toes, brain, and hematologic cells.⁴³

Apoptosis also helps to maintain the homeostatic balance between cell proliferation and death. Although it has been evident for some time that failures to control proliferation can lead to the accumulation of clonally related cells (a neoplasm), it has been only within the last decade that the regulation of cell death is recognized as equally important in the carcinogenesis process.

Survival of somatic cells requires the input of survival signals to suppress apoptosis. The control of apoptosis pathways involves a number of known oncogenes and TSGs. The components of the apoptotic machinery generally function to sense extracellular and intracellular normality or damage. External factors that can mediate apoptotic sensitivity are growth factors, cytokines, interleukins, glucocorticoids, androgens, estrogens, and neurotransmitters. Internal factors include DNA damage, signaling imbalances, survival factor insufficiency, and hypoxia. Any of these can affect the balance of proapoptosis and antiapoptosis forces. These sen-

sor molecules transmit the information to other molecules that effect apoptotic death.

The *Bcl-2* family of oncogenic proteins is key to apoptotic control. The members of the family with proapoptotic action are *Bax*, *Bak*, *Bid*, and *Bim*. Those with antiapoptotic action are *Bcl-2*, *Bcl-XL*, and *Bcl-W*. *Bcl-2* overexpression is capable of inhibiting cell death. The tumor suppressor protein *p53* can elicit apoptosis by upregulating expression of *Bax*, which, in turn, governs mitochondrial death through release of cytochrome C that begins the apoptosis cascade through caspases.⁴⁴

Caspases are a group of intracellular protease proteins that have the final role in accomplishing apoptosis.⁴⁵ Certain caspases are activated by death receptors, such as the FAS protein or directly by the cytochrome C released from the mitochondria. The sensor caspases trigger more than a dozen effector caspases to complete the apoptosis program with destruction of the genetic material and subcellular organelles.

There are clear clinical examples of specific failures of the apoptotic process leading to malignancy. Follicular lymphoma is an example of cancer associated with defective apoptosis.

Due to a chromosomal translocation, the *Bcl-2* gene is disrupted. This gene's expression is normally tightly controlled. Affected individuals inappropriately express *Bcl-2* in B lymphocytes, thus bypassing the normal apoptotic pathway and allowing the cell a survival advantage that can lead to lymphoma.⁴⁶

The role of apoptosis has more generalized applications to cancer biology as well, because apoptosis serves as a central mechanism to preventing the proliferation of cells with harmful mutations. Furthermore, inhibition of apoptosis may lead to the accumulation of cells with a higher mutation rate, thus accelerating malignant transformations. Cell-cycle checkpoints as described above play an essential role in detecting and eliminating abnormal cells through apoptotic paths.

4. Limitless Replicative Potential

Generally cultured human cells have a finite replicative potential of 60–70 cell doubling cycles.⁴⁷ Once cells have reached that point, they stop growing, a process termed *senescence*. The cellular determinant of senescence is telomere shortening. Telomeres are the end of the chromosomes, composed of several thousand repeats of a short 6-basepair (bp) sequence element. As a cell moves through the mitosis process, it normally loses between 50 and 100 of these bps from the ends of every chromosome. This progressive erosion of the telomeres through successive cell cycles eventually causes them to lose the ability to protect the ends of the chromosomal DNA, which leads to end-to-end chromosomal fusions, cytogenetic chaos, and ultimately, cell death. The successive decrease in the size of the telomeres could be likened to a biological clock ticking off the finite number of DNA replications the cell has left before it dies.

In contrast, the vast majority of cancer cells do not lose telomeric DNA.⁴⁸ It is likely that control of cell aging and death is orchestrated by multiple genes and gene products. One protein in particular, the enzyme telomerase, is responsible for 85–90% of telomere maintenance with other rare cases maintained through an alternative mechanism involving chromosomal recombination.^{49–50} Telomerase is present in fetal development and then is not generally expressed in most somatic cells after birth.

