7

Neoplasia

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Cancer is the second leading cause of death in the United States; only cardiovascular diseases exact a higher toll. Even more agonizing than the mortality rate is the emotional and physical suffering inflicted by neoplasms. Patients and the public often ask, "When will there be a cure for cancer?" The answer to this simple question is difficult, because cancer is not one disease but many disorders that share a profound growth dysregulation. Some cancers, such as Hodgkin lymphoma, are curable, whereas others, such as pancreatic adenocarcinoma, have a high mortality. The only hope for controlling cancer lies in learning more about its cause and pathogenesis, and great strides have been made in understanding its molecular basis. Indeed, some good news has emerged: cancer mortality for both men and women in the United States declined during the last decade of the twentieth century and has continued its downward course in the 21st.[1] The discussion that follows deals with both benign and malignant tumors, focusing on the basic morphologic and biologic properties of tumors and the molecular basis of carcinogenesis. We also discuss the interactions of the tumor with the host and the host response to tumors.

Nomenclature

Neoplasia means "new growth," and a new growth is called a *neoplasm. Tumor* originally applied to the swelling caused by inflammation, but the non-neoplastic usage of *tumor* has almost vanished; thus, the term is now equated with neoplasm. *Oncology* (Greek *oncos* = tumor) is the study of tumors or neoplasms.

Although all physicians know what they mean when they use the term *neoplasm,* it has been surprisingly difficult to develop an accurate definition. The eminent British oncologist Willis[2] has come closest: "A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change." We know that the persistence of tumors, even after the inciting stimulus is gone, results from *genetic alterations that are passed down to the progeny of the tumor cells. These genetic changes allow excessive and unregulated proliferation that becomes autonomous (independent of physiologic growth stimuli),* although tumors generally remain dependent on the host for their nutrition and blood supply. As we shall discuss later, the entire population of neoplastic cells within an individual tumor arises from a single cell that has incurred genetic change, and hence tumors are said to be *clonal.*

A tumor is said to be *benign* when its microscopic and gross characteristics are considered relatively innocent, implying that it will remain localized, it cannot spread to other sites, and it is generally amenable to local surgical removal; the patient generally survives. It should be noted, however, that benign tumors can produce more than localized lumps, and sometimes they are responsible for serious disease.

Malignant tumors are collectively referred to as *cancers,* derived from the Latin word for *crab,* because they adhere to any part that they seize on in an obstinate manner, similar to a crab. *Malignant,* as applied to a neoplasm, implies that the lesion can invade and destroy adjacent structures and spread to distant sites (metastasize) to cause death. Not all cancers pursue so deadly a course. Some are discovered early and are treated successfully, but the designation *malignant* always raises a red flag.

All tumors, benign and malignant, have two basic components: (1) clonal neoplastic cells that constitute their *parenchyma* and (2) reactive *stroma* made up of connective tissue, blood vessels, and variable numbers of macrophages and lymphocytes. Although the neoplastic cells largely determine a tumor's behavior and pathologic consequences, their growth and evolution is critically dependent on their stroma. An adequate stromal blood supply is requisite for the tumor cells to live and divide, and the stromal connective tissue provides the structural framework essential for the growing cells. In addition, there is cross-talk between tumor cells and stromal cells that directly influences the growth of tumors. In some tumors, the stromal support is scant and so the neoplasm is soft and fleshy. In other cases the parenchymal cells stimulate the formation of an abundant collagenous stroma, referred to as *desmoplasia.* Some demoplastic tumors—for example, some cancers of the female breast—are stony hard or *scirrhous.* The nomenclature of tumors and their biologic behavior are based primarily on the parenchymal component.

Benign Tumors. In general, benign tumors are designated by attaching the suffix *-oma* to the cell of origin. Tumors of mesenchymal cells generally follow this rule. For example, a benign tumor arising in fibrous tisssue is called a *fibroma,* whereas a benign cartilaginous tumor is a *chondroma.* In contrast, the nomenclature of benign epithelial tumors is more complex. These are variously classified, some based on their cells of origin, others on microscopic pattern, and still others on their macroscopic architecture.

Adenoma is applied to a benign epithelial neoplasm derived from glands, although they may or may not form glandular structures. On this basis, a benign epithelial neoplasm that arises from renal tubular cells growing in the form of numerous tightly clustered small glands would be termed an *adenoma,* as would a heterogeneous mass of adrenal cortical cells growing as a solid sheet. Benign epithelial neoplasms producing microscopically or macroscopically visible finger-like or warty projections from epithelial surfaces are referred to as *papillomas.* Those that form large cystic masses, as in the ovary, are referred to as *cystadenomas.* Some tumors produce papillary patterns that protrude into cystic

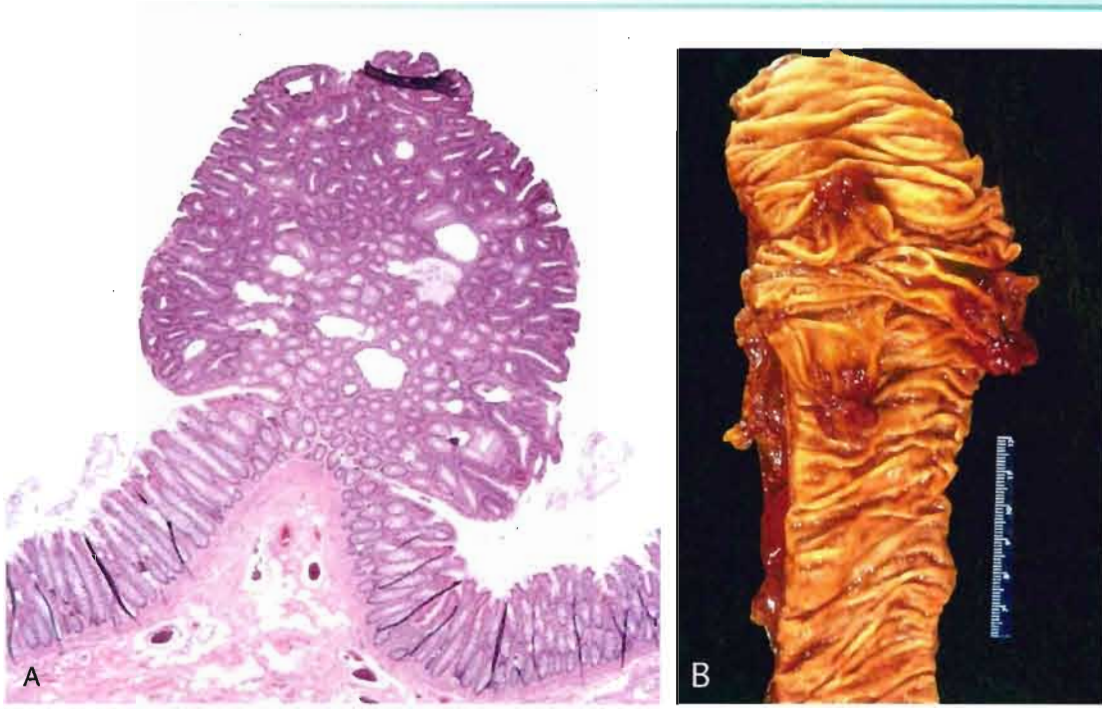


FIGURE 7–1 Colonic polyp. **A,** This benign glandular tumor (adenoma) is projecting into the colonic lumen and is attached to the mucosa by a distinct stalk. **B,** Gross appearance of several colonic polyps.

spaces and are called *papillary cystadenomas.* When a neoplasm, benign or malignant, produces a macroscopically visible projection above a mucosal surface and projects, for example, into the gastric or colonic lumen, it is termed a *polyp* (Fig. 7–1).

Malignant Tumors. The nomenclature of malignant tumors essentially follows the same schema used for benign neoplasms, with certain additions. *Malignant tumors arising in mesenchymal tissue are usually called sarcomas* (Greek *sar* = fleshy), because they have little connective tissue stroma and so are fleshy (e.g., fibrosarcoma, chondrosarcoma, leiomyosarcoma, and rhabdomyosarcoma). Malignant neoplasms of epithelial cell origin, derived from any of the three germ layers, are called *carcinomas.* Thus, cancer arising in the epidermis of ectodermal origin is a carcinoma, as is a cancer arising in the mesodermally derived cells of the renal tubules and the endodermally derived cells of the lining of the gastrointestinal tract. Carcinomas may be further qualified. *Squamous cell carcinoma* would denote a cancer in which the tumor cells resemble stratified squamous epithelium, and *adenocarcinoma* denotes a lesion in which the neoplastic epithelial cells grow in glandular patterns. Sometimes the tissue or organ of origin can be identified, as in the designation of renal cell adenocarcinoma or bronchogenic squamous cell carcinoma. Not infrequently, however, a cancer is composed of undifferentiated cells of unknown tissue origin, and must be designated merely as an undifferentiated malignant tumor.

In many benign and malignant neoplasms, the parenchymal cells bear a close resemblance to each other, as though all were derived from a single cell. Indeed, neoplasms are of monoclonal origin, as is documented later. Infrequently, divergent differentiation of a single neoplastic clone along two lineages creates what are called *mixed tumors.* The best example of this is the *mixed tumor of salivary gland origin.* These tumors contain epithelial components scattered within a myxoid stroma that sometimes contains islands of cartilage or bone (Fig. 7–2). All these elements, it is believed, arise from a single clone capable of giving rise to epithelial and myoepithelial cells; thus, the preferred designation of these neoplasms is *pleomorphic adenoma.* The great majority of neoplasms, even mixed tumors, are composed of cells representative of a single germ layer. The multifaceted mixed tumors should not be confused with a *teratoma,* which contains recognizable mature or immature cells or tissues representative of more than one germ cell layer and sometimes all three. Teratomas originate from totipotential cells such as those normally present in the ovary and testis and sometimes abnormally present in

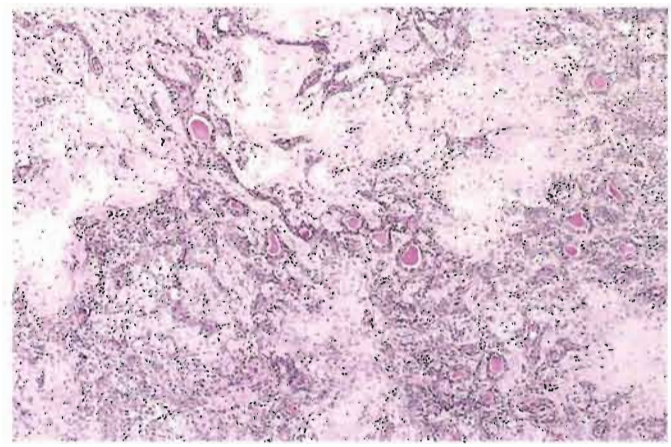


FIGURE 7–2 This mixed tumor of the parotid gland contains epithelial cells forming ducts and myxoid stroma that resembles cartilage. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

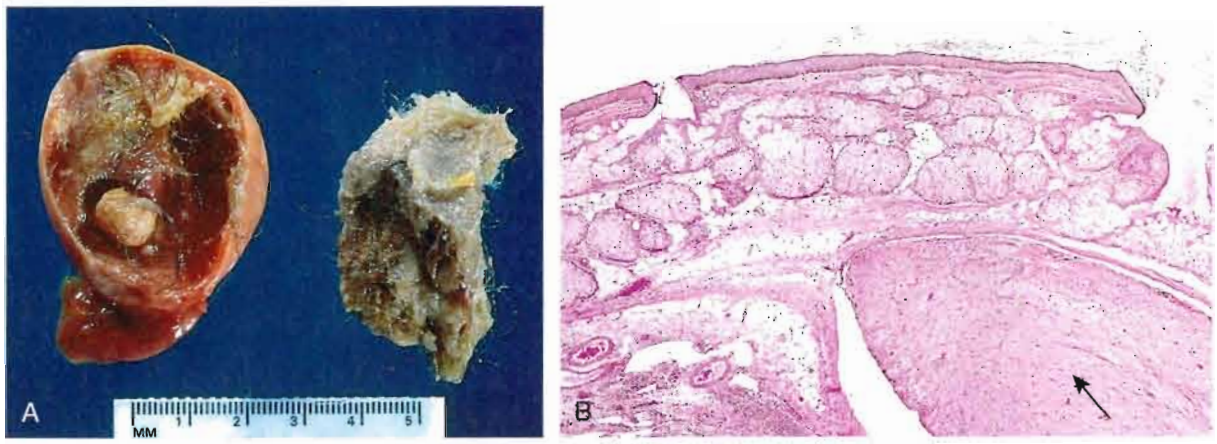


FIGURE 7–3 **A,** Gross appearance of an opened cystic teratoma of the ovary. Note the presence of hair, sebaceous material, and tooth. **B,** A microscopic view of a similar tumor shows skin, sebaceous glands, fat cells, and a tract of neural tissue *(arrow).*

sequestered midline embryonic rests. Such cells have the capacity to differentiate into any of the cell types found in the adult body and so, not surprisingly, may give rise to neoplasms that mimic, in a helter-skelter fashion, bits of bone, epithelium, muscle, fat, nerve, and other tissues. When all the component parts are well differentiated, it is a *benign (mature) teratoma*; when less well differentiated, it is an immature, potentially or overtly, *malignant teratoma.* A particularly common pattern is seen in the ovarian *cystic teratoma* (dermoid cyst), which differentiates principally along ectodermal lines to create a cystic tumor lined by skin replete with hair, sebaceous glands, and tooth structures (Fig. 7–3).

