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INTRODUCTION

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WHAT GOES WRONG IN CANCER

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INTRODUCTION

In this chapter we give a brief overview of current ideas about the biology of cancer. We paint with a broad brush and illustrate what goes wrong, using as examples some of the disease's more dramatic and central processes. We then summarize newer genetic and biochemical information and how it is being used in treatment. These ideas are discussed in more detail in later chapters.

THE DISEASE

About a third of humans develop cancer in a lifetime. Cancer starts as an abnormal cell which grows with time into a mass of cells, some of which can spread to other

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locations in the body (*metastasize*), where they grow and upset normal bodily functions. It is one of the most frequent causes of human death. The rate of death varies greatly for different types of cancer. Lung and pancreatic cancer are the worst, usually fatal within a year. But not all cancers are fatal: Only one-fifth of breast cases result in death. Successful treatments utilize surgery, radiation, drugs, and immunology.

Cancer is a complicated set of diseases. About 200 varieties have been described, whose properties and treatments are different. There are three main types. *Carcinomas* (90%) are solid cancers (e.g., solid tumors) that arise from the epithelial cells that cover our inner and outer surfaces. *Sarcomas* are solid cancers developed from the connective tissue cells that form body structures such as muscle and bone. *Leukemias* and *lymphomas* are cancers of white blood cells. Leukemias of early childhood differ from adult leukemias in their properties and treatments. Cancers are named according to the organ from which they came. Retinoblastoma is mainly a cancer of the eye, osteosarcoma of bone, and melanoma of skin pigment cells. Lung, colon, prostate, and breast cancers are the most common.

Frequencies of various cancers vary greatly between countries. These differences are not inherited but are environmental; second-generation Japanese in California have a tenfold higher death rate from prostate cancer than do Japanese in Japan. Studies of population environments reveal carcinogenic agents: for example, particular diets high in calories, fat, and meat are bad, and diets high in fibers and fruits are good. Colon cancer is tenfold higher in women from countries in which high quantities of meat ($\frac{1}{2}$ pound per day) are eaten. Japanese have a high level of stomach cancer, related to the fern fronds that they eat. Lung cancer correlates with increased smoking; it is five times higher in Britain than in Norway, where only one-fourth as many cigarettes are smoked per person. It increased in men about 15-fold since 1930, when smoking became prevalent, but increased much later in women. Skin cancer develops based on excessive exposure to sunlight, especially for races with light skin pigmentation. The probability of getting cancer can be decreased by avoiding smoking, a high-meat diet, and excessive sun exposure. Leukemias are frequently developed following exposure to radiation.

GENES, MUTATIONS, AND CANCER

These connections with environmental factors suggest that some cancers could originate from agents that change a cell's genetic material (mutation). Each of the more than 100 trillion cells in a human body carries its genetic information in *deoxyribonucleic acid* (DNA), composed of long double-helical strands (see Figure 2) made of sequences of four building blocks (bases) linked in pairs. It is packaged in 23 pairs of chromosomes which can be seen with a microscope. The DNA in each cell carries information equal to the letters in 600 encyclopedia volumes. *Genes* are sequences of DNA that code for individual proteins.

Mutations are errors in DNA structure that alter this genetic information. Most mutations arise spontaneously, possibly from mistakes that arise while DNA duplicates during cell growth. Experiments have shown that foods contain many chemicals that cause carcinogenic damage to DNA. Errors can also be produced by damage from toxic chemicals (*carcinogens*) or radiation. Cell growth is stopped when molecular mechanisms termed *checkpoints* sense the damage, recruit the molecules to rectify the problem, and give time for corrections to be made. Then enzymes for repair are activated, and the cell may recover if the damage was not too severe. Genes designated *BRCA1* and *BRCA2* are involved in DNA repair and are mutated in some breast and ovarian cancers. The inability to repair damaged DNA may result in cancer.

Visible changes in the structure of chromosomes in cancer cells provide direct evidence for the genetic basis of cancer. Rearrangements at many definite positions have been observed repeatedly in many types of cancers (Figure 1). At the molecular level are found miscoding changes, including substitutions, deletions, duplications, and rearrangements of DNA building blocks. For example, in a recent study of breast and colon cancers, 189 genes (average 11 per tumor) were frequently found to be mutated. In several cancers, mutations change the functioning of genes located at their positions. DNA is often altered in human chromosome 6 at position p21, where the cancer-related K-*ras oncogene*, a gene that may modify cell growth aberrantly and lead to cancer, is located. Additional copies of a particular gene make a cell resistant to the anticancer drug methotrexate. Rare cancers are produced by virus infection; for example, introduction of genetic material by the human papilloma virus causes cervical cancers. This provides further evidence for the genetic basis of cancer.

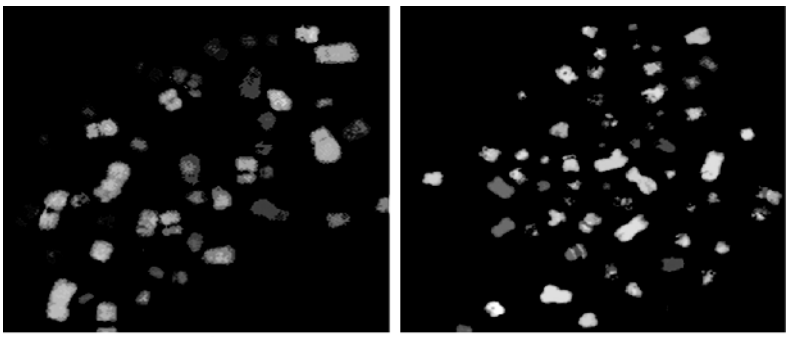


Figure 1. Cancer-related chromosomal aberrations. In early stages of cancer, chromosomes break and join with segments of other chromosomes that are not adjacent in normal cells (chromosomal translocations). As a consequence, genes that control cell growth and specialized properties of cells are frequently rearranged. Other cancer-related alterations that result from reorganization are in cell adhesion and motility as well as in capacity to invade and grow in tissues at distant sites from the initial tumor (metastasis). (A) normal chromosomes (note that each chromosome is a single color); (B) chromosomes in cancer cells that contain fused segments of multiple chromosomes (note multicolored chromosomes). (See insert for color representation.)