Senescence, like apoptosis, reflects a protective mechanism against cancer under normal circumstances. Cancer cells with disrupted senescence processes achieve immortality that allows them to continue to proliferate and divide beyond the point when normal cells would age and die. Antitelomerase therapy may be a future focus for the treatment of cancer.⁵¹

5. Sustained Angiogenesis

Tumor expansion is dependent on angiogenesis, ie, on the production of new blood vessels to bring oxygen and other nutrients to cells. The current belief is that most cells within rapidly proliferating tumors lack the capacity for angiogenesis. To progress to a larger size, early neoplastic tissue must develop this capacity.

As with all other biologic systems described above, there are factors that promote and factors that inhibit angiogenesis. There are a variety of signals involved in promoting angiogenesis, including soluble factors and their receptors on endothelial cells, integrins and cell adhesion molecules, and direct cell-to-cell signals.^{52–54}

There are more than a dozen angiogenesis inhibitors. Thrombospondin-1 is a prototypical example.⁵⁴ It acts through a transmembrane receptor on endothelial cells that is coupled to intracellular tyrosine kinases.

Angiogenesis is promoted by more than a dozen peptide growth factors, of which vascular endothelial growth factor (VEGF) is one of the most selective and potent. In normal cells, VEGF is regulated by multiple factors, including hypoxia. In response to hypoxia, the VEGF is upregulated to signal production of more blood vessels. Cells in fast-growing tumors that become hypoxic require additional blood vessels to supply nutrients to the expanding tumor mass.

Angiogenesis induction and sustenance is essential for tumor growth.⁵⁵ Tumor explants will not grow without it, and anti-VEGF antibodies are able to impair neovascularization and growth of subcutaneous tumors in mice.⁵⁶ There are now available many antiangiogenic substances that impair the growth of tumor cells in mice. A therapeutic approach incorporating some of these biological agents underlies a number of clinical trials in humans.

6. Tissue Invasion and Metastasis

Tumor cells relate to their surroundings in ways that differ from normal. Lacking contact inhibition, they continue moving in their own course, migrating over adjacent cells, and growing in chaotic ways. Eventually some may invade adjacent tissues or travel to biologically distant sites establishing new neoplasms. In addition to all of the mechanisms discussed above, the invasive and metastatic cells must acquire additional capacities. Although these are not completely understood, there are some hints. For example, both invasion and metastases involve changes in the physical coupling of cells to each other and to their microenvironment.¹² They also have activation of extracellular proteases.

There are several types of molecules involved in coupling of cells: cell-cell adhesion molecules (CAMs) and integrins.⁵⁷ E-cadherin is a CAM that is expressed on epithelial cells. E-cadherin forms bridges between adjacent cells that allow for transmission of intracellular signals that interact with Beta-catenin. E-cadherin function is often lost in most epithelial tumors by a variety of mechanisms, including mutation of the E-cadherin or Beta-catenin genes, transcriptional repression, or proteolysis of the extracellular cadherin domain.⁵⁸ With the loss of the E-cadherin pathway function as an inhibitor of invasion and metastases, these processes may move forward.

The balance between the expression of the alpha and beta forms of integrin molecules can also play a role in changing the tissue microenvironment for invading and metastasizing cancer cells. The larger number of integrin genes and of het-

erodimeric receipts has made it difficult to determine all of the cellular effects of integrins.

Extracellular proteases break down proteins in cells walls, a necessary step in cell invasion and metastasis. Again, the process must involve increases in protease expression and decreases in protease inhibitor expression or action. The proteases may be produced not only by epithelial cancer cells but also by cooperating stromal and inflammatory cells.⁴¹

■ Genetic Alterations and Cancer

Many processes that maintain the fidelity of DNA and chromosomal replication and repair become increasingly erratic in cancer cells. Genomic instability refers to the multiple changes that occur in genetic material due to an underlying problem. The instability problem is circular. Cells acquire initial changes that cause more generalized instability, which cause more changes and so forth. Most agree that this instability is a prerequisite for all of the other changes that normal cells go through in the process of becoming malignant.⁵⁹⁻⁶¹ Normally, the DNA of cells is incorporated in the 46 chromosomes in each cell. When a cell divides, it duplicates its genetic material and intracellular components, then divides it evenly among the daughter cells. Sometimes errors occur. In healthy tissue, cells with defective genetic material are repaired or eliminated quickly. Thus, most cells have a genetic makeup that is consistent and stable.