The nomenclature of the more common forms of neoplasia is presented in Table 7–1. It is evident from this compilation that there are some inappropriate but deeply entrenched usages. For generations, benign-sounding designations such as lymphoma, melanoma, mesothelioma, and seminoma have been used for certain malignant neoplasms. The converse is also true; ominous terms may be applied to trivial lesions. *Hamartomas* present as disorganized but benign-appearing masses composed of cells indigenous to the particular site. They were once thought to be a developmental malformation, unworthy of the -oma designation. For example, pulmonary chondroid harmatoma contains islands of disorganized, but histologically normal cartilage, bronchi, and vessels. However, many hamartomas, including pulmonary chondroid hamartoma, have clonal, recurrent translocations involving genes encoding certain chromatin proteins.[3] Thus, through molecular biology they have finally earned their -oma designation. Another misnomer is the term *choristoma.* This congenital anomaly is better described as a *heterotopic rest* of cells. For example, a small nodule of well-developed and normally organized pancreatic substance may be found in the submucosa of the stomach, duodenum, or small intestine. This heterotopic rest may be replete with islets of Langerhans and exocrine glands. The term *choristoma,* connoting a neoplasm, imparts to the heterotopic rest a gravity far beyond its usual trivial significance. Although regrettably the terminology of neoplasms is not simple, it is important because it is the language by which the nature and significance of tumors are categorized.

Characteristics of Benign and Malignant Neoplasms

Nothing is more important to the individual with a tumor than being told "It is benign," and so the differentiation between benign and malignant tumors is one of the most important distinctions a pathologist can make. In the great majority of instances, a benign tumor may be distinguished from a malignant tumor with considerable confidence on the basis of morphology. Occasionally, despite the pathologist's best efforts, a neoplasm defies categorization. Certain anatomic features may suggest innocence, whereas others point toward cancerous potential. In a few instances there is not perfect concordance between the appearance of a neoplasm and its biologic behavior. In these cases molecular profiling (see below) or other molecular ancillary tests may provide useful information. Although an innocent face may mask an ugly nature, in general, benign and malignant tumors can be distinguished on the basis of differentiation and anaplasia, rate of growth, local invasion, and metastasis.

DIFFERENTIATION AND ANAPLASIA

Differentiation refers to the extent to which neoplastic parenchymal cells resemble the corresponding normal parenchymal cells, both morphologically and functionally; lack of differentiation is called anaplasia. In general, benign tumors are well differentiated (Figs. 7–4 and 7–5). The neoplastic cell in a benign adipocyte tumor—a lipoma—so closely resembles the normal cell that it may be impossible to recognize it as a tumor by microscopic examination of individual cells. Only the growth of these cells into a discrete mass discloses the neoplastic nature of the lesion. One may get so close to the tree that one loses sight of the forest. In well-differentiated benign tumors, mitoses are extremely scant in number and are of normal configuration.

Malignant neoplasms are characterized by a wide range of parenchymal cell differentiation, from surprisingly well differentiated (Fig. 7–6) to completely undifferentiated. Certain well-differentiated adenocarcinomas of the thyroid, for

TABLE 7–1 Nomenclature of Tumors

Tissue of Origin	Benign	Malignant
COMPOSED OF ONE PARENCHYMAL CELL TYPE		
Tumors of Mesenchymal Origin		
Connective tissue and derivatives	Fibroma Lipoma Chondroma Osteoma	Fibrosarcoma Liposarcoma Chondrosarcoma Osteogenic sarcoma
Endothelial and Related Tissues		
Blood vessels	Hemangioma	Angiosarcoma
Lymph vessels	Lymphangioma	Lymphangiosarcoma
Synovium		Synovial sarcoma
Mesothelium		Mesothelioma
Brain coverings	Meningioma	Invasive meningioma
Blood Cells and Related Cells		
Hematopoietic cells Lymphoid tissue		Leukemias Lymphomas
Muscle		
Smooth	Leiomyoma	Leiomyosarcoma
Striated	Rhabdomyoma	Rhabdomyosarcoma
Tumors of Epithelial Origin		
Stratified squamous Basal cells of skin or adnexa	Squamous cell papilloma	Squamous cell carcinoma Basal cell carcinoma
Epithelial lining of glands or ducts	Adenoma Papilloma Cystadenoma	Adenocarcinoma Papillary carcinomas Cystadenocarcinoma
Respiratory passages	Bronchial adenoma	Bronchogenic carcinoma
Renal epithelium	Renal tubular adenoma	Renal cell carcinoma
Liver cells	Liver cell adenoma	Hepatocellular carcinoma
Urinary tract epithelium (transitional)	Transitional-cell papilloma	Transitional-cell carcinoma
Placental epithelium	Hydatidiform mole	Choriocarcinoma
Testicular epithelium (germ cells)		Seminoma Embryonal carcinoma
Tumors of Melanocytes	Nevus	Malignant melanoma
MORE THAN ONE NEOPLASTIC CELL TYPE—MIXED TUMORS, USUALLY DERIVED FROM ONE GERM CELL LAYER		
Salivary glands	Pleomorphic adenoma (mixed tumor of salivary origin)	Malignant mixed tumor of salivary gland origin
Renal anlage		Wilms tumor
MORE THAN ONE NEOPLASTIC CELL TYPE DERIVED FROM MORE THAN ONE GERM CELL LAYER—TERATOGENOUS		
Totipotential cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma, teratocarcinoma

example, may form normal-appearing follicles, and some squamous cell carcinomas contain cells that do not differ cytologically from normal squamous epithelial cells (Fig. 7–7). Thus, the morphologic diagnosis of malignancy in well-differentiated tumors may sometimes be quite difficult. In between the two extremes lie tumors that are loosely referred to as *moderately well differentiated.*

Malignant neoplasms that are composed of poorly differentiated cells are said to be *anaplastic.* Lack of differentiation, or anaplasia, is considered a hallmark of malignancy. The term *anaplasia* literally means "to form backward," implying a reversal of differentiation to a more primitive level. It is believed, however, that most cancers do not represent "reverse differentiation" of mature normal cells but, in fact, arise from less mature cells with "stem-cell-like" properties, such as tissue stem cells (Chapter 3). In well-differentiated tumors (Fig. 7–7), daughter cells derived from these "cancer stem cells" retain the capacity for differentiation, whereas in poorly differentiated tumors that capacity is lost.

Lack of differentiation, or anaplasia, is often associated with many other morphologic changes.

- *Pleomorphism.* Both the cells and the nuclei characteristically display *pleomorphism*—variation in size and shape (Fig. 7–8). Thus, cells within the same tumor are not uniform, but range from large cells, many times larger than their neighbors, to extremely small and primitive appearing.
- *Abnormal nuclear morphology.* Characteristically the nuclei contain abundant chromatin and are dark staining *(hyperchromatic).* The nuclei are disproportionately large

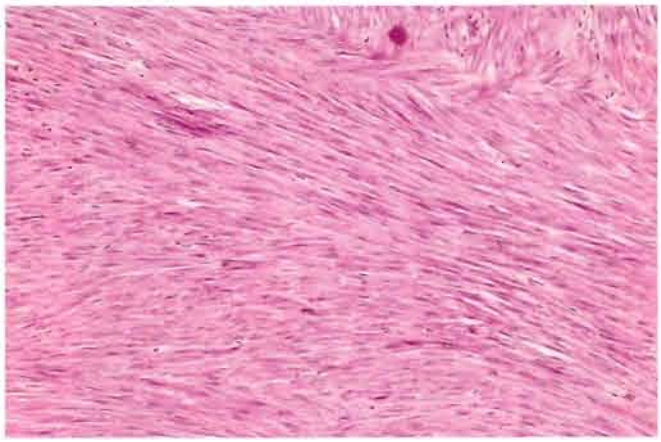


FIGURE 7–4 Leiomyoma of the uterus. This benign, well-differentiated tumor contains interlacing bundles of neoplastic smooth muscle cells that are virtually identical in appearance to normal smooth muscle cells in the myometrium.

for the cell, and the nuclear-to-cytoplasm ratio may approach 1 : 1 instead of the normal 1 : 4 or 1 : 6. The nuclear shape is variable and often irregular, and the chromatin is often coarsely clumped and distributed along the nuclear membrane. Large nucleoli are usually present in these nuclei.

- *Mitoses.* As compared with benign tumors and some well-differentiated malignant neoplasms, undifferentiated tumors usually possess large numbers of mitoses, reflecting the higher proliferative activity of the parenchymal cells. *The presence of mitoses, however, does not necessarily indicate that a tumor is malignant or that the tissue is neoplastic.* Many normal tissues exhibiting rapid turnover, such as bone marrow, have numerous mitoses, and non-neoplastic proliferations such as hyperplasias contain many cells in mitosis. More important as a morphologic feature of malignancy are atypical, bizarre mitotic figures, sometimes producing tripolar, quadripolar, or multipolar spindles (Fig. 7–9).

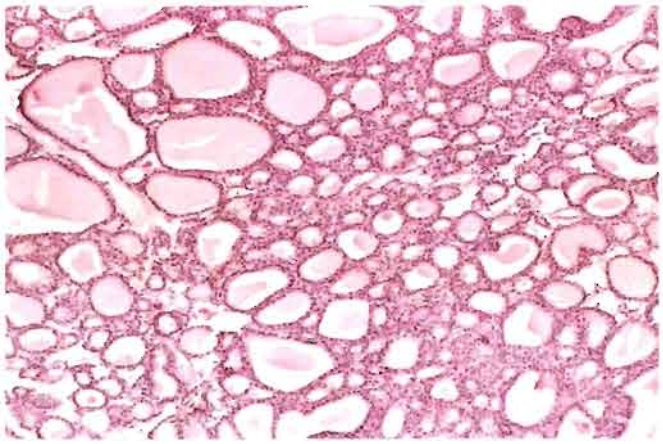


FIGURE 7–5 Benign tumor (adenoma) of the thyroid. Note the normal-looking (well-differentiated), colloid-filled thyroid follicles. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

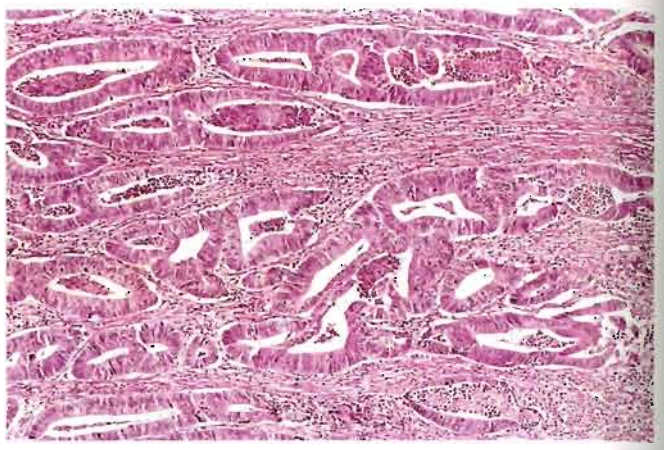


FIGURE 7–6 Malignant tumor (adenocarcinoma) of the colon. Note that compared with the well-formed and normal-looking glands characteristic of a benign tumor (see Fig. 7–5), the cancerous glands are irregular in shape and size and do not resemble the normal colonic glands. This tumor is considered differentiated because gland formation can be seen. The malignant glands have invaded the muscular layer of the colon. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

- *Loss of polarity.* In addition to the cytologic abnormalities, the *orientation of anaplastic cells is markedly disturbed (i.e., they lose normal polarity).* Sheets or large masses of tumor cells grow in an anarchic, disorganized fashion.
- *Other changes.* Another feature of anaplasia is the formation of *tumor giant cells*, some possessing only a single huge polymorphic nucleus and others having two or more large, hyperchromatic nuclei (Fig. 7–8). These giant cells are not to be confused with inflammatory Langhans or foreign body giant cells, which are derived from macrophages and contain many small, normal-appearing nuclei. Although growing tumor cells obviously require a blood supply, often the vascular stroma is scant, and in many anaplastic tumors, large central areas undergo ischemic *necrosis.*