Some people who are related genetically carry a DNA defect that might cause a relatively rare inherited cancer, of which there are about 30 types. Seven percent of breast and ovarian cancers are hereditary; mutations of breast cancer–associated genes (BRCAs) are a common cause (50%). The hereditary autosomal polyposis gene (APC) causes growths (polyps) in the colon that develop into cancers. Spontaneous mutations of APCs are also found in nonhereditary cancers. Prostate cancer is more frequent in persons descended from Africans, whose sequence of a gene that involves the male hormone differs from that of Europeans.

A *tumor* is an excessive localized growth of cells which is usually not fatal if detected early and immediately treated. But many of its properties progress from bad to worse with time, from a series of mutations followed by selection of those multimutated cells that grow faster; hence, it develops into a mass of differently mutated cells. This multiple-step process must alter perhaps a half-dozen genes to produce a clinical cancer. Furthermore, mutational ''hits'' on both of a pair of genes are usually necessary for a biological effect, because one mutation can be masked by functioning of the nonmutated partner. In cases of such multiple hits, cells can lose control of their growth and their ability to develop into specialized cells (to *differentiate*). The consequences of mutations that set the stage for metastasis are particularly devastating. Tumors become lethal (*malignant*) cancers that spread to other locations in the body (metastasis), where their cells interfere with normal body functions. They can also release molecules that modify other cells. Metastasis causes 90% of cancer deaths.

After a tumor is initiated, it can take 20 to 30 years to become clinically apparent. Accumulation of all the mutations takes time, so human cancers can develop over decades. Cancer deaths increase dramatically (exponentially) with age; they are five times higher for 80-year-olds than for 50-year-olds. The normal mutation rate is not high enough to produce the several required mutations in a lifetime in even one of a person's 100 trillion cells. Some mutations can speed up the mutational process 25-fold or so. This accelerated mutation rate creates genetic instability, due to inactivation of DNA repair genes or changes at the ends (*telomeres*) of chromosomes that prevent their proper separation between cells at division.

CANCER CELL BIOLOGY

Cells are the units of life. Normal cells act on each other to control their growth and other properties in balance with the entire organism. They are closely regulated by a variety of genetic and biochemical processes. For example, biological feedbacks act in much the same way that a thermostat controls heat production by a furnace. Cancer is a disease of ''outlaw'' cells, cells that have lost their normal relationship to the whole organism. A tumor originates when single normal cells mutate and develop into cancer cells, termed *transformed cells*. Mutations produce defects in

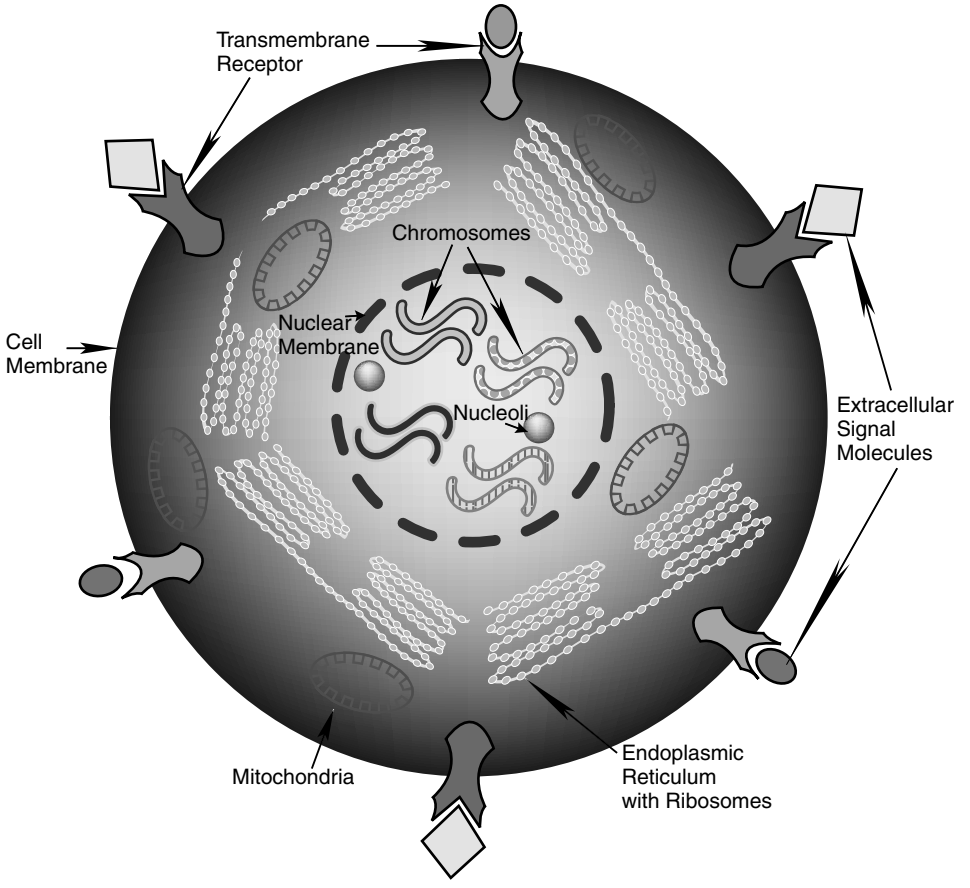
their cellular regulatory mechanisms, changing their biochemistry and biology so that they differ from normal cells in structure and functioning and grow at the wrong times and in the wrong places (Figure 2).