There are a variety of ways that damage and possible instability can occur. Disruptions in DNA replication and repair mechanisms happen naturally with aging. Genetic instability can also occur because of exposure to occupational, lifestyle, and environmental carcinogens; due to inherited problems; or by chance.

Problems with genetic fidelity can occur on either the chromosomal or the gene level.¹⁷⁻⁶¹ A variety of problems can occur as listed in Figure 11. There can be changes in chromosome number leading to aneuploid cells with too many or too few chromosomes. Frequent translocations occur in other cancers, with specific translocations associated with specific hematologic malignancies. There can also be more subtle gene alterations, such as small deletions, insertions, and single bp substitutions/mutations. Some oncogenes can undergo amplifications from fivefold to hundredfold multiplications of a sin-

- Chromosomal aneuploidy
- Chromosomal rearrangements
- Gene alterations—gatekeeper genes
- Gene alterations—caretaker genes
- Gene amplification
- Exogenous sequence insertion
- Epigenetic modification of gene expression—imprinting

Figure 11 ■ Types of genetic alterations observed in malignancies.

gle gene or small region of a chromosome. These may result in pieces of amplified DNA known as double minutes (dmin) and homogeneously staining regions (HSRs) on cytogenetic analysis or may not be detected without molecular diagnostics or immunohistochemistry. Finally, there can be insertion of exogenous sequences, such as contributed viral genes, that result in abnormal cell growth.

There is currently debate among scientists regarding which of the genetic changes is more fundamental.⁶² There are investigators who believe that chromosomal aneuploidy is the most fundamental characteristic of a cancer cell. There are others who concede that aneuploidy is often present but may be brought about by defects in genes controlling the mitotic apparatus that result in cell progeny with chromosomal imbalance. Although some carcinogens, such as asbestos, can directly target the spindle apparatus of the dividing cells or fragment the chromosomes directly, most act by inducing mutations. Most believe that the cancer process is driven by a collective genetic disruption of a number of key cellular processes.

The following section describes some of these categories of genetic alterations in more detail.

■ Specific Types of Genetic Alterations in Cancer

Cytogenetics and Chromosomal Alterations in Malignancies

Cytogenetics is the field that focuses on study of the chromosomes. Gross chromosomal changes such as aneuploidy, translocations, deletions, inversions, and amplifications can disrupt genes that play critical roles in the carcinogenesis process.⁶³

The recurring sites of chromosomal change are not random but occur at sites that disrupt genes that normally participate in regulation of cell growth and other critical processes. For those with an interest, there are a number of catalogs of chromosomal alterations in cancer.⁶⁴⁻⁶⁶

Solid tumors often demonstrate multiple clonal structural and numeric chromosome rearrangements. It is believed that these represent byproducts of molecular events that participate in the generation or progression of the malignancy.⁶⁷ In general, metastases have more alterations than primary tumors, reflecting the increasing level of genetic chaos as carcinogenesis progresses.

Information about cytogenetic status often has prognostic implications. Therefore, cytogenetics and immunohistochemistry tests are widely ordered on specimens of potentially malignant tissue. Chromosomal aneuploidies (either more or less than the normal number of 46) are manifestations of genetic instability at the largest end of the scale and can be seen on standard cytogenetic analysis. Aneuploidy can also be detected by flow cytometry. Structural abnormalities of the chromosomes are also possible. A large segment of genetic information found on one of the chromosomes can be broken,

inverted, deleted, duplicated, or translocated to another chromosome. These rearrangements can be detected through cytogenetic studies or through techniques that combine molecular technologies with cytogenetics, such as fluorescent in situ hybridization (FISH),⁶⁸ comparative genomic hybridization (CGH),^{69,70} and spectral karyotyping (SKY).⁷¹ These types of genetic rearrangements frequently result in the disruption of a gene that can eventually lead to malignancy.