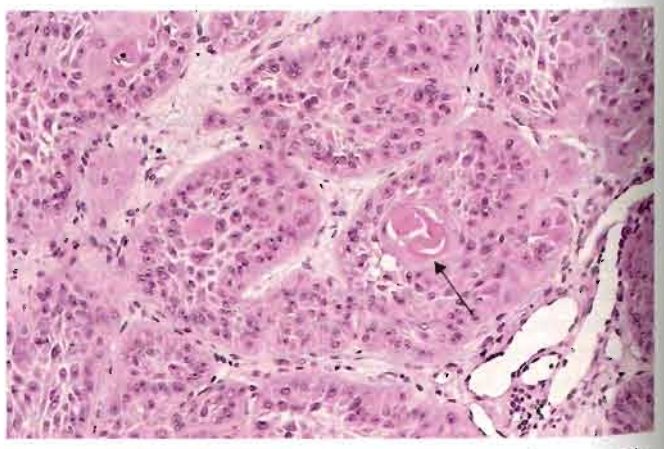


FIGURE 7–7 Well-differentiated squamous cell carcinoma of the skin. The tumor cells are strikingly similar to normal squamous epithelial cells, with intercellular bridges and nests of keratin pearls *(arrow).* (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

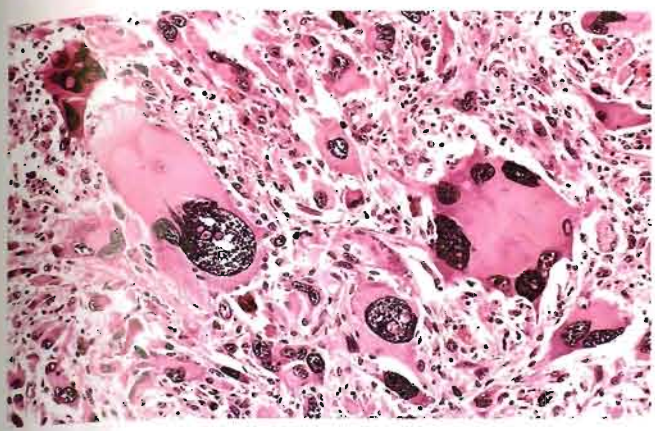


FIGURE 7–8 Anaplastic tumor of the skeletal muscle (rhabdomyosarcoma). Note the marked cellular and nuclear pleomorphism, hyperchromatic nuclei, and tumor giant cells. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

Before we leave the subject of differentiation and anaplasia, we should discuss metaplasia and dysplasia. *Metaplasia is defined as the replacement of one type of cell with another type.* Metaplasia is nearly always found in association with tissue damage, repair, and regeneration. Often the replacing cell type is more suited to a change in environment. For example, gastroesophageal reflux damages the squamous epithelium of the esophagus, leading to its replacement by glandular (gastric or intestinal) epithelium, more suited to the acidic environment. *Dysplasia* is a term that literally means disordered growth. Dysplasia often occurs in metaplastic epithelium, but not all metaplastic epithelium is also dysplastic. Dysplasia is encountered principally in epithelia, and it is characterized by a constellation of changes that *include a loss in the uniformity of the individual cells as well as a loss in their architectural orientation.* Dysplastic cells exhibit considerable pleomorphism and often contain large hyperchromatic nuclei with a high nuclear-to-cytoplasmic ratio. The architecture of the tissue may be disorderly. For example, in squamous epithelium the usual progressive maturation of tall cells in the basal layer to flattened squames on the surface may be lost and replaced by a scrambling of dark basal-appearing cells throughout the epithelium. Mitotic figures are more abundant than usual, although almost invariably they have a normal configuration. Frequently, however, the mitoses appear in abnormal locations within the epithelium. For example, in dysplastic stratified squamous epithelium, mitoses are not confined to the basal layers but instead may appear at all levels, including surface cells. When dysplastic changes are marked and involve the entire thickness of the epithelium but the lesion remains confined by the basement membrane, it is considered a preinvasive neoplasm and is referred to as *carcinoma in situ* (Fig. 7–10). Once the tumor cells breach the basement membrane, the tumor is said to be *invasive.* Dysplastic changes are often found adjacent to foci of invasive carcinoma, and in some situations, such as in long-term cigarette smokers and persons with Barrett esophagus, severe epithelial dysplasia frequently antedates the appearance of cancer. However, *dysplasia does not necessarily progress to cancer.* Mild to moderate changes that do not involve the entire thickness of epithelium may be reversible, and with removal of the inciting causes the epithelium may revert to normal. Even carcinoma in situ may take years to become invasive.

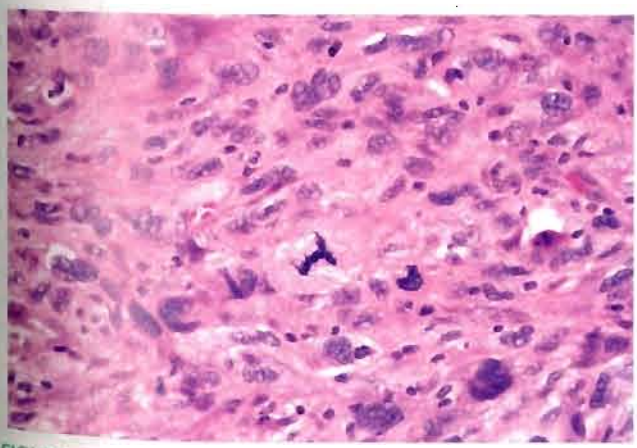


FIGURE 7–9 Anaplastic tumor showing cellular and nuclear variation in size and shape. The prominent cell in the center field has an abnormal tripolar spindle.

As you might presume, the better the differentiation of the transformed cell, the more completely it retains the functional capabilities found in its normal counterparts. Thus, benign neoplasms and well-differentiated carcinomas of endocrine glands frequently elaborate the hormones characteristic of their origin. Increased levels of these hormones in the blood are used clinically to detect and follow such tumors. Well-differentiated squamous cell carcinomas of the epidermis elaborate keratin, just as well-differentiated hepatocellular carcinomas elaborate bile. Highly anaplastic undifferentiated cells, whatever their tissue of origin, lose their resemblance to the normal cells from which they have arisen. In some instances, new and unanticipated functions emerge. Some tumors may elaborate fetal proteins not produced by comparable cells in the adult. Carcinomas of nonendocrine origin may produce a variety of hormones. For example, bronchogenic carcinomas may produce corticotropin, parathyroid-like hormone, insulin, and glucagon, as well as others. *Despite exceptions, the more rapidly growing and the more anaplastic a tumor, the less likely it will have specialized functional activity. The cells in benign tumors are almost always well differentiated and resemble their normal cells of origin; the cells in cancer are more or less differentiated, but some derangement of differentiation is always present.*

RATES OF GROWTH

A fundamental issue in tumor biology is to understand the factors that affect the growth rates of tumors and their influence on clinical outcome and therapeutic responses. One can begin the consideration of tumor cell kinetics by asking the question: How long does it take to produce a clinically overt tumor mass? It is a reasonable estimate the original transformed cell (approximately 10 μm in diameter) must undergo at least 30 population doublings to produce 10^9 cells (weighing approximately 1 gm), which is the smallest clinically detectable mass. In contrast, only 10 additional doubling cycles are required to produce a tumor containing 10^{12} cells (weighing ~1 kg), which is usually

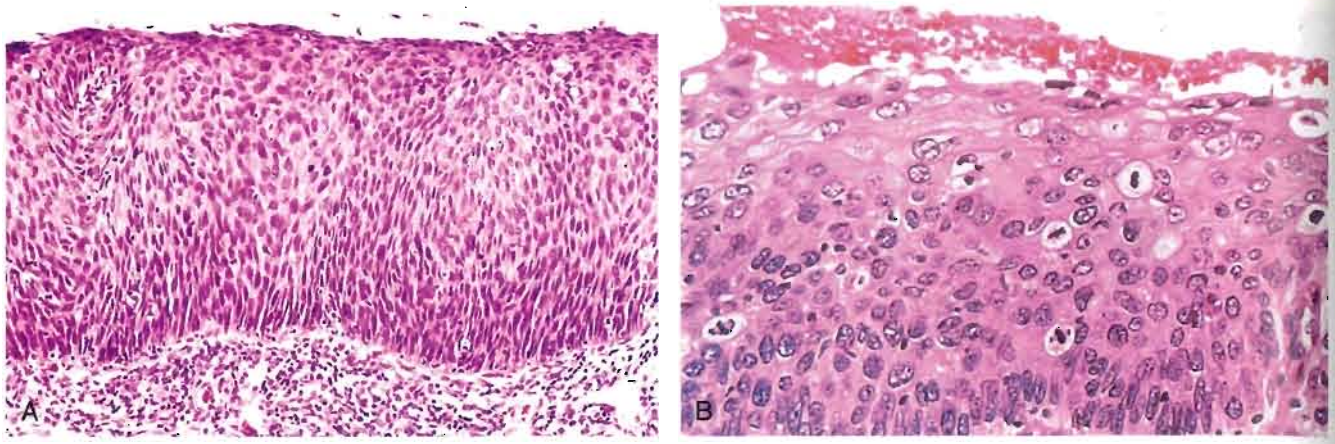


FIGURE 7–10 **A,** Carcinoma in situ. This low-power view shows that the entire thickness of the epithelium is replaced by atypical dysplastic cells. There is no orderly differentiation of squamous cells. The basement membrane is intact, and there is no tumor in the subepithelial stroma. **B,** A high-power view of another region shows failure of normal differentiation, marked nuclear and cellular pleomorphism, and numerous mitotic figures extending toward the surface. The basement membrane is not seen in this section.

the maximal size compatible with life. These are minimal estimates, based on the assumption that all descendants of the transformed cell retain the ability to divide and that there is no loss of cells from the replicative pool. This concept of tumor as a "pathologic dynamo" is not entirely correct, as we discuss subsequently. Nevertheless, this calculation highlights an extremely important concept about tumor growth: *By the time a solid tumor is clinically detected, it has already completed a major portion of its life span.* This is a major impediment in the treatment of cancer and underscores the need to develop diagnostic markers to detect early cancers.

The rate of growth of a tumor is determined by three main factors: the doubling time of tumor cells, the fraction of tumor cells that are in the replicative pool, and the rate at which cells are shed or die. Because cell cycle controls are deranged in most tumors, tumor cells can be triggered to cycle without the usual restraints. The dividing cells, however, do not necessarily complete the cell cycle more rapidly than do normal cells. In reality, total cell cycle time for many tumors is equal to or longer than that of corresponding normal cells. Thus, it can be safely concluded that growth of tumors is not commonly associated with a shortening of cell cycle time.

The proportion of cells within the tumor population that are in the proliferative pool is referred to as the *growth fraction.* Clinical and experimental studies suggest that during the early, submicroscopic phase of tumor growth, the vast majority of transformed cells are in the proliferative pool (Fig. 7–11). As tumors continue to grow, cells leave the proliferative pool in ever-increasing numbers as a result of shedding, lack of nutrients, necrosis, apoptosis, differentiation, and reversion to the nonproliferative phase of the cell cycle (G_0). Thus, by the time a tumor is clinically detectable, most cells are not in the replicative pool. Even in some rapidly growing tumors, the growth fraction is only about 20% or less.

Ultimately the progressive growth of tumors and the rate at which they grow are determined by an *excess of cell production over cell loss.* In some tumors, especially those with a relatively high growth fraction, the imbalance is large, resulting in more rapid growth than in those in which cell production exceeds cell loss by only a small margin. Some leukemias and lymphomas and certain lung cancers (i.e., small-cell carcinoma) have a relatively high growth fraction, and their clinical course is rapid. By comparison, many common tumors, such as cancers of the colon and breast, have low growth fractions, and cell production exceeds cell loss by only about 10%; they tend to grow at a much slower pace.

Several important conceptual and practical lessons can be learned from studies of tumor cell kinetics:

- Fast-growing tumors may have a high *cell turnover*, implying that rates of both proliferation and apoptosis are high. Obviously if the tumor is to grow, the rate of proliferation must exceed that of cell death.
- The growth fraction of tumor cells has a profound effect on their susceptibility to cancer chemotherapy. Because most anticancer agents act on cells that are in cycle, it is not difficult to imagine that a tumor that contains 5% of all cells in the replicative pool will be slow growing but relatively refractory to treatment with drugs that kill dividing cells. One strategy used in the treatment of tumors with low growth fraction (e.g., cancer of colon and breast) is first to shift tumor cells from G_0 into the cell cycle. This can be

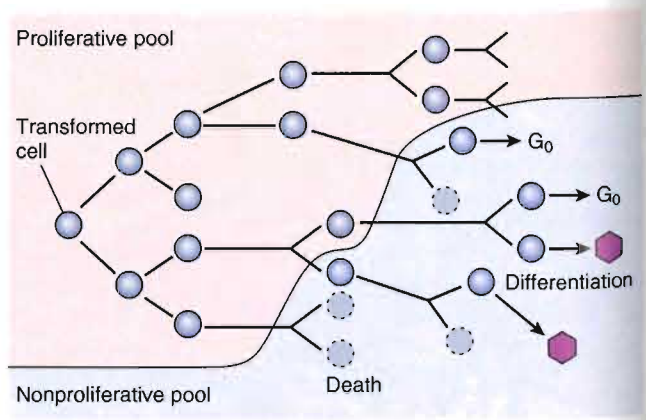


FIGURE 7–11 Schematic representation of tumor growth. As the cell population expands, a progressively higher percentage of tumor cells leaves the replicative pool by reversion to G_0, differentiation, and death.

accomplished by debulking the tumor with surgery or radiation. The surviving tumor cells tend to enter the cell cycle and thus become susceptible to drug therapy. Such considerations form the basis of combined-modality treatment. Some aggressive tumors (such as certain lymphomas and leukemias) that contain a large pool of dividing cells literally melt away with chemotherapy and may even be cured.