Briefly, each cell is surrounded by a membrane that separates it from its surroundings, which include other cells, nutrients, and molecules that regulate growth and other functions. Within the cell is a fluid, *cytoplasm*, containing proteins and structures, including mitochondria (the source of energy for a cell), that produce chemical energy and the machinery (ribosomes) that synthesizes proteins. The nucleus, which contains the genetic material, sits in the middle of the cell. Location within a cell can determine a molecule's possible biochemical interactions and effects. Cells of cancers develop into disorganized arrangements, and their nuclear shapes are abnormal, properties that are scrutinized carefully during diagnosis and are used to classify the *stage* of a cancer.

Regulatory machinery in the cells is organized architecturally within the nucleus and cytoplasm as well as in the plasma membrane that surrounds the cell and in the nuclear membrane that separates the nucleus from the cytoplasm (Figure 2). Solid tumors, leukemias, and lymphomas exhibit striking changes in cell and tissue structure that are linked to the onset and progression of cancer. Modifications occur in the cell size and shape; in the compartmentalization (packaging/location) of factors that control gene expression, replication, repair, protein synthesis, and exchange of regulatory signals; and in the representation and organization of cells within tumors.

General and tumor-type specific modifications in nuclear organization are long-standing indications of cancer. Many cancers have alterations in the number and composition of *nucleoli* (see the small orange spheres in the cell nucleus in Figure 2), the focal sites within the cell nucleus for ribosomal gene expression that supports protein synthesis. Chromosomal rearrangements are prevalent in cancer. Modifications in plasma membrane–associated receptors modify responses of the tumor cell to growth factors. Changes in *integrins*, molecules that mediate communication between the extracellular environment and the cytoplasm within a cancer cell, influence the transmission of information (Figure 3). Cancer-related alterations occur in the exchange of signals between the cell nucleus and cytoplasm, which are critical for control of cell regulatory machinery. These changes provide insight into cellular and molecular parameters of cancer that facilitate tumor diagnosis and are targets for therapy. Effects of mutation that are found in most cancer cells are failures of molecular mechanisms that limit growth and differentiation into specialized cells, causing their death (apoptosis), their movement out of the tumor (metastasis), and the activation of a blood supply, which is required to feed the tumor (angiogenesis).

Normal cells of an adult animal usually are not growing. They can be stimulated to increase in number (proliferate) upon changes in external conditions such as increased concentration of growth factor proteins or hormones, or elimination of contacting cells through death or by wounding. A single cell must double all its parts and divide to produce two daughter cells. This is a sequence of events termed


Transmembrane
Receptor
Chromosomes
Nuclear
Membrane
Cell
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Nucleoli
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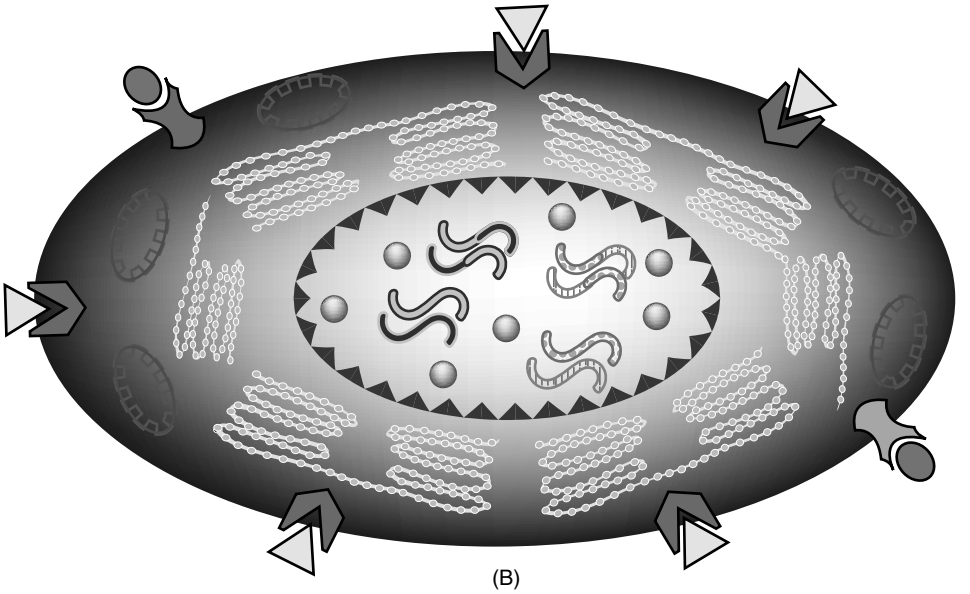
with Ribosomes

(A)



(B)

the *cell cycle* (Figure 4). It is similar in content and timing for normal and cancer cells. This process is repeated many times to produce the many cells of an organism. Cancers grow because they can initiate their cell cycles independent of external growth factors and inhibitions by contacting cells, or they are stimulated by their mutated internal machinery. Elimination by mutation or inactivation of inhibitory tumor suppressors (such as the retinoblastoma protein) releases constraints on proliferation, and control of cell division is lost (see below). Dozens of these genes are misregulated in cancers.

Stem cells are *multipotential* in nature. They can develop into *any* type of tissue (differentiate) and thereby create various tissues and eventually ''build'' organs such as muscle, liver, or blood. The differentiated cells function in specialized ways, and most of them stop growing. Stem cells fail to stop dividing. Tumors contain immature cells that exhibit differentiation failures. Such a rare defectively differentiated subset of stem cells in tissues has been proposed as the origin of tumor cells. An example is acute promyelocytic leukemia, where stem cells have been blocked from achieving differentiation.