CHROMOSOMAL CHANGES IN LEUKEMIAS AND LYMPHOMAS

Chromosomal changes are very common in hematologic malignancies such as leukemias and lymphomas. Somatic acquired chromosome translocations activate proto-oncogenes in more than half of all leukemias described to date and in substantial proportions of lymphomas. In most instances, chromosomal translocation fuse sequences of a transcription factor or receptor tyrosine kinase gene to those of a normal gene, resulting in a chimeric protein with oncogenic properties.⁷²

In leukemia, the white blood cells often show rearrangements, duplications, and deletions of portions of their chromosomes. The identification of the specific chromosome rearrangement can assist with the diagnosis of a specific type of leukemia. For example, the Philadelphia chromosome, seen in chronic myelogenous leukemia, is characterized by the translocation of genetic material between chromosomes 9 and 22, leading to a genetic rearrangement in which the *abl* proto-oncogene from chromosome 9 becomes fused with the *bcr* gene from chromosome 22. The result of this translocation is the production of an abnormal combined *bcr/abl* protein, which contributes to the development of leukemia. Treatment implications based on targeting this *bcr/abl* chimeric protein are discussed in a subsequent module in this series.⁷³

CHROMOSOMAL CHANGES IN HUMAN SOLID TUMORS

Many solid tumors feature an abnormal number or arrangement of chromosomes in the malignant cells.⁶⁷ Tumor-specific chromosome rearrangements have been identified in human solid tumors, especially for a variety of human sarcomas. In general, these translocations juxtapose segments of 2 genes that give risk to chimeric fusion transcripts with oncogenic properties. For example, the 2 closely related genes of *EWS* on chromosome 22 and *FUS* on chromosome 16 have participated in tumor-specific translocations in several sarcomas. In each case, the *EWS* or *FUS* acquires a DNA-binding domain from the translocation partner. This enables the new protein to have transcription activity and participate in malignant transformation.⁶⁷

A more complex genetic involvement can be seen in breast cancer, where numerous cytogenetic alterations have been documented. Among the most frequent changes in breast carcinomas are the loss of chromosome 17 in primary tumors and the amplification in metastatic tumors of the portion of chro-

sosome 17 that encodes the *HER-2/neu* oncogene. More commonly, structural alterations of chromosome 1,3,7, and 11 are seen.

In further elucidation of somatic cytogenetic changes in breast cancers, investigators performed a genome-wide survey by comparative genomic hybridization on breast cancers from 21 *BRCA1* mutation carriers, 15 *BRCA2* mutation carriers, and 55 unselected controls.⁷⁴ The total number of genetic changes was almost two times higher in tumors with the inherited mutations in these TSGs than in the control group. Furthermore, the cytogenetic losses in *BRCA1* tumors differed from those with *BRCA2* tumors and both differed from the control group. It seems that accumulation of somatic genetic changes during tumor progression may follow unique pathways, depending on the underlying inherited genotype of the individual.

In keeping with the current trend to tie cytogenetic findings to underlying molecular mechanisms, gene-expression profile studies were undertaken in these same populations of hereditary and sporadic breast cancers.⁷⁵ Analysis revealed 176 genes that were differentially expressed in tumors with *BRCA1* mutations and tumors with *BRCA2* mutations. The researchers concluded that a heritable mutation influences not only the cytogenetic profile but also the gene-expression profile of the breast cancer.