We can now return to the question posed earlier: How long does it take for one transformed cell to produce a clinically detectable tumor containing 10^9 cells? If every one of the daughter cells remained in cell cycle and no cells were shed or lost, we could anticipate the answer to be 90 days (30 population doublings, with a cell cycle time of 3 days). In reality, *the latent period before which a tumor becomes clinically detectable is unpredictable but typically much longer than 90 days, as long as many years for most solid tumors, emphasizing once again that human cancers are diagnosed only after they are fairly advanced in their life cycle.* After they become clinically detectable, the average volume-doubling time for such common killers as cancer of the lung and colon is about 2 to 3 months. As might be anticipated from the discussion of the variables that affect growth rate, however, the range of doubling time values is extremely broad, varying from less than 1 month for some childhood cancers to more than 1 year for certain salivary gland tumors. Cancer is indeed an unpredictable group of disorders.

In general, *the growth rate of tumors correlates with their level of differentiation, and thus most malignant tumors grow more rapidly than do benign lesions.* There are, however, many exceptions to such an oversimplification. Some benign tumors have a higher growth rate than malignant tumors. Moreover, the rate of growth of benign as well as malignant neoplasms may not be constant over time. Factors such as hormonal stimulation, adequacy of blood supply, and unknown influences may affect their growth. For example, the growth of uterine leiomyomas (benign smooth muscle tumors) may change over time because of hormonal variations. Not infrequently, repeated clinical examination of women bearing such neoplasms over the span of decades discloses no significant increase in size. After menopause the neoplasms may atrophy and may be replaced largely by collagenous, sometimes calcified, tissue. During pregnancy leiomyomas frequently enter a growth spurt. Such changes reflect the responsiveness of the tumor cells to circulating levels of steroid hormones, particularly estrogens. Cancers show a wide range of growth. Some malignant tumors grow slowly for years and then suddenly increase in size, explosively disseminating to cause death within a few months of discovery. It is possible that such behavior results from the emergence of an aggressive subclone of transformed cells. At the other extreme are malignant neoplasms that grow more slowly than do benign tumors and may even enter periods of dormancy lasting for years. On occasion, cancers decrease in size and even spontaneously disappear, but such "miracles" are rare enough that they remain intriguing curiosities.

CANCER STEM CELLS AND CANCER CELL LINEAGES

The continued growth and maintenance of many tissues that contain short-lived cells, such as the formed elements of the blood and the epithelial cells of the gastrointestinal tract and skin, require a resident population of tissue stem cells that are long-lived and capable of self-renewal. Tissue stem cells are rare and exist in a niche created by support cells, which produce paracrine factors that sustain the stem cell.[4] Recall from Chapter 3 that tissue stem cells divide asymmetrically to produce two types of daughter cells—those with limited proliferative potential, which undergo terminal differentiation and die, and those that retain stem cell potential.

Cancers are immortal and have limitless proliferative capacity, indicating that like normal tissues, they also must contain cells with "stemlike" properties.[5,6] The concept of cancer stem cells has several important implications. Most notably, if cancer stem cells are essential for tumor persistence, it follows that these cells must be eliminated to cure the affected patient. It is hypothesized that like normal stem cells, cancer stem cells have a high intrinsic resistance to conventional therapies, because of their low rate of cell division and the expression of factors, such as multiple drug resistance-1 (MDR1), that counteract the effects of chemotherapeutic drugs.[5,6] Thus, the limited success of current therapies may in part be explained by their failure to kill the malignant stem cells that lie at the root of cancer. Cancer stem cells could arise from normal tissue stem cells or from more differentiated cells that, as part of the transformation process, acquire the property of self-renewal. Studies of certain leukemias (Chapter 13) support both of these possibilities. For example, chronic myelogenous leukemia (CML) originates from the malignant counterpart of a normal hematopoietic stem cell, whereas certain acute myeloid leukemias (AMLs) are derived from more differentiated myeloid precursors that acquire an abnormal capacity for self-renewal. The identification of "leukemia stem cells" has spurred the search for cancer stem cells in solid tumors. Most such studies have focused on the identification of tumor-initiating cells (T-ICs), which are defined as cells that allow a human tumor to grow and maintain itself indefinitely when transplanted into an immunodeficient mouse. T-ICs have been identified in several human tumors, including breast carcinoma, glioblastoma multiforme, colon cancer, and AML,[5–8] in which they constitute 0.1% to 2% of the total cellularity.

More recent studies have shown that in some cancers, T-ICs are very common, representing 25% of the total cellularity.[9] Thus some tumors may have a small number of T-ICs that then "differentiate" to form the bulk of the tumor, while other tumors may be primarily composed of T-ICs. In the future, it will be important to identify the tumorigenic population in each tumor to direct therapy against tumor stem cells. An emerging theme is that the genes and pathways that maintain cancer stem cells are the same as those that regulate normal tissue stem cell homeostasis. Examples include BMI1, a component of the polycomb chromatin-remodeling complex that promotes "stem-ness" in both normal hematopoietic and leukemic stem cells; and the WNT pathway, a key regulator of normal colonic crypt stem cells that has been implicated in the maintenance of colonic adenocarcinoma "stem cells."[9,10] Important remaining questions revolve around whether T-ICs are an accurate measure of cancer stem cells, if cancer stem cells remain dependent on the "niche" that supports normal stem cells, and if it will be possible to selectively target cancer cell "stem-ness" factors.

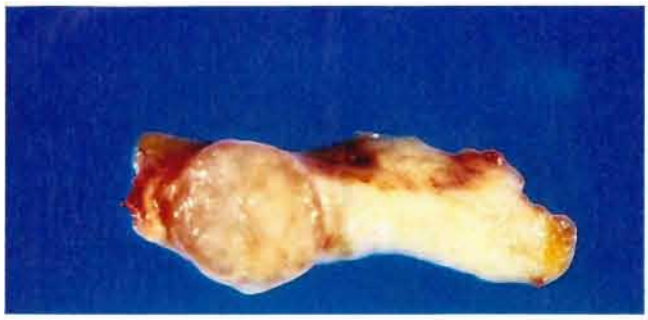


FIGURE 7–12 Fibroadenoma of the breast. The tan-colored, encapsulated small tumor is sharply demarcated from the whiter breast tissue.

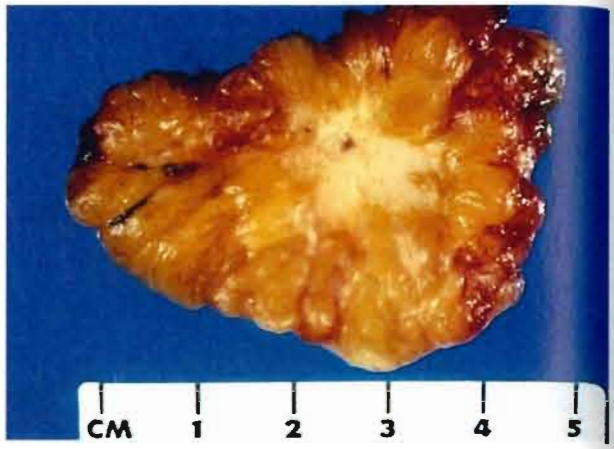


FIGURE 7–14 Cut section of an invasive ductal carcinoma of the breast. The lesion is retracted, infiltrating the surrounding breast substance, and would be stony hard on palpation.

LOCAL INVASION

Nearly all benign tumors grow as cohesive expansile masses that remain localized to their site of origin and do not have the capacity to infiltrate, invade, or metastasize to distant sites, as do malignant tumors. Because they grow and expand slowly, they usually develop a rim of compressed connective tissue, sometimes called a fibrous *capsule,* which separates them from the host tissue. This capsule is derived largely from the extracellular matrix of the native tissue due to atrophy of normal parenchymal cells under the pressure of an expanding tumor. Such encapsulation does not prevent tumor growth, but it keeps the benign neoplasm as a discrete, readily palpable, and easily movable mass that can be surgically enucleated (Figs. 7–12 and 7–13). Although a well-defined cleavage plane exists around most benign tumors, in some it is lacking. For example, hemangiomas (neoplasms composed of tangled blood vessels) are often unencapsulated and may appear to permeate the site in which they arise (commonly the dermis of the skin).

The growth of cancers is accompanied by progressive infiltration, invasion, and destruction of the surrounding tissue. In general, malignant tumors are poorly demarcated from the surrounding normal tissue, and a well-defined cleavage plane is lacking (Figs. 7–14 and 7–15). Slowly expanding malignant tumors, however, may develop an apparently enclosing fibrous capsule and may push along a broad front into adjacent normal structures. Histologic examination of such pseudoencapsulated masses almost always shows rows of cells penetrating the margin and infiltrating the adjacent structures, a crablike pattern of growth that constitutes the popular image of cancer.

Most malignant tumors are obviously invasive and can be expected to penetrate the wall of the colon or uterus, for example, or fungate through the surface of the skin. They recognize no normal anatomic boundaries. Such invasiveness makes their surgical resection difficult or impossible, and even if the tumor appears well circumscribed it is necessary to remove a considerable margin of apparently normal tissues adjacent to the infiltrative neoplasm. *Next to the development*

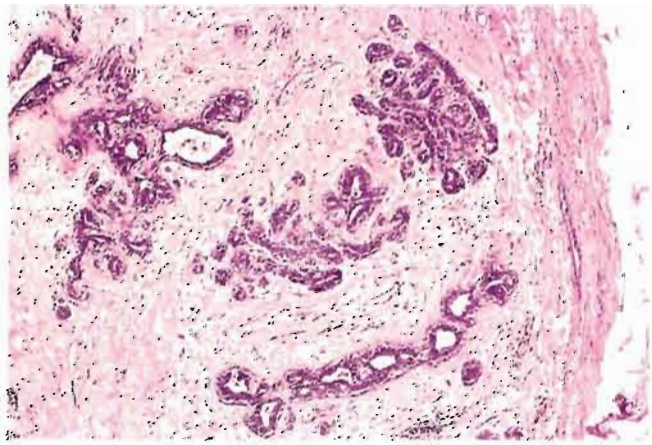


FIGURE 7–13 Microscopic view of fibroadenoma of the breast seen in Figure 7–12. The fibrous capsule *(right)* delimits the tumor from the surrounding tissue. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

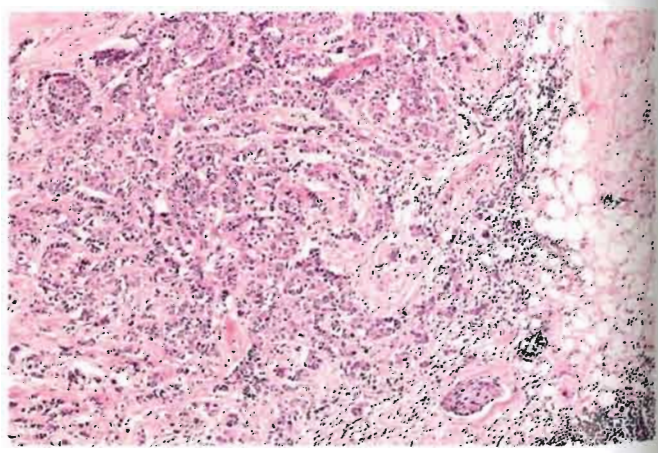


FIGURE 7–15 The microscopic view of the breast carcinoma seen in Figure 7–14 illustrates the invasion of breast stroma and fat by nests and cords of tumor cells (compare with fibroadenoma shown in Fig. 7–13). The absence of a well-defined capsule should be noted. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

of metastases, invasiveness is the most reliable feature that differentiates malignant from benign tumors. We noted earlier that some cancers seem to evolve from a preinvasive stage referred to as *carcinoma in situ.* This commonly occurs in carcinomas of the skin, breast, and certain other sites and is best illustrated by carcinoma of the uterine cervix (Chapter 22). *In situ epithelial cancers display the cytologic features of malignancy without invasion of the basement membrane.* They may be considered one step removed from invasive cancer; with time, most penetrate the basement membrane and invade the subepithelial stroma.

METASTASIS

Metastases are tumor implants discontinuous with the primary tumor. *Metastasis unequivocally marks a tumor as malignant because benign neoplasms do not metastasize.* The invasiveness of cancers permits them to penetrate into blood vessels, lymphatics, and body cavities, providing the opportunity for spread. *With few exceptions, all malignant tumors can metastasize.* The major exceptions are most malignant neoplasms of the glial cells in the central nervous system, called *gliomas*, and basal cell carcinomas of the skin. Both are locally invasive forms of cancer, but they rarely metastasize. It is evident then that the properties of invasion and metastasis are separable.