Normal cells can stop growing permanently, a process of arrest that is designated cell *senescence*. Cells survive for varying lengths of times. At one extreme, brain cells might last a lifetime, but white cells in blood survive for only about two months. Cancer cells, in contrast, are immortalized and have an unlimited potential to proliferate. This indefinite proliferation requires activity of *telomerase*, an enzyme that at each cycle of the cell adds back DNA sequences of telomeres to the ends of chromosomes. Telomerase is active in malignant cancer cells but not in normal cells.

Cells sense defects in their functioning, which causes them to commit suicide, called programmed cell death or *apoptosis*, thereby removing defective cells. In fact, many cells of a tumor die spontaneously; the tumor's size increases because proliferation exceeds death. Cancer cells very often become mutated to decrease apoptosis. The p53 tumor suppressor gene is the most often altered growth regulatory gene in cancers. Because the p53 gene is central to activating apoptosis, it has been called the guardian of the genome. Again, in this struggle for survival the cancer cells that resist apoptosis are selected; they have an advantage for

Figure 2. Structure and organization of regulatory machinery in (A) normal and (B) cancer cells. The organization and location of machinery that controls genes is modified during the onset and progression of cancer. The transition from a round or cuboidal to an elongated cell is characteristic of early-stage tumors. Changes that are frequently observed in tumor cells are in the cell membrane, receptors for transduction of signals from the outside to the inside of the cell (the cell's communication system), exchange of chromosome segments, and an increased number of nucleoli which support synthesis of proteins. Often, the nuclear membrane that controls exchange of information between the cell nucleus and cytoplasm is modified (see the purple dashed-line circle surrounding the center of the ''normal cell''). These changes in cell structure and location of genes and regulatory molecules result in the development and spread of cancer. The altered organization of the cell's regulatory machinery is important for cancer diagnosis and provides targets for treatment. (See insert for color representation.)

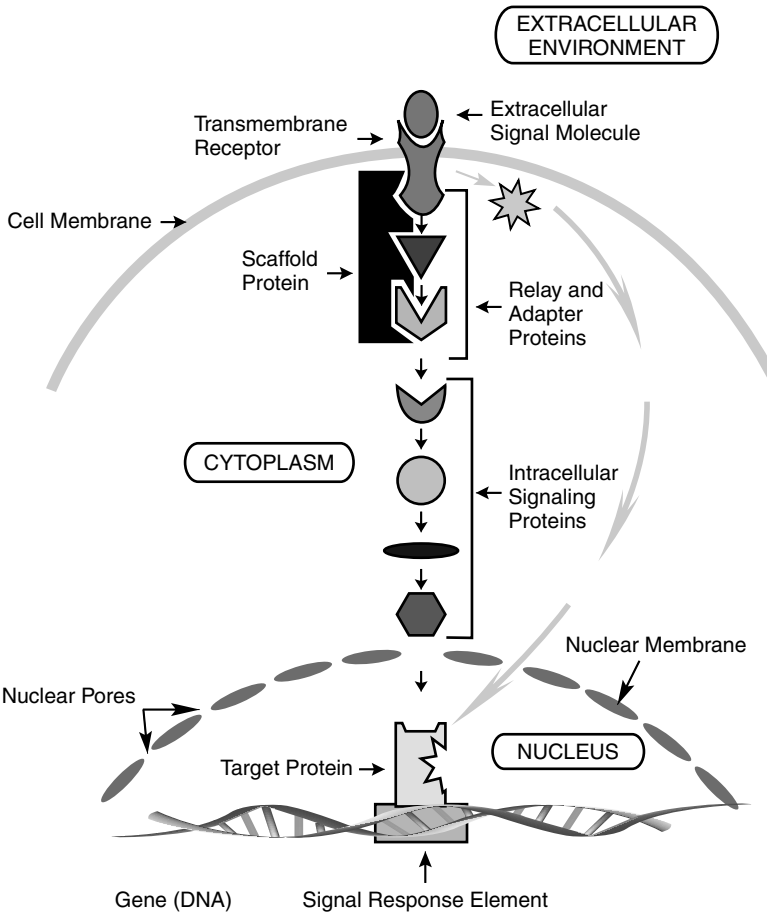


Figure 3. Cell signaling. Cells communicate and respond to the extracellular environment through a process designated *signal transduction*. Signal molecules bind to transmembrane receptors that span the cell membrane. The interaction of signal molecules with components of receptors located outside the cell modifies the intracellular components of the receptors. An environmental signal is thereby transduced into a cascade of regulatory steps that control genes which control cell proliferation and specialized properties of cells.

In some signaling pathways, scaffold proteins assemble signaling molecules into complexes for the initial passage of information from the transmembrane receptor to relay and adaptor proteins. Subsequent steps in the signaling process amplify and integrate signals. A chain of intracellular signaling proteins processes regulatory information through the cytoplasm and into the cell nucleus to activate or suppress genes. In other signaling pathways the regulatory cascades are abbreviated. The transduction of regulatory information from the intracellular component of the transmembrane receptor is more direct, circumventing intermediary steps in information transfer. At an early stage in the signaling process a signaling protein enters the nucleus and interacts directly with genes to modify expression.

Many cancer cells exhibit defects in one or more steps of signaling cascades that alter control of cell growth, specialized cell properties, cell–cell communication, cell motility, and cell adhesion. The components of signaling pathways that are modified in tumor cells are targets for treatments that are effective and specific. (See insert for color representation.)