Increased Chromosomal Breakage and Defective Deoxyribonucleic Acid Repair

In contrast to the acquired somatic chromosomal aberrations described above, there are a variety of inherited disorders that are characterized by intrinsic abnormalities in the mechanisms that repair chromosomal and DNA damage. These are all associated with increased risks for congenital abnormalities as well as cancer. Examples include defective DNA repair in Ataxia Telangiectasia,⁷⁶ Xeroderma Pigmentosa (XP), Cockayne syndrome (CS) and trichothiodystrophy (TID),⁷⁷ Bloom syndrome,⁷⁸ and Fanconi anemia.⁷⁹⁻⁸¹ All these involve mutations in one or more genes that code for proteins necessary for maintaining stability and fidelity of the chromosomes and/or genes. For example, people with nucleotide excision repair defects causing XP, CS, or TID have an extremely acute sensitivity to sunlight. This is due to the inability to remove a wide array of DNA problems related to exposure of short wave ultraviolet (UV) light. Defective repair also results in genetic instability leading to increased chromosome abnormalities and mutagenesis that may predispose to skin cancer. People with Bloom syndrome have spontaneous hypermutability on the molecular gene level and on the microscopic chromosomal level. This is manifest in cells of patients with Bloom syndrome by visible chromatid gaps, breaks, and rearrangements as well as mutations in specific loci. Cells from patients with Fanconi anemia show characteristic chromosome breakage, especially when challenged with certain chemicals. Patients with these types of defects are prone to birth defects, developmental problems, and malignancies of hematologic and other types,

thus demonstrating the multiple roles of these types of genes in development and differentiation as well as mature cell functioning.

Mismatch Repair Genes, Replication Errors, Associated With Hereditary Nonpolyposis Colorectal Cancer

The discovery of the genes causing mismatch repair (MMR) followed a proposal that a hypermutable phenotype resulting in instability at certain DNA locations would be necessary for some types of tumors to develop.⁵⁹⁻⁸² In 1993, an unusual form of somatic mutations predicted by this hypothesis was discovered independently by three laboratories.⁸³⁻⁸⁵ The phenomenon was originally called replication errors (RER) by some and MIN by others but is now known by scientific consensus as microsatellite instability (MIS) by most researchers.⁸⁶ Within the year, several groups reported mapping and then the first of the MMR genes, *MSH2*, a human homologue to a yeast DNA repair gene.⁸⁷⁻⁸⁹ Shortly after the first locus was mapped to chromosome 2p, a second HNPCC locus was discovered on 3p coding for *MLH1*.⁹⁰⁻⁹³ Conceptualizations of the mechanisms by which the MMR genes act are available.^{94,95} Since then, other related genes have been discovered, including *PMS2*, *MSH3*, and *MSH6*.⁹⁶⁻⁹⁸ Mutations in *MSH2* and *MLH1* are the most common and probably make roughly equal contributions to the increased risk of cancer due to HNPCC.

The MMR genes perform “caretaker” roles in the genome in that they repair the DNA mismatches that are sometimes created during new strand synthesis by DNA polymerase. These mismatches deform the DNA helix and are recognized by *MSH2* and *MSH6*. These form a complex with the other MMR elements to excise and repair the mismatch. When elements of this complex are abnormal, efficient repair does not occur and there is several-fold increase in mismatches.⁹⁹ This results in very large numbers of small deletions or insertions at microsatellite sequences, a phenomenon known as high microsatellite instability or MSI-H. Mutations in *MSH6* have a slightly different molecular and clinical phenotype, which may not include high levels of MSI. Reviews of the complete molecular genetics of mismatch repair are available elsewhere.^{100,101}

The impact of mutations in MMR genes is manifest at both the molecular and the clinical levels. Mutations in any one of several MMR genes cause a genetic syndrome known as Hereditary Non-Polyposis Colon Cancer (HNPCC), also known as Lynch syndrome.¹⁰² This clinical entity is characterized by colorectal, endometrial, and other cancers. Persons who inherit an inactivating germline mutation in one of the MMR genes carry one copy of the normal and one copy of the mutated allele. Once the wild-type allele is lost in a somatic cell, it acquires the hypermutable phenotype that facilitates the rapid accumulation of other mutations that are permissive of malignancy, either directly or through mutating additional oncogenes and TSGs. Thus, genetic testing can be incorporated into clinical care of people in families with HNPCC.¹⁰³