In general, the more aggressive, the more rapidly growing, and the larger the primary neoplasm, the greater the likelihood that it will metastasize or already has metastasized. There are innumerable exceptions, however. Small, well-differentiated, slowly growing lesions sometimes metastasize widely; conversely, some rapidly growing, large lesions remain localized for years. Many factors relating to both invader and host are involved.

Approximately 30% of newly diagnosed individuals with solid tumors (excluding skin cancers other than melanomas) present with metastases. Metastatic spread strongly reduces the possibility of cure; hence, short of prevention of cancer, no achievement would be of greater benefit to patients than methods to block metastases.

Pathways of Spread

Dissemination of cancers may occur through one of three pathways: (1) direct seeding of body cavities or surfaces, (2) lymphatic spread, and (3) hematogenous spread. Although direct transplantation of tumor cells, as for example on surgical instruments, may theoretically occur, it is rare and we do not discuss this artificial mode of dissemination further. Each of the three major pathways is described separately.

Seeding of Body Cavities and Surfaces. Seeding of body cavities and surfaces may occur whenever a malignant neoplasm penetrates into a natural "open field." Most often involved is the peritoneal cavity (Fig. 7–16), but any other cavity—pleural, pericardial, subarachnoid, and joint space—may be affected. Such seeding is particularly characteristic of carcinomas arising in the ovaries, when, not infrequently, all peritoneal surfaces become coated with a heavy layer of cancerous glaze. Remarkably, the tumor cells may remain confined to the surface of the coated abdominal viscera without penetrating into the substance. Sometimes mucus-secreting appendiceal carcinomas fill the peritoneal cavity with a gelatinous neoplastic mass referred to as *pseudomyxoma peritonei.*

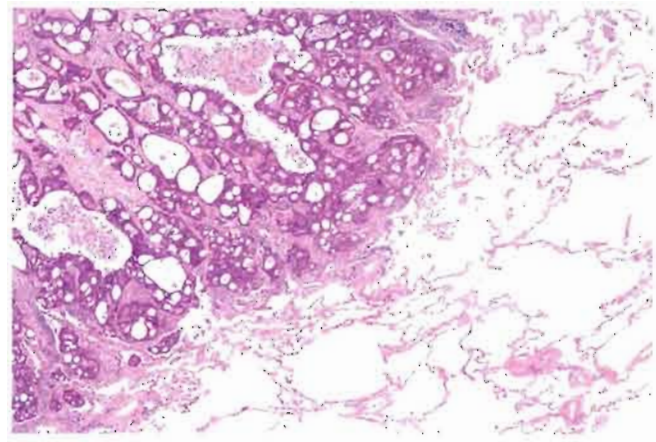


FIGURE 7–16 Colon carcinoma invading pericolonic adipose tissue. (Courtesy of Dr. Melissa Upton, University of Washington, Seattle, WA.)

Lymphatic Spread. Transport through lymphatics is the most common pathway for the initial dissemination of carcinomas (Fig. 7–17), and sarcomas may also use this route. Tumors do not contain functional lymphatics, but lymphatic vessels located at the tumor margins are apparently sufficient for the lymphatic spread of tumor cells.[11] The emphasis on lymphatic spread for carcinomas and hematogenous spread for sarcomas is misleading, because ultimately there are numerous interconnections between the vascular and the lymphatic systems. *The pattern of lymph node involvement follows the natural routes of lymphatic drainage.* Because carcinomas of the breast usually arise in the upper outer quadrants, they generally disseminate first to the axillary lymph nodes. Cancers of the inner quadrants drain to the nodes along the internal mammary arteries. Thereafter the infraclavicular and supraclavicular nodes may become involved. Carcinomas of the lung arising in the major respiratory passages metastasize first to the perihilar tracheobronchial and mediastinal

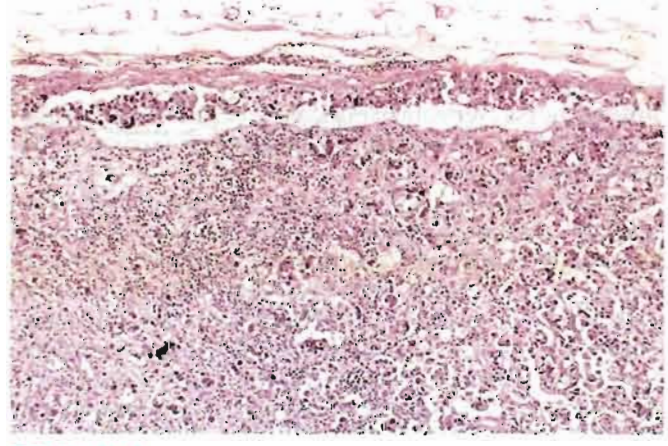


FIGURE 7–17 Axillary lymph node with metastatic breast carcinoma. The subcapsular sinus *(top)* is distended with tumor cells. Nests of tumor cells have also invaded the subcapsular cortex. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)

nodes. Local lymph nodes, however, may be bypassed—so-called "skip metastasis"—because of venous-lymphatic anastomoses or because inflammation or radiation has obliterated lymphatic channels.

In breast cancer, determining the involvement of axillary lymph nodes is very important for assessing the future course of the disease and for selecting suitable therapeutic strategies. To avoid the considerable surgical morbidity associated with a full axillary lymph node dissection, *biopsy of sentinel nodes* is often used to assess the presence or absence of metastatic lesions in the lymph nodes. A sentinel lymph node is defined as "the first node in a regional lymphatic basin that receives lymph flow from the primary tumor."[12] Sentinel node mapping can be done by injection of radiolabeled tracers and blue dyes, and the use of frozen section upon the sentinel lymph node at the time of surgery can guide the surgeon to the appropriate therapy. Sentinel node biopsy has also been used for detecting the spread of melanomas, colon cancers, and other tumors.[12,13]

In many cases the regional nodes serve as effective barriers to further dissemination of the tumor, at least for a while. Conceivably the cells, after arrest within the node, may be destroyed by a tumor-specific immune response. Drainage of tumor cell debris or tumor antigens, or both, also induces reactive changes within nodes. Thus, enlargement of nodes may be caused by (1) the spread and growth of cancer cells or (2) reactive hyperplasia (Chapter 13). Therefore, *nodal enlargement in proximity to a cancer, while it must arouse suspicion, does not necessarily mean dissemination of the primary lesion.*

Hematogenous Spread. Hematogenous spread is typical of sarcomas but is also seen with carcinomas. Arteries, with their thicker walls, are less readily penetrated than are veins. Arterial spread may occur, however, when tumor cells pass through the pulmonary capillary beds or pulmonary arteriovenous shunts or when pulmonary metastases themselves give rise to additional tumor emboli. In such vascular spread, several factors influence the patterns of distribution of the metastases. With venous invasion the blood-borne cells follow the venous flow draining the site of the neoplasm, and the tumor cells often come to rest in the first capillary bed they encounter. Understandably the liver and lungs are most frequently involved in such hematogenous dissemination (Figs. 7–18 and 7–19), because all portal area drainage flows to the liver and all caval blood flows to the lungs. Cancers arising in close proximity to the vertebral column often embolize through the paravertebral plexus, and this pathway is involved in the frequent vertebral metastases of carcinomas of the thyroid and prostate.

Certain cancers have a propensity for invasion of veins. Renal cell carcinoma often invades the branches of the renal vein and then the renal vein itself to grow in a snakelike fashion up the inferior vena cava, sometimes reaching the right side of the heart. Hepatocellular carcinomas often penetrate portal and hepatic radicles to grow within them into the main venous channels. Remarkably, such intravenous growth may not be accompanied by widespread dissemination. Histologic evidence of penetration of small vessels at the site of the primary neoplasm is obviously an ominous feature. Such changes, however, must be viewed guardedly because, for reasons discussed later, they do not indicate the inevitable development of metastases.

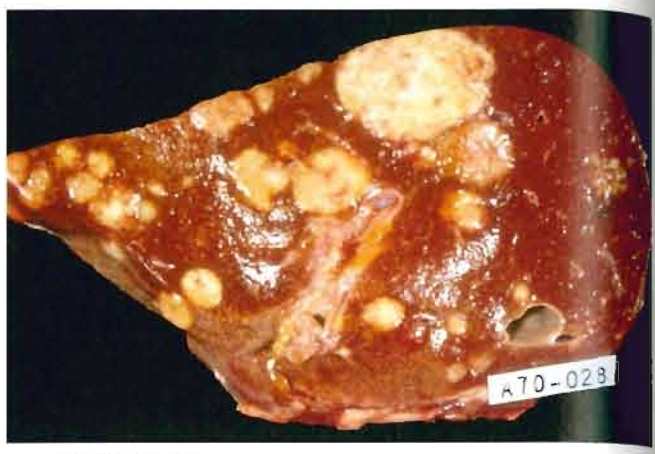


FIGURE 7–18 A liver studded with metastatic cancer.

Many observations suggest that mere anatomic localization of the neoplasm and natural pathways of venous drainage do not wholly explain the systemic distributions of metastases. For example, breast carcinoma preferentially spreads to bone, bronchogenic carcinomas tend to involve the adrenals and the brain, and neuroblastomas spread to the liver and bones. Conversely, skeletal muscles and the spleen, despite the large percentage of blood flow they receive and the enormous vascular beds present, are rarely the site of secondary deposits. The probable basis of such tissue-specific homing of tumor cells is discussed later.

The distinguishing features of benign and malignant tumors discussed in this overview are summarized in Table 7–2 and Figure 7–20. With this background on the structure and behavior of neoplasms, we now discuss the origin of tumors, starting with insights gained from the epidemiology of cancer and followed by the molecular basis of carcinogenesis.

Epidemiology

Because cancer is a disorder of cell growth and behavior, its ultimate cause has to be defined at the cellular and subcellular levels. Study of cancer patterns in populations, however, can

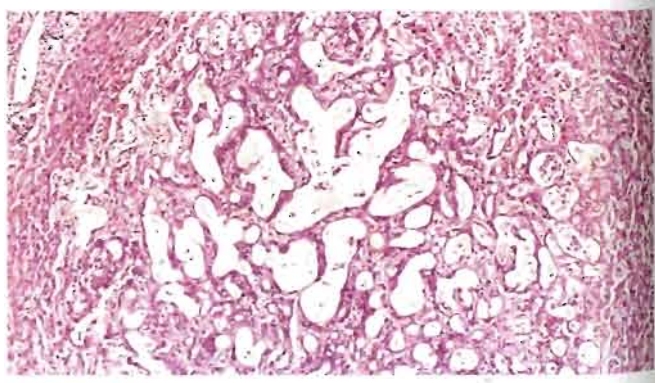


FIGURE 7–19 Microscopic view of liver metastasis. A pancreatic adenocarcinoma has formed a metastatic nodule in the liver. (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallax, TX.)

TABLE 7-2 Comparisons between Benign and Malignant Tumors

Characteristics	Benign	Malignant
Differentiation/anaplasia	Well differentiated; structure sometimes typical of tissue of origin	Some lack of differentiation with anaplasia; structure often atypical
Rate of growth	Usually progressive and slow; may come to a standstill or regress; mitotic figures rare and normal	Erratic and may be slow to rapid; mitotic figures may be numerous and abnormal
Local invasion	Usually cohesive expansile well-demarcated masses that do not invade or infiltrate surrounding normal tissues	Locally invasive, infiltrating surrounding tissue; sometimes may be seemingly cohesive and expansile
Metastasis	Absent	Frequently present; the larger and more undifferentiated the primary, the more likely are metastases

contribute substantially to knowledge about the origins of cancer. Epidemiologic studies have established the causative link between smoking and lung cancer, and comparison of diet and cancer rates in the Western world and Africa has implicated high dietary fat and low fiber in the development of colon cancer. Major insights into the causes of cancer can be obtained by epidemiologic studies that relate particular environmental, racial (possibly hereditary), and cultural influences to the occurrence of specific neoplasms. Certain diseases associated with an increased risk of developing cancer (preneoplastic disorders) also provide clues to the pathogenesis of cancer. In the following discussion we first summarize the overall incidence of cancer to gain an insight into the magnitude of the cancer problem, then we review some factors relating to the patient and environment that influence the predisposition to cancer.