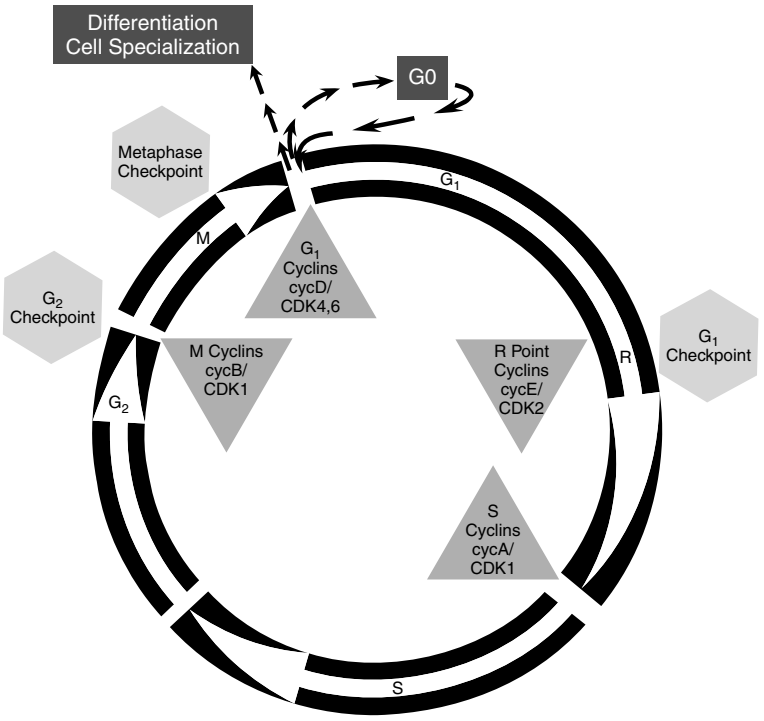
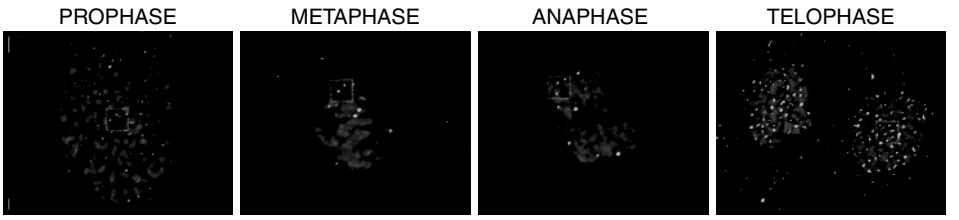


Figure 4. (A) The stages of the cell cycle. G1 is the period following cell division (M; mitosis) and precedes the S phase, the period when genes (DNA) are duplicated to provide an identical set of genes for progeny cells. Following gene duplication, the G2 period provides the cell time to prepare for cell division. The cell is regulated by cyclins (cys) and cyclin-dependent kinases (cdk) that regulate genes during each period. Early G1 is controlled by cyclin D, cdk4, and cdk6. At the restriction point (R point) late in G1, competency for gene replication and cell cycle progression is established. Control is by cyclin E and cdk2. During the S phase and into mitosis, control is by cyclin A and cdk1. During mitosis, control is by cyclin B and cdk1.

Checkpoints at three strategic locations during the cell cycle provide surveillance for effectiveness of the process. Progression of the cell cycle occurs only if fidelity of control is confirmed. The cell cycle is delayed or terminates if problems are encountered that cannot be corrected. The G1 checkpoint assesses DNA damage by chemicals or radiation and monitors adequacy of conditions to support DNA synthesis (gene duplication) and permits entry into the S phase. The G2 checkpoint determines that DNA replication has occurred after DNA is damaged and permits entry into mitosis. The metaphase checkpoint assures that chromosomes are attached to the mitotic spindle, the apparatus for distribution of genes to progeny cells during mitotic division.

(B) The stages of mitosis, the process of cell division. During prophase, the initial stage of mitosis, DNA in the nucleus (2 yards of DNA in each nucleus) is organized into chromosomes (two sets of 23 chromosomes in every human cell). The chromosomes attach to the spindle and at the completion of prophase, the membrane surrounding the nucleus disassembles. During metaphase the chromosomes attach to the mitotic spindle and align in the center of the cell. During anaphase the chromosomes move along the spindle fibers to the opposite poles of the cell. Identical sets of chromosomes (genes) are distributed to progeny cells that will be formed at the completion of cell division. Telophase, the last phase of mitosis (cell division), is initiated when the chromosomes reach the poles. The compact chromosomes now begin to disassemble. A cleavage furrow forms and deepens, culminating in the formation of two cells, each genetically, structurally, and functionally equivalent to the parent cell. (See insert for color representation.)



(B)

Figure 4. (*Continued*)

survival in the tumor environment. As an example, prostate cells undergo apoptosis if androgen (male sex hormone) is lowered. Prostate cancers that lose androgen dependence develop resistance to apoptosis.

Metastasis, which causes 90% of cancer deaths, makes surgery and radiation far less effective, because these treatments are local. Advanced cancers whose cells have undergone many different mutations develop metastases. A cell loses control of proliferation and multiplies into a primary tumor mass. Then additional mutations produce cells that escape and move through the blood or lymphatic system to other places in the body. The biology of metastasis is complex and is incompletely understood. Its several steps include increased cell migration, motility, escape into the blood, settling into a new site, and proliferation. Genes and proteins responsible for events of metastasis are being discovered; for example, cell–cell adhesion molecules that facilitate cell–cell interactions have antimetastatic activities. These adhesion molecules are dissolved by enzymes termed *proteases*, which increase in cancers, and their inhibitors are removed. Tumor cells are thereby released and enabled to populate sites that may be considerably distant from the primary tumor. A protein (maspin) that inhibits these proteases is eliminated as breast cancers progress. Metastatic cells may spread only to a specific organ in which normal cells and conditions permit attachment and growth into secondary tumors. For example, prostate cancer cells frequently metastasize to bone. This is the ''seed and soil'' hypothesis of metastasis, a framework for understanding relationships between the tumor and the tissues at the metastatic site.