Gene Amplification

The “volume” of a normal gene message can be turned up or amplified. This may occur through either gene amplification or increased gene expression. Gene amplification is generally a characteristic of certain oncogenes. Gene amplification occurs when errors in replication lead to the presence of multiple copies of an oncogene, which give the cell extra messages to grow. Cells containing an amplified oncogene may thus have an advantage over surrounding cells and contribute to the aggressive growth of the malignancy.¹⁰⁴ Recurrent oncogene amplifications are common in a wide variety of human cancers. Genes that are commonly amplified in cancer tissues include members of the *Myc* and *Ras* families of oncogenes, growth factor receptors such as *Her-2/neu*, and genes involved in cell-cycle regulation. Evidence for gene amplification can have clinical significance, in terms of both prognosis and treatment. A specific example of clinically relevant genetic evaluation is the association of amplification of the *N-myc* oncogene with the most aggressive cases of neuroblastoma. Another example is *Her-2/neu* amplification in breast cancer being targeted for rationale chemotherapy strategies.

Epigenetic Changes: Imprinting, Hypermethylation, and Cancer

Genomic imprinting is another way that genetic material can be altered in cancer and other conditions.¹⁰⁵ It is defined as a “an epigenetic modification of a specific parental allele of a gene, or the chromosome on which it resides, in the gamete or zygote leading to differential expression of the two alleles of the gene in somatic cells of the offspring.”^{106(p95)} In other words, some genes are chemically marked or imprinted so that they will be expressed differently, depending on whether they are inherited from the mother or the father. The imprinting is referred to as epigenetic because it does not involve a change in DNA sequence when changing allele expression. Imprinting of certain genes is essential in normal human development.

The mechanism of imprinting is believed to involve DNA methylation of cytosine or of CpG islands. These chromosomal areas rich in CpG dinucleotides are believed to be important in control of gene transcription.

Several chromosomes show parental origin-specific alterations in cancer. Beckwith-Wiedemann syndrome, which involves both developmental abnormalities and malignancy, sometimes involves parental origin-specific germ-line chromosomal rearrangements. Adult cancers, such as leukemias, also demonstrate evidence of imprinting.

Loss of imprinting (LOI) is a genetic alteration that involves loss of parental origin-specific gene expression. Often in cancer it is the paternal allele that is silenced but not always. Loss of imprinting may result in activation of the normally silent copy of oncogenes and/or down-regulation of TSGs. These changes can be seen in a number of conditions such as Wilms’ tumor; bilateral retinoblastoma; embryonal rhabdomyosarcoma; hepatoblastoma; osteosarcoma; Ewing sarcoma; paraganglioma, neurofibromato-

sis type I; Multiple Endocrine Neoplasia, type 2B; uterine, cervical, esophageal, prostate, lung, and colon cancers; choriocarcinoma; and germ-cell tumors.

An important clinical implication of discovery of LOI in human cancer is that it is potentially reversible. This may have therapeutic implications.

Viruses and Cancer

RNA and DNA viruses have been implicated in the development of several cancers, such as cervical, hepatic, Kaposi, and Burkett's lymphoma.¹⁰⁷⁻¹⁰⁹ Although the infections may play an initiating or contributing role to the process of carcinogenesis, additional changes in the cellular (host) DNA are usually required before the full cancer phenotype is demonstrated. The mechanisms by which the RNA or DNA viruses impact the development of cancers is through their promotion of cellular proliferation, disruption of host cell cycle control, DNA repair mechanisms, and apoptosis, in addition to causing host immunosuppression. A full description of the biology of RNA and DNA viruses and their associated cancers is beyond the scope of this chapter. Table 1 presents a list of the various RNA and DNA viruses, the cancers with which they are associated, and the disruptive processes that have been described for each virus.

Conclusion

Studying malignancies at the single gene level has provided dramatic understanding of genes involved in basic biological

processes, such as cell-cycle control, aging, and proliferation. Models of carcinogenesis incorporate views of biological changes through various malignant stages accompanied by causative genetics changes. The process of identifying cancer-relevant genes will undoubtedly continue into the foreseeable future.

In addition, a new stream of cancer genetics research is beginning to gain momentum, that of development of technologies that foster understanding of multiple biological factors acting in concert. This type of research is happening on the DNA, RNA, and protein levels through integration of sophisticated genetics, molecular biology, pathology, biochemistry, use of transgenic and knock-out animal models, and bioinformatics advances.