CANCER INCIDENCE

In some measure, an individual's likelihood of developing a cancer is expressed by national incidence and mortality rates. For example, residents of the United States have about a one in five chance of dying of cancer. There were, it is estimated, about 1,437,180 new cancer cases and 565,650 deaths from cancer in 2008, representing 23% of all mortality,[1] a frequency surpassed only by deaths caused by cardiovascular diseases. These data do not include an additional 1 million, for the most part readily curable, non-melanoma cancers of the skin and

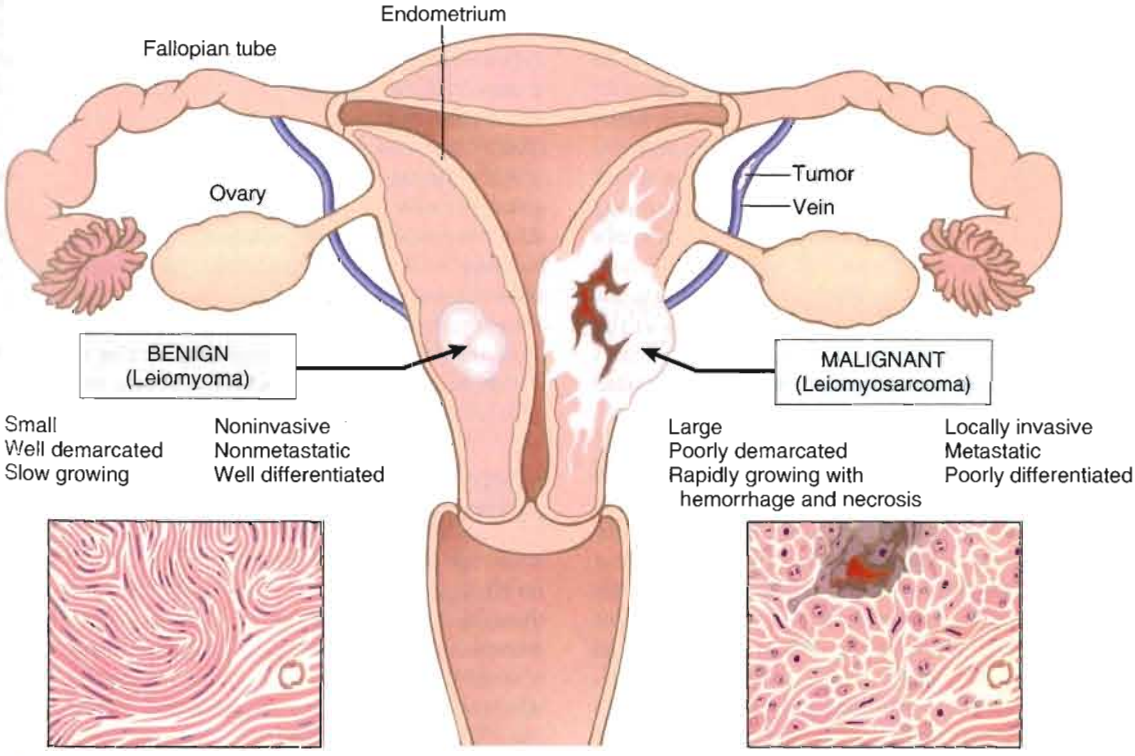


FIGURE 7-20 Comparison between a benign tumor of the myometrium (leiomyoma) and a malignant tumor of the same origin (leiomyosarcoma).

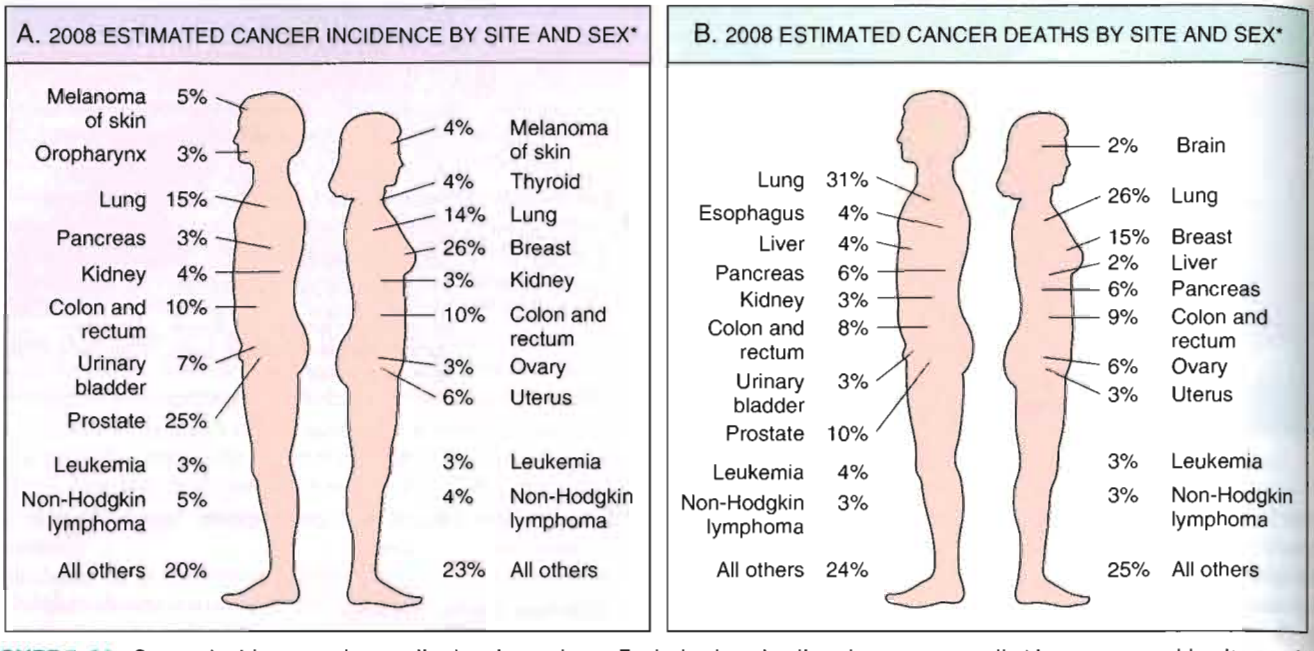


FIGURE 7–21 Cancer incidence and mortality by site and sex. Excludes basal cell and squamous cell skin cancers and in situ carcinomas, except urinary bladder. (Adapted from Jemal A et al.: Cancer statistics, 2008. CA Cancer J Clin 58:2, 2008.)

122,000 cases of carcinoma in situ, largely of the female breast and melanomas.[1] The major organ sites affected and the estimated frequency of cancer deaths are shown in Figure 7–21. The most common tumors in men arise in the prostate, lung, and colorectum. In women, cancers of the breast, lung, and colon and rectum are the most frequent. Cancers of the lung, female breast, prostate, and colon/rectum constitute more than 50% of cancer diagnoses and cancer deaths in the U.S. population.[1]

The age-adjusted death rates (number of deaths per 100,000 population) for many forms of cancer have significantly changed over the years. Many of the long-term comparisons are noteworthy. Over the last 50 years of the twentieth century, the overall age-adjusted cancer death rate significantly increased in both men and women. However, since 1995 the cancer incidence rate in men has stabilized and since 1990 the cancer death rate in men has decreased 18.4%.[1] In women the cancer incidence rate stabilized in 1995, and the cancer death rate has decreased 10.4% since 1991.[1] Among men nearly 80% of the total decrease in cancer death rates is accounted for by decreases in death rates from lung, prostate, and colorectal cancers since 1990.[1] Among women nearly 60% of the decrease in cancer death rates is due to reductions in death rates from breast and colorectal cancers.[1] Nearly 40% of the sex-specific decreases in cancer death rates is accounted for by a reduction in lung cancer deaths in men and breast cancer deaths in women.[1] Decreased use of tobacco products is responsible for the reduction in lung cancer deaths, while improved detection and treatment are responsible for the decrease in death rates for colorectal, female breast, and prostate cancer.[1] The last half century has seen a decline in the number of deaths caused by cervical cancer that relates to earlier diagnosis made possible by the Papanicolaou (Pap) smear. The downward trend in deaths from stomach cancer has been attributed to a decrease in some dietary carcinogens, as a consequence of better food preservation or changes in dietary habits. Unfortunately, between 1990–1991 and 2004, lung cancer death rates in women, and liver and intrahepatic bile duct cancer death rates in men, increased substantially, offsetting some of the improvement in survival from other cancers.[1] Indeed, although in women carcinomas of the breast occur about 2.5 times more frequently than those of the lung, lung cancer has become the leading cause of cancer deaths in women. Deaths from primary liver cancers, which declined between 1930 and 1970, have approximately doubled during the past 30 years. This number is expected to increase over the coming decades, as the large number of individuals infected with the hepatitis C virus (HCV) begin to develop hepatocellular carcinoma.

Although race is not a strict biologic category, it can define groups at risk for certain cancers.[14,15] The disparity in cancer mortality rates between white and black Americans persists, but African Americans had the largest decline in cancer mortality during the past decade. Hispanics living in the United States have a lower frequency of the most common tumors than the white non-Hispanic population but a higher incidence of tumors of the stomach, liver, uterine cervix, and gallbladder, as well as certain childhood leukemias.

GEOGRAPHIC AND ENVIRONMENTAL FACTORS

Although genetics and environmental triggers both play a role in the pathogenesis of cancer, environmental factors are thought to be the more significant contributors in most common sporadic cancers. In one large study the proportion of risk from environmental causes was found to be 65%, whereas heritable factors contributed 26% to 42% of cancer risk. Remarkable differences found in the incidence and death rates of specific forms of cancer around the world also suggest a role for environmental factors.[16,17] For example, the

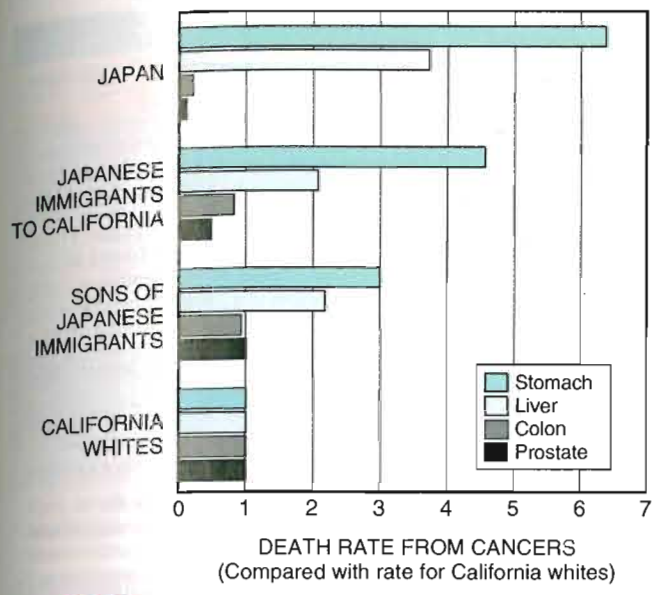


FIGURE 7–22 The change in incidence of various cancers with migration from Japan to the United States provides evidence that the occurrence of cancers is related to components of the environment that differ in the two countries. The incidence of each kind of cancer is expressed as the ratio of the death rate in the population being considered to that in a hypothetical population of California whites with the same age distribution; the death rates for whites are thus defined as 1. The death rates among immigrants and immigrants' sons tend consistently toward California norms. (From Cairns J: The cancer problem. In Readings from Scientific American—Cancer Biology. New York, WH Freeman, 1986, p 13.)

death rate for stomach carcinoma in both men and women is seven to eight times higher in Japan than in the United States. In contrast, the death rate from carcinoma of the lung is slightly more than twice as great in the United States as in Japan. Although racial predispositions cannot be ruled out, it is generally believed that most of these geographic differences are the consequence of environmental influences. Indeed, comparing mortality rates for Japanese immigrants to the United States and Japanese born in the United States of immigrant parents (Nisei) with those of long-term residents of both countries shows that cancer mortality rates for first-generation Japanese immigrants are intermediate between those of natives of Japan and natives of California, and the two rates come closer with each passing generation (Fig. 7–22). This points strongly to environmental and cultural factors rather than genetic predisposition.

There is no paucity of carcinogenic environmental factors: they lurk in the ambient environment, in the workplace, in food, and in personal practices. Individuals may be exposed to carcinogenic factors when they go outside (ultraviolet [UV] rays, smog), in their medication (methotrexate), at work (asbestos, vinyl chloride; Table 7–3), or at home (high-fat diet, alcohol). Overall, mortality data indicate that the most overweight individuals in the U.S. population have a 52% (men) and 62% (women) higher death rate from cancer than do their slimmer counterparts. Indeed, obesity is associated with approximately 14% of cancer deaths in men and 20% in women.[18] Alcohol abuse alone increases the risk of carcinomas of the oropharynx (excluding lip), larynx, and esophagus and, by the development of alcoholic cirrhosis, hepatocellular carcinoma. Smoking, particularly of cigarettes, has been implicated in cancer of the mouth, pharynx, larynx, esophagus, pancreas, bladder, and most significantly, about 90% of lung cancer deaths (Chapter 9). Cigarette smoking has been called the single most important environmental factor contributing to premature death in the United States. Alcohol and tobacco together synergistically increase the danger of incurring cancers in the upper aerodigestive tract. The risk of cervical cancer is linked to age at first intercourse and the number of sex partners, and it is now known that infection by venereally transmitted human papillomavirus (HPV) contributes to cervical dysplasia and cancer. It appears that almost everything one does to gain a livelihood or for pleasure is fattening, immoral, illegal, or, even worse, oncogenic.

AGE

Age has an important influence on the likelihood of being afflicted with cancer. Most carcinomas occur in the later years of life (>55 years). Cancer is the main cause of death among women aged 40 to 79 and among men aged 60 to 79; the decline in deaths after age 80 is due to the lower number of individuals who reach this age. The rising incidence with age may be explained by the accumulation of somatic mutations associated with the emergence of malignant neoplasms (discussed later). The decline in immune competence that accompanies aging may also be a factor.