Until traveling cancer cells are able to get the food and other molecules needed for growth into large cancers, they remain micrometastases. Once a critical mass of aggregate tumor cells develops in one location, a solid tumor is formed that requires sustenance to survive. *Angiogenesis* is the production by a tumor of a new blood vessel system for the purpose of providing nutrients to the tumor. Therapies for inhibiting angiogenesis are being investigated.

BASIC CANCER RESEARCH

Spectacular advances are being made in understanding mechanisms that self-regulate normal cells and defects in cancer cells. This helps us to understand the

initiation and progression of the disease and can provide targets for its treatment. In cancer, there are numerous altered structures, and amounts and degrees of cell functioning of DNA, ribonucleic acid (RNA), and proteins. Mutations can alter a gene's expression of a protein by changing controls, both genetic and biochemical. There may be cancer-related alterations that modify structure and affect the rate of a protein's (or its messenger RNA's) synthesis and degradation, and its characteristics.

Studies with humans have taught us a great deal about cancer. But this research is limited by ethics and by the requirement for immediate surgical removal of the tumor, or chemotherapy or radiation treatment, to optimize survival and quality of life. Therefore, most fundamental research is performed with animals. Among the many living creatures used for cancer research are yeasts, flies, nematode worms, sea urchins, frogs, zebrafish, mice, and rats. Mice are the animals most frequently used for basic studies, including tumor growth, metastasis, and effects of gene modifications and drugs. In these animals, injected cancer cells respond based on interactions with adjacent normal cells. An animal provides comparisons of treatment outcomes as they relate to toxicity and growth within the context of cancer cells and normal cells. Many of the insights into growth control that have come from animal studies translate directly to elucidation of control of proliferation in human cells. Human and animal cancers are similar but of course not identical, so applications to humans have to be tested in human clinical trials. Yeasts have regulatory mechanisms similar to those of human cells and are much easier to investigate genetically. Yeast studies have provided important basic information about cell proliferation.

Much fundamental research is done with cells in culture dishes, in which they can be studied conveniently. In this setting, it is easy to provide or remove molecules, including nutrients, vitamins, inhibitors, and growth factor proteins such as insulin. By comparison, difficulties are encountered with cells in animals because of their internal environment. Normal cell growth and survival require adhesion to a surface (anchorage). Cells increase in number until they cover the surface of the culture dish. When cells come into close contact with neighboring cells, they stop proliferating. This arrest is called *density-dependent growth* or *contact inhibition of growth*. A protein coating can be applied to the culture dish to provide a more natural surface than plastic, to which a set of regulatory proteins located on the cell's surface binds. Three-dimensional culture in protein gels is even more physiological (i.e., more closely represents the three-dimensional environment in the body). In contrast, tumor cells can grow in suspension, without any of the supportive mechanisms required by normal cells.

CANCER MOLECULAR BIOLOGY AND BIOCHEMISTRY

An understanding of functioning at genetic and molecular levels is very important for comprehending the workings of cancer cells and for finding therapeutic *targets*.

Most genes are inactive most of the time. Molecules that enter the cell nucleus can activate specific genes to be copied into messenger RNAs (mRNAs), which act as the working blueprints that determine sequences of the amino acids in proteins. Proteins of many types are the cell's working machinery. These proteins have many functions, including forming structures, transfering nutrients and external signals into cells, activating gene expression, acting as enzymes that speed up biochemical reactions, and performing as regulators of these reactions.

Biochemistry is organized into many sequences of enzyme-catalyzed reactions, which can produce and degrade molecules and provide energy. These reactions are tightly regulated, primarily through molecules that bind to enzymes. For example, inhibitors are small molecules that can attach loosely to enzymes and decrease their activities. A very important component of regulation is the chemical attachment of a phosphate group to a protein; this can increase or decrease an enzyme's activity, affecting its location and even its degradation. These reactions are catalyzed by a set of enzymes called *kinases*, hundreds of which target different proteins. Kinases are balanced by *phosphatases*, enzymes that remove phosphates from proteins. For example, PI3K kinase is countered by PTEN phosphatase, a tumor suppressor which when lost frequently causes cancer and activates tumor progression.

Cell proliferation usually starts with binding of external molecules to receptors of the cell. For example, estrogen binding turns on expression of genes that release normal breast and ovarian cells from the nondividing "quiescent" state. Excessive estrogen receptors overactivate proliferation in 70% of breast cancers. Chemicals such as tamoxifen, used for breast cancer therapy, compete with estrogen for this binding. Cell proliferation can also be initiated by proteins called growth factors that bind to their receptors on the cell surface. Epidermal growth factor proteins activate excessive receptors on many breast cancers. These are more aggressive and more difficult to treat than are those activated by estrogen. Drugs and antibodies that block this interaction are being used clinically. An example is the antibody herceptin (Trastuzumab), which decreases by half the risk of reappearance of hormone-independent breast cancer.

Activating signals must pass through the cell's cytoplasm and into the nucleus within the cell, where genes are located. Molecular signals convey regulatory information into and out of cells as well as between cells. There are three major signaling pathways, each composed of a cascade of kinase reactions that move and amplify signals (Figure 3). Various mutant genes in cancers turn on these processes. Changes in the multiple steps in signaling cascades often occur during the onset and progression of cancer, providing options for cancer detection and are also targets for treatment.

Of primary interest to cancer research is the cycle of events during a cell's duplication (Figure 4). A growing cell can pass though the cell cycle in a day or so to produce two daughter cells. At different times during this cycle all components of the cell must be duplicated. DNA is duplicated near midcycle, which divides the cycle into four biochemically different phases (G1, S, G2, and M), followed by division.