Molecular profiling of human tumors using microarray technologies have shown that there may be dozens or even hundreds of genetic alterations that accumulate along the path from a cell being normal to malignant.¹¹⁰ For example, DNA microarrays allow for simultaneous detection of somatic mutations or polymorphisms in multiple alleles from a tumor. Gene expression arrays are used to detect which genes are turned on and which turned off in an given tumor cells compared with ones that are either normal or from premalignant tissue. Gene expression profiles can predict the aggressive behavior of breast cancer cells.¹¹¹ There is preliminary evidence that an artificial intelligence-based algorithm can be used to analyze multiple proteomic data points to produce recognition patterns with high accuracy for detecting presence of biopsy proven prostate or ovarian cancers.¹¹² One laboratory recently introduced the integrated genomic and proteomic analyses of a systematically

Table 1 • Viruses Associated With Malignancies

Viral Agent	Related Cancer	Proposed Mechanism of Carcinogenesis Induction
Deoxyribonucleic acid viruses		
Hepatitis B Virus	Hepatocellular carcinoma	Persistent viremia (5% of infected individuals) produces prolonged liver cell proliferation, in response to latent infection, and increases opportunities for mutations to occur
Human Papilloma Virus (HPV)	Genital tract cancers	Encode oncoproteins E6 and E7 that bind to cell regulatory proteins p53 and RB
Epstein-Barr Virus (EBV)	Burkitt Lymphoma	Latent infection causes increases in the host's NFκ-B, c-myc, immune globulin, and RAG gene expression and prevents apoptosis
Kaposi Sarcoma-Associated Herpesvirus (HHV-8)	Kaposi Sarcoma	Prolonged spindle cell proliferation related to latent infection with HHV-8. Prolonged proliferation increases the chance of mutation occurring.
RNA viruses		
HIV/Kaposi Sarcoma	Kaposi Sarcoma	As above with addition of immunosuppression due to HIV infection
HIV/HPV	Genital tract cancers	Same
HIV/EBV	Burkitt lymphoma	Same
Human T-Cell Leukemia Virus Type I	Adult T-cell leukemia	Tax protein interacts with host cellular proteins involved in transcription, cell-cycle regulation, cell proliferation, and apoptosis causing immortalization and propagation of cells, which increase the chance of a transformed clone to emerge.
Hepatitis C Virus	Hepatocellular carcinoma	Not entirely clear. May act indirectly as a result of inflammatory responses that lead to liver cell destruction, regeneration and fibrosis.

perturbed metabolic pathway through analysis of DNA microarrays, quantitative proteomics, and databases of known physical interactions.¹¹³ If these types of genomic and proteomic procedures are validated in future studies, they will profoundly alter each phase of oncology care.

We now know that the carcinogenic process is more complex than the linear accumulation of deleterious mutations. For a potentially premalignant clone to evolve into cancer, it must not only proliferate faster than its neighbors but also overcome the natural growth-inhibiting mechanisms, such as apoptosis and senescence, that are present in normal cells.¹¹⁴

Furthermore, it is not just the cancer cell that is disturbed in the carcinogenic process but the ancillary cells that also collaborate in the process. Hanahan and Weinberg¹² use the term *heterotypic cell biology* to refer to the process by which mutant cancer cells have conscripted and used normal cell types (eg, fibroblasts and endothelial cells) to support the neoplastic process via paracrine signals from neighboring cells, systemic endocrine signals, and/or cell-to-cell signals. Liotta and Kohn cite evidence that “invasion occurs within a tumor–host microecology, where stroma and tumor cells exchange enzymes and cytokines that modify the local extracellular matrix, stimulate migration, and promote proliferation and survival.”^{115(p375)}

For more detail on applications of cancer genetics to oncology practice, see upcoming module 4 on cancer susceptibility testing²⁵ and module 5 on genetic applications in cancer prevention, diagnosis, prognosis, treatment, and monitoring.⁷³

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