However, children are not spared; cancer accounts for slightly more than 10% of all deaths in children under age 15 in the United States, second only to accidents. However, the types of cancers that predominate in children are significantly different from those seen in adults. Carcinomas, the most common general category of tumor in adults, are extraordinarily rare among children. Instead, acute leukemia and primitive neoplasms of the central nervous system are responsible for approximately 60% of childhood cancer deaths. The common neoplasms of infancy and childhood include the so-called small round blue cell tumors such as neuroblastoma, Wilms tumor, retinoblastoma, acute leukemias, and rhabdomyosarcomas. These are discussed in Chapter 10 and elsewhere in the text.

GENETIC PREDISPOSITION TO CANCER

One frequently asked question is: "My mother and father both died of cancer. Does that mean I am doomed to get it?" Based on current knowledge, the answer must be carefully qualified.[19,20] Evidence now indicates that for a large number of cancer types, including the most common forms, there exist not only environmental influences but also hereditary predispositions. For example, lung cancer is in most instances clearly related to cigarette smoking, yet mortality from lung cancer has been shown to be four times greater among nonsmoking relatives (parents and siblings) of lung cancer patients than among nonsmoking relatives of controls (the effects of secondhand smoke may confound some of these results). Less than 10% of cancer patients have inherited mutations that predispose to cancer, and the frequency is even lower (around 0.1%) for certain types of tumors. Despite the low frequency, the

TABLE 7–3 Occupational Cancers

Agents or Groups of Agents	Human Cancer Site for Which Reasonable Evidence Is Available	Typical Use or Occurrence
Arsenic and arsenic compounds	Lung, skin, hemangiosarcoma	Byproduct of metal smelting; component of alloys, electrical and semiconductor devices, medications and herbicides, fungicides, and animal dips
Asbestos	Lung, mesothelioma; gastrointestinal tract (esophagus, stomach, large intestine)	Formerly used for many applications because of fire, heat, and friction resistance; still found in existing construction as well as fire-resistant textiles, friction materials (i.e., brake linings), underlayment and roofing papers, and floor tiles
Benzene	Leukemia, Hodgkin lymphoma	Principal component of light oil; despite known risk, many applications exist in printing and lithography, paint, rubber, dry cleaning, adhesives and coatings, and detergents; formerly widely used as solvent and fumigant
Beryllium and beryllium compounds	Lung	Missile fuel and space vehicles; hardener for lightweight metal alloys, particularly in aerospace applications and nuclear reactors
Cadmium and cadmium compounds	Prostate	Uses include yellow pigments and phosphors; found in solders; used in batteries and as alloy and in metal platings and coatings
Chromium compounds	Lung	Component of metal alloys, paints, pigments, and preservatives
Nickel compounds	Nose, lung	Nickel plating; component of ferrous alloys, ceramics, and batteries; by-product of stainless-steel arc welding
Radon and its decay products	Lung	From decay of minerals containing uranium; potentially serious hazard in quarries and underground mines
Vinyl chloride	Angiosarcoma, liver	Refrigerant; monomer for vinyl polymers; adhesive for plastics; formerly inert aerosol propellant in pressurized containers

Modified from Stellman JM, Stellman SD: Cancer and workplace. CA Cancer J Clin 46:70, 1996.

recognition of inherited predisposition to cancer has had a major impact on the understanding of cancer pathogenesis. Moreover, genes that are causally associated with cancers that have a strong hereditary component are generally also involved in the much more common sporadic forms of the same tumor. Genetic predisposition to cancer can be divided into three categories (Table 7–4).

Autosomal Dominant Inherited Cancer Syndromes. Inherited cancer syndromes include several well-defined cancers in which inheritance of a single autosomal dominant mutant gene greatly increases the risk of developing a tumor. The inherited mutation is usually a point mutation occurring in a single allele of a tumor suppressor gene. The silencing of the second allele occurs in somatic cells, generally as a consequence of deletion or recombination. Childhood *retinoblastoma* is the most striking example in this category. Approximately 40% of retinoblastomas are inherited. Carriers of a mutant of the *RB tumor suppressor gene* have a 10,000-fold increased risk of developing retinoblastoma, usually bilateral. They also have a greatly increased risk of developing a second cancer, particularly osteosarcoma. Familial adenomatous polyposis is an autosomal dominant hereditary disorder caused by mutation of the adenomatous polyposis coli (*APC*) tumor suppressor gene. Other autosomal dominant cancer syndromes include Li-Fraumeni syndrome resulting from germline mutations of the *p53* gene; multiple endocrine neoplasia types 1 and 2 (MEN-1 and MEN-2) caused by mutation in the genes that encode the menin transcription factor and the RET tyrosine kinase, respectively; hereditary nonpolyposis colon cancer (HNPCC), a condition caused by inactivation of a DNA mismatch repair gene (also listed below among repair defects); and several others listed in Table 7–4.

There are several features that characterize inherited cancer syndromes:

- In each syndrome, tumors tend to arise in specific sites and tissues, although they may involve more than one site. There is no increase in predisposition to cancers in general. For example, in MEN-2, thyroid, parathyroid, and adrenals are involved, while in MEN-1, the pituitary, parathyroid, and pancreas are involved. Patients with familial adenomatous polyposis develop innumerable polypoid adenomas of the colon, and virtually 100% of those affected develop a colonic adenocarcinoma by age 50. The one exception to this tumor specific tissue involvement is Li-Fraumeni syndrome.
- Tumors within this group are often associated with a specific marker phenotype. For example, there may be multiple

TABLE 7–4 Examples of Inherited Predisposition to Cancer

Gene	*Inherited Predisposition*
INHERITED CANCER SYNDROMES (AUTOSOMAL DOMINANT)	
RB	Retinoblastoma
p53	Li-Fraumeni syndrome (various tumors)
p16/INK4A	Melanoma
APC	Familial adenomatous polyposis/colon cancer
NF1, NF2	Neurofibromatosis 1 and 2
BRCA1, BRCA2	Breast and ovarian tumors
MEN1, RET	Multiple endocrine neoplasia 1 and 2
MSH2, MLH1, MSH6	Hereditary nonpolyposis colon cancer
PTCH	Nevoid basal cell carcinoma syndrome
PTEN	Cowden syndrome (epithelial cancers)
LKB1	Peutz-Jegher syndrome (epithelial cancers)
VHL	Renal cell carcinomas
INHERITED AUTOSOMAL RECESSIVE SYNDROMES OF DEFECTIVE DNA REPAIR	
Xeroderma pigmentosum	
Ataxia-telangiectasia	
Bloom syndrome	
Fanconi anemia	
FAMILIAL CANCERS	
Familial clustering of cases, but role of inherited predisposition not clear for each individual	
Breast cancer	
Ovarian cancer	
Pancreatic cancer	

benign tumors in the affected tissue, as occurs in familial polyposis of the colon and in MEN. Sometimes, there are abnormalities in tissue that are not the target of transformation (e.g., Lisch nodules and café-au-lait spots in neurofibromatosis type 1; see Chapter 27).

As in other autosomal dominant conditions, both incomplete penetrance and variable expressivity occur.

Defective DNA-Repair Syndromes. Besides the dominantly inherited precancerous conditions, a group of cancer-predisposing conditions is collectively characterized by defects in DNA repair and resultant DNA instability. These conditions generally have an autosomal recessive pattern of inheritance. Included in this group are xeroderma pigmentosum, ataxia-telangiectasia, and Bloom syndrome, all rare diseases characterized by genetic instability resulting from defects in DNA-repair genes. Also included here is HNPCC, an autosomal dominant condition caused by inactivation of a DNA mismatch repair gene.[21] HNPCC is the most common cancer predisposition syndrome, increasing the susceptibility of cancer of the colon, the small intestine, endometrium, and ovary (Chapter 17).

Familial Cancers. Besides the inherited syndromes of cancer susceptibility, cancer may occur at higher frequency in certain families without a clearly defined pattern of transmission. Virtually all the common types of cancers that occur sporadically have also been reported to occur in familial forms. Examples include carcinomas of colon, breast, ovary, and brain, as well as melanomas and lymphomas. *Features that characterize familial cancers include early age at onset, tumors arising in two or more close relatives of the index case, and sometimes, multiple or bilateral tumors.* Familial cancers are not associated with specific marker phenotypes. For example, in contrast to the familial adenomatous polyp syndrome, familial colonic cancers do not arise in preexisting benign polyps. The transmission pattern of familial cancers is not clear. In general, siblings have a risk between two and three times greater than unrelated individuals. Segregation analyses of large families usually show that predisposition to the tumors is dominant, but multifactorial inheritance cannot be easily ruled out. It is likely that familial susceptibility to cancer may depend on multiple low-penetrance alleles, each contributing to only a small increase in the risk of tumor development. Genome-wide association studies show great promise in identifying such alleles (Chapter 5).[22] It has been estimated that 10% to 20% of patients with breast or ovarian cancer have a first- or second-degree relative with one of these tumors. Although two breast cancer susceptibility genes, named *BRCA1* and *BRCA2*, have been identified, mutation of these genes occurs in no more than 3% of breast cancers.[20] A similar situation occurs in familial melanomas, in which a mutation of the *p16* tumor suppressor gene has been identified. However, mutation in this gene accounts for only about 20% of familial melanoma kindreds, suggesting that other factors are involved in the familial predisposition.[23]

Interactions between Genetic and Nongenetic Factors. What can be said about the influence of heredity on the majority of malignant neoplasms? It could be argued that they are largely of environmental origin, but lack of family history does not preclude an inherited component. It is generally difficult to sort out the hereditary and acquired basis of a tumor, because these factors often interact closely. The interaction between genetic and nongenetic factors is particularly complex when tumor development depends on the action of multiple contributory genes. Even in tumors with a well-defined inherited component, the risk of developing the tumor can be

greatly influenced by nongenetic factors. For instance, breast cancer risk in female carriers of *BRCA1* or *BRCA2* mutations is almost threefold higher for women born after 1940, as compared with the risks for women born before that year.[20] Furthermore, the genotype can significantly influence the likelihood of developing environmentally induced cancers. Inherited variations (polymorphisms) of enzymes that metabolize procarcinogens to their active carcinogenic forms (see "Initiation of Carcinogenesis") can influence the susceptibility to cancer. Of interest in this regard are genes that encode the cytochrome P-450 enzymes. As discussed later under "Chemical Carcinogenesis," polymorphism at one of the P-450 loci confers inherited susceptibility to lung cancers in cigarette smokers. More such associations are likely to be found.

NONHEREDITARY PREDISPOSING CONDITIONS

The only certain way of avoiding cancer is not to be born; to live is to incur the risk. Certain predisposing influences, such as environment, behaviors, and clinical conditions, can increase that risk, however. For example, regenerative, metaplastic, hyperplastic, and dysplastic proliferations are fertile soil for the origin of a malignant tumor, because cell replication is involved in neoplastic transformation. Indeed, proliferation may be required for neoplastic transformation in some settings, since it is proliferating cells that accumulate the genetic lesions required for carcinogenesis.

Chronic Inflammation and Cancer. In 1863 Virchow proposed that *cancer develops at sites of chronic inflammation*, and the potential relationships between cancer and inflammation have been studied since then.[24] This is exemplified by the increased risk of cancer in individuals affected by a variety of chronic inflammatory diseases of the gastrointestinal tract (Table 7–5). These include ulcerative colitis, *Helicobacter pylori* gastritis, viral hepatitis, and chronic pancreatitis. Although the precise mechanisms that link inflammation and cancer development have not been established, recent work has demonstrated that in the setting of unresolved chronic inflammation, as occurs in viral hepatitis or chronic gastritis, the immune response may become maladaptive, promoting tumorigenesis.[24] As with any cause of tissue injury, there is a compensatory proliferation of cells so as to repair the damage. This regenerative process is aided and abetted by a plethora of growth factors, cytokines, chemokines, and other bioactive substances produced by activated immune cells that promote cell survival, tissue remodeling, and angiogenesis. In some cases, chronic inflammation may increase the pool of tissue stem cells, which become subject to the effect of mutagens. These mediators also cause genomic stress and mutations; additionally the activated immune cells produce reactive oxygen species that are directly genotoxic. To add insult to injury, many of these mediators promote cell survival, even in the face of genomic damage. In the short term this can be adaptive; the organism must survive, and the damaged cells can be repaired or eliminated later. However, in chronic inflammation such behavior is maladaptive, since it allows the creation and fixation of such mutations, eventually leading to cancer. Whatever the precise mechanism, the link between chronic inflammation and cancer has practical implications. For instance, expression of the enzyme *cyclooxygenase-2 (COX-2)*, which brings about the conversion of arachidonic acid into prostaglandins (Chapter 2), is induced by inflammatory stimuli and is increased in colon cancers and other tumors.[25] The development of COX-2 inhibitors for cancer treatment is an active area of research.[26]

Precancerous Conditions. Certain non-neoplastic disorders—*the chronic atrophic gastritis of pernicious anemia, solar keratosis of the skin, chronic ulcerative colitis, and leukoplakia of the oral cavity, vulva, and penis*—have such a well-defined association with cancer that they have been termed *precancerous conditions*. This designation is somewhat unfortunate, because in the great majority of these lesions no malignant neoplasm emerges. Nonetheless, the term persists because it calls attention to the increased risk. Certain forms of benign neoplasia also constitute precancerous conditions. The villous adenoma of the colon, as it increases in size, becomes malignant in up to 50% of cases. It might be asked: Is there not a risk with all benign neoplasms? Although some risk may be inherent, a large cumulative experience indicates that most benign neoplasms do not become cancerous. Nonetheless, numerous examples could be offered of cancers arising, albeit rarely, in benign tumors—for example, a leiomyosarcoma beginning in a leiomyoma, and carcinoma appearing in long-standing pleomorphic adenomas. Generalization is impossible, because each type of benign neoplasm is associated with a particular level of risk ranging from virtually never to frequently. Only follow-up studies of large series of each neoplasm can establish the level of risk, and always the question remains: Did the cancer arise from a nonmalignant cell in the benign tumor, or did the benign tumor contain, from the outset, a silent or indolent malignant focus?