In the initial phase (G1) of the cell cycle, molecular machinery for DNA synthesis is produced and activated. The regulating circuitry is complicated; details are discussed in later chapters. Briefly, *cyclins*, a series of short-lived proteins that increase and then decrease at definite times in the cell cycle, are central to regulating all stages of cell proliferation. Their defects are important in cancer. Cyclin D1, which appears early in the G1 phase of the cell cycle, activates a cyclin-dependent kinase (Cdk) that adds phosphate molecules to proteins that control progression through the cell cycle. It is overexpressed in many types of human tumors (e.g., 50% of human breast cancers). This causes contact-independent growth by the cells and thereby increases the risk of early metastasis. Breast cancers that have progressed to overproduce altered cyclin E are no longer responsive to drug treatment. Cyclin A1 activity is a variant form of cyclin A that is elevated in cancer cells but is normally produced only in embryos. Other proteins, such as INK4, inhibit Cdks, are negative regulators of proliferation, and are frequently mutated to be inactive or are lost in cancers.

The *restriction point* is a most critical event for control of the cell cycle. Normal cells in the G1 phase select between continuing to proliferate or returning to quiescence, which depends on whether external conditions are suitable for growth. The cell must accumulate enough of a protein to activate a Cdk that phosphorylates retinoblastoma protein (pRb), a regulatory factor that suppresses cell growth. Thereby, pRb releases the gene-activating protein that is designated E2F-1. Enzymes are then produced that catalyze DNA synthesis. Restriction point control is diminished in cancers, and tumor cells therefore proliferate under conditions that would stop normal cells from dividing. pRb, a principal regulator of growth control, is mutated in the majority of human cancers. Loss of growth regulatory proteins makes the cells insensitive to antigrowth factors and to the requirement for Cdk-cyclins. Under these conditions, the ability of a cell to control proliferation is out of reach.

The second cell cycle phase is S, named for the synthesis of DNA. During this period of the cell cycle, proteins designated *histones* are synthesized to package newly replicated DNA into chromosomes. Then follows the short G2 phase, which is preparatory for the cell to enter mitosis (M phase). In mitosis the duplicated chromosomes are distributed equally to the two progeny cells produced by cell division. Errors of chromosome separation take place in a cancer cell during mitosis and cell division, especially if their DNA has been damaged, which creates further mutations and advances the cancer.

As a summary of self-regulation, a mutation alters the structure of a gene, which can change a regulatory protein and in turn cause the loss of control of cellular processes such as proliferation, leading to cancer. The mutations that cause cancer alter structures and positions of genes controlling biochemical balances in cells. These, in turn, change the amounts and properties of critical regulatory proteins. These proteins are specifically designed to modify the function of an enzyme or another protein involved in cell regulation. Proteins do this by binding to a location of the target protein other than the normal site for activity, thereby changing its

structure. Regulation mechanisms are even more complex. The structure and function of an active protein or its regulatory partner are often modified by strong bonding of a small molecule, such as phosphate or acetate, as mentioned above, or by weaker binding of other molecules. Among the most frequently mutated proteins are the cyclins, which activate cyclin-dependent kinases; the retinoblastoma protein, which inactivates protein E2F-1 and thereby disrupts the copying of many genes into their mRNAs; and the p53 protein, which activates the programmed death mechanism, which can eliminate cancer cells.

CHEMOTHERAPY

Surgery is the primary method of treating cancer. For example, the standard for patients with early breast cancer is surgical removal with a wide local margin, followed by x-radiation. Why are they not cured? Surgery can be effective only if the cancer has not already metastasized. A cancer that does not get early treatment is likely to reappear. These procedures are therefore often followed by chemotherapy (often referred to as *adjuvent chemotherapy*). Chemotherapy poses many difficult problems. Anticancer drugs are poisons that must kill most of the cancer cells but also must not kill too many normal cells and thereby the patient. Selection is difficult because the cells are similar. Cells can develop resistance to drugs, so a carefully tested small set of drugs is applied to the tumor, chosen from the several dozen currently available. These drugs must be applied with proper dosage and schedule and be supervised carefully. Illnesses can develop from treatment. Also, not all drugs can help all patients, since each person and each tumor is genetically different. Some cells treated by chemotherapy can survive and grow into drug-resistant cancers. Treatment-related diseases can develop later. Many of the drugs that kill tumors can cause mutations that transform normal cells to cancer. Quality of life for the cancer survivor after completion of treatment is an important consideration. Cancer prevention and reduction in the risk of recurrence can significantly influence the life-threatening consequences of cancer. Ongoing research is determining methods of cancer prevention (e.g., intake of substances such as antioxidants that can prevent cancer or tumor recurrences, management of diet, and use of natural products or synthetic compounds that can decrease the risk of mutations).

Research on drug treatments is a continuous competition of human inventiveness against the constantly changing defenses of cancer cells. Discovery of differences between normal and tumor cells is necessary to provide a selective drug target. A cell is in a dynamic ‘‘steady-state’’ balance between positive and negative control systems, similar to the motor and brakes that control movement of an automobile. These regulatory balances operate at many levels: genetic, biochemical, structural, and cellular. Examples are cell proliferation versus death, oncogenes and tumor suppressor genes, syntheses versus removal of proteins

and mRNAs, and kinases versus phosphatases. These complex components of cellular control are different in normal and cancer cells.

The molecular changes that create cancers have been found to provide targets for therapy. This is the Achilles' heel principle: an advantage can create a weakness. A major difference in cancer cells from normal cells is that cancer cells frequently make DNA to support the rapid rate of proliferation. This has provided a therapeutic target for drugs that attack the DNA replication process. These include the DNA building block analogs that disrupt DNA replication (e.g., 5-fluorodeoxyuridine, cytarabine) and inhibitors of DNA synthesis (e.g., methotrexate). Cells in the S phase are more sensitive to therapeutic agents that damage DNA (e.g., radiation, cyclophosphamide, *cis*-platinum). A therapeutic challenge here is that normal blood-forming and intestinal cells also divide frequently, so they too are killed. Destruction of these healthy cells results in side effects from chemotherapy that can cause considerable discomfort for cancer patients undergoing treatment. Another difference between normal and tumor cells may be the inability of tumor cells to repair DNA damage correctly. In this way, cancer cells become mutated. Cancer cells also frequently enter mitosis, and compounds that block mitosis (e.g., taxol, vincristine) preferentially kill them. Hormone-dependent cancer can be treated by decreasing the hormone's level or its activity with a competing compound. But cancer cells can become independent of hormones and resistant to such hormone-based therapies. Another treatment is the application of an antibody that targets a growth factor's receptor on the cell surface, blocking the passage of signals for proliferation, as mentioned above.

New ways to treat cancers are being investigated. Twenty-six drugs were approved by the U.S. Food and Drug Administration in the past decade. Some are based on new knowledge about targets, often discovered in academic centers and in biotechnology companies. Newly developed therapeutic inhibitors are being applied, such as Gleevec, which inhibits the oncogenic growth regulatory factor Abl kinase, which is overexpressed in some cancers. A problem frequently encountered in cancer therapy is low specificity (i.e., the drug targets normal as well as cancer cells). Toxicity to vital organs can result from treatment with such drugs. Other therapeutic targeted drugs are modifications of established compounds. Many are marginally effective, extending life only for months. They might be most effective in combination with a classic generally toxic drug.

Novel targets are being investigated. Several mechanisms are responsible for resistance of cancer cells to apoptosis. Certain drugs can reactivate apoptosis of cancer cells and thereby make them die. For example, proteasomes are large enzymes that degrade proteins, including the p53 tumor suppressor. As a consequence, a reaction is blocked, which prevents the killing of cancer cells. Velcade inhibits proteasomes, which increases p53 tumor suppressor levels and reactivates apoptosis. Velcade is used effectively to treat various cancers, including multiple myeloma.

Expression of genes provides new therapeutic targets. The structure and function of chromatin are altered by attaching acetyl groups to the histone proteins that package genes (DNA) within the nucleus of the cell. For example, the compound SAHA inhibits removal of these acetyl groups, restores differentiation (cell specialization), and inhibits cancers. SAHA has been approved for clinical use. A major development (rewarded by the Nobel Prize in Physiology for 2006) is the discovery of naturally existing small RNA molecules that inhibit expression of specific genes. An exciting possibility is the use of these inhibitory small RNA molecules or related synthetic molecules clinically to block expressions of oncogenes.

Cancer prevention is an active area of investigation to which clinical trials are being applied, especially with persons who are at high risk, those with a premalignant condition, or those who have been treated for an initial cancer. For example, the antiestrogen drug tamoxifen has decreased the incidence of hormone-dependent breast cancer by about 50%. It has not been used widely as a preventive agent for women who are not at high risk because of problematic side effects. Compounds found in plant foods such as green tea are being tested for chemoprevention. Such plant compounds can affect cells by removing or blocking carcinogens. It is important to develop molecular tests to determine the effectiveness of chemoprevention compounds on tumors.

EARLIER DETECTION

There is no substitute for early detection. This is the patient's best opportunity for a competitive advantage against a tumor. Cancers are often detected when the disease has progressed beyond an early stage. Tumors of 100 million cells can be detected clinically, are palpable at 1 billion cells (pea size), and lethal at 1 trillion cells. Physical detection methods include x-rays and computerized tomography (CT) scan. Tumors are more likely to be treated successfully in early stages. This is demonstrated by the Papanicolaou tissue test (Pap smear), which detects early cervical cancer and has saved many lives. Other examples are mammographic breast examination and prostate-specific antigen protein tests (PSA is a protein that appears in the blood of men with prostate cancer, the level of which changes during treatment and recurrence). Methods for earlier detection of most cancers are being developed. These methods are based on detecting molecular markers such as changed DNA, mRNAs, or proteins and identifying them in tissues, blood, and other body fluids. An example is the detection of excess or structurally altered cyclin E protein, which controls cell growth during breast cancer progression and correlates with unsuccessful treatment. Simultaneous assessment of dozens or even hundreds of frequently altered biomarkers that provide a ''signature'' for a tumor will be needed. The sensitivity of these tests must be sufficient to reveal the few cancer cells among many normal cells.

SUMMARY

Cell growth and death are closely regulated by special mechanisms. The controlling molecules are specialized and are additional to a cell's functional molecules. A variety of these molecules act at multiple molecular levels to keep normal cells in balance metabolically. Cancer arises from mutations that damage these mechanisms and thereby allow cells to multiply when and where they should not. Solid cancers, leukemias, lymphomas, and myelomas exhibit changes in cell size and shape, ability to grow, compartmentalization of regulatory machinery, and competency for motility. Metastatic cancer cells result, which can be lethal. Intense scrutiny of the differences between normal and cancer cells provides targets for therapy.

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DETAILED REVIEWS OF MATERIAL IN THIS CHAPTER

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Weinberg RA. 2006. *The Biology of Cancer.* New York: Garland Sciences.

SOURCES OF FURTHER INFORMATION

National Institutes of Health website

PubMed (http://www.ncbi.nlm.nih.gov/entrez), especially for searches on reviews and key word combinations

Local libraries

Yellow Pages under ''Social Services and Health Agencies''

Cancer Information Service Hotline, 1-800-4-CANCER. National Cancer Institute, Building 31, Bethesda MD 20892

American Cancer Society, 1-800-ACS-2345. 1599 Clifton Rd. N.E., Atlanta, GA 30329.