Molecular Basis of Cancer

The literature on the molecular basis of cancer continues to proliferate at such a rapid pace that it is easy to get lost in the growing forest of information. We list some fundamental principles before delving into the details of the molecular basis of cancer.

- *Nonlethal genetic damage lies at the heart of carcinogenesis.* Such genetic damage (or mutation) may be acquired by the action of environmental agents, such as chemicals, radiation, or viruses, or it may be inherited in the germ line.[26] The term *environmental*, used in this context, involves any acquired defect caused by exogenous agents or endogenous products of cell metabolism. Not all mutations, however, are "environmentally" induced. Some may be spontaneous and stochastic, falling into the category of bad luck.
- *A tumor is formed by the clonal expansion of a single precursor cell that has incurred genetic damage (i.e., tumors are monoclonal).* Clonality of tumors can be assessed in women who are heterozygous for polymorphic X-linked markers, such as the androgen receptor. The principle underlying such an analysis is illustrated in Figure 7–23. The most commonly used method to determine tumor clonality involves the analysis of methylation patterns adjacent to the highly polymorphic locus of the human androgen receptor gene, *AR*.[27] The frequency of such polymorphisms in the general population is more than 90%, so it is easy to estab-

TABLE 7–5 Chronic Inflammatory States and Cancer

Pathologic Condition	Associated Neoplasm(s)	Etiologic Agent
Asbestosis, silicosis	Mesothelioma, lung carcinoma	Asbestos fibers, silica particles
Bronchitis	Lung carcinoma	Silica, asbestos, smoking (nitrosamines, peroxides)
Cystitis, bladder inflammation	Bladder carcinoma	Chronic indwelling urinary catheters
Gingivitis, lichen planus	Oral squamous cell carcinoma	
Inflammatory bowel disease	Colorectal carcinoma	
Lichen sclerosis	Vulvar squamous cell carcinoma	
Chronic pancreatitis	Pancreatic carcinoma	Alcoholism
Hereditary pancreatitis	Pancreatic carcinoma	Mutation in trypsinogen gene
Reflux esophagitis, Barrett esophagus	Esophageal carcinoma	Gastric acids
Sialadenitis	Salivary gland carcinoma	
Sjögren syndrome, Hashimoto thyroiditis	MALT lymphoma	
CANCERS ASSOCIATED WITH INFECTIOUS AGENTS		
Opisthorchis, cholangitis	Cholangiosarcoma, colon carcinoma	Liver flukes (*Opisthorchis viverrini*)
		Bile acids
Chronic cholecystitis	Gallbladder cancer	Bacteria, gallbladder stones
Gastritis/ulcers	Gastric adenocarcinoma, MALT	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Mononucleosis	B-cell non-Hodgkin lymphoma and Hodgkin lymphoma	Epstein-Barr virus
AIDS	Non-Hodgkin lymphoma, squamous cell carcinoma, Kaposi sarcoma	Human immunodeficiency virus, human herpesvirus type 8
Osteomyelitis	Carcinoma in draining sinuses	Bacterial infection
Pelvic inflammatory disease, chronic cervicitis	Ovrian carcinoma, cervical/anal carcinoma	Gonorrhea, chlamydia, human papillomavirus
Chronic cystitis	Bladder, liver, rectal carcinoma	Schistosomiasis

Adapted from Tlsty TD, Coussens LM: Tumor stroma and regulation of cancer development. Ann Rev Pathol Mech Dis 1:119, 2006.

lish clonality by showing that all the cells in a tumor express the same allele. For tumors with acquired cytogenetic aberrations of any type (e.g., a translocation) their presence can be taken as evidence that the proliferation is clonal. Immunoglobulin receptor and T-cell receptor gene rearrangements serve as markers of clonality in B- and T-cell lymphomas, respectively.

- *Four classes of normal regulatory genes—the growth-promoting proto-oncogenes, the growth-inhibiting tumor suppressor genes, genes that regulate programmed cell death (apoptosis), and genes involved in DNA repair—are the principal targets of genetic damage.* Mutant alleles of proto-oncogenes are considered dominant, because they transform cells despite the presence of a normal counterpart. In contrast, typically, both normal alleles of the tumor suppressor genes must be damaged before transformation can occur. However, there are exceptions to this rule; sometimes, loss of a single allele of a tumor suppressor gene reduces levels or activity of the protein enough that the brakes on cell proliferation and survival are released. Loss of gene function caused by damage to a single allele is called *haploinsufficiency.* Such a finding indicates that dosage of the gene is important, and that two copies are required for normal function.[28] Genes that regulate apoptosis may behave as proto-oncogenes or tumor suppressor genes. Mutations of DNA repair genes do not directly transform cells by affecting proliferation or apoptosis. Instead, DNA-repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including proto-oncogenes, tumor suppressor genes, and genes that regulate apoptosis. A disability in the DNA-repair genes can predispose cells to widespread mutations in the genome and thus to neoplastic transformation. Cells with mutations in DNA repair genes are said to have developed a *mutator phenotype.*[29] Interestingly, a new class of regulatory molecules, called microRNAs (miRNAs), has recently been discovered (Chapter 5). Even though they do not encode proteins, different families of miRNAs have been shown to act as either oncogenes or tumor suppressors.[29,30] They do so by affecting the translation of other genes as will be discussed later.
- *Carcinogenesis is a multistep process at both the phenotypic and the genetic levels, resulting from the accumulation of multiple mutations.*[31] As discussed earlier, malignant neoplasms have several phenotypic attributes, such as excessive growth, local invasiveness, and the ability to form distant metastases. Furthermore, it is well established that over a period of time many tumors become more aggressive and acquire greater malignant potential. This phenomenon is referred to as tumor progression and is not simply a function of an increase in tumor size. Careful clinical and experimental studies reveal that increasing malignancy is often acquired in an incremental fashion. At the molecular level, tumor progression and associated heterogeneity most likely result from multiple mutations that accumulate independently in different cells, generating subclones with varying abilities to grow, invade, metastasize, and resist (or respond to) therapy (Fig. 7–24). Some of the mutations may be lethal; others may spur cell growth by affecting additional proto-oncogenes or tumor suppressor genes. *Even though most malignant tumors are monoclonal in origin, by the time they become clinically evident their constituent cells are*

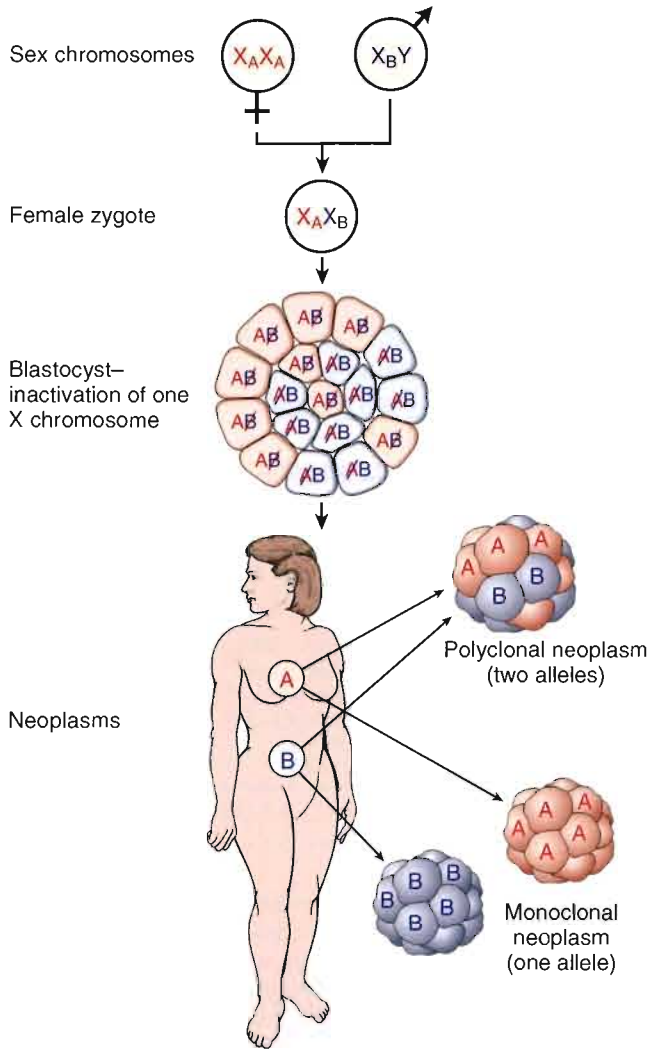


FIGURE 7–23 The use of X-linked markers as evidence of the monoclonality of neoplasms. Because of random X inactivation, all females are mosaics with two cell populations (with different alleles for the androgen receptor labeled A and B in this case). When neoplasms that arise in women who are heterozygous for X-linked markers are analyzed, they are made up of cells that contain the active maternal (X_A) or the paternal (X_B) X chromosome but not both.

extremely heterogeneous. During progression, tumor cells are subjected to immune and nonimmune selection pressures. For example, cells that are highly antigenic are destroyed by host defenses, whereas those with reduced growth factor requirements are positively selected. A growing tumor therefore tends to be enriched for subclones that "beat the odds" and are adept at survival, growth, invasion, and metastasis.

ESSENTIAL ALTERATIONS FOR MALIGNANT TRANSFORMATION

With this overview we can now address in some detail the molecular pathogenesis of cancer and then discuss the carcinogenic agents that inflict genetic damage. Over the past two decades, hundreds of cancer-associated genes have been discovered. Some, such as *p53*, are mutated in many different cancers; others, such as *ABL1*, are affected only in one or few. Each of the cancer-associated genes has a specific function, the dysregulation of which contributes to the origin or progression of malignancy. It is traditional to describe cancer-associated genes on the basis of their presumed function. It is beneficial, however, to consider cancer-related genes in the context of *seven fundamental changes in cell physiology that together determine malignant phenotype.*[32] (Another important change for tumor development is *escape from immune attack.* This property is discussed later in this chapter.) The seven key changes are the following:

- *Self-sufficiency in growth signals*: Tumors have the capacity to proliferate without external stimuli, usually as a consequence of oncogene activation.
- *Insensitivity to growth-inhibitory signals*: Tumors may not respond to molecules that are inhibitory to the proliferation of normal cells such as transforming growth factor β (TGF-β) and direct inhibitors of cyclin-dependent kinases (CDKIs).
- *Evasion of apoptosis*: Tumors may be resistant to programmed cell death, as a consequence of inactivation of *p53* or activation of anti-apoptotic genes.
- *Limitless replicative potential*: Tumor cells have unrestricted proliferative capacity, avoiding cellular senescence and mitotic catastrophe.
- *Sustained angiogenesis*: Tumor cells, like normal cells, are not able to grow without formation of a vascular supply to bring nutrients and oxygen and remove waste products. Hence, tumors must induce angiogenesis.
- *Ability to invade and metastasize*: Tumor metastases are the cause of the vast majority of cancer deaths and depend on processes that are intrinsic to the cell or are initiated by signals from the tissue environment.
- *Defects in DNA repair*: Tumors may fail to repair DNA damage caused by carcinogens or incurred during unregulated cellular proliferation, leading to genomic instability and mutations in proto-oncogenes and tumor suppressor genes.

Mutations in one or more genes that regulate these cellular traits are seen in every cancer. However, the precise genetic pathways that give rise to these attributes differ between individual cancers, even within the same organ. It is widely believed that the occurrence of mutations in cancer-related genes is conditioned by the robustness of the DNA-repair machinery, as well as protective mechanisms such as apoptosis and senescence that prevent the proliferation of cells with damaged DNA. Indeed, recent studies in a variety of human tumors, such as melanoma and prostate adenocarcinoma, have shown that oncogene-induced senescence, wherein mutation of a proto-oncogene drives cells into senescence rather than proliferation, is an important barrier to carcinogenesis.[33] Some limits to neoplastic growth are even physical. If a tumor is to grow larger than 1 to 2 mm, mechanisms that allow the delivery of nutrients and the elimination of waste products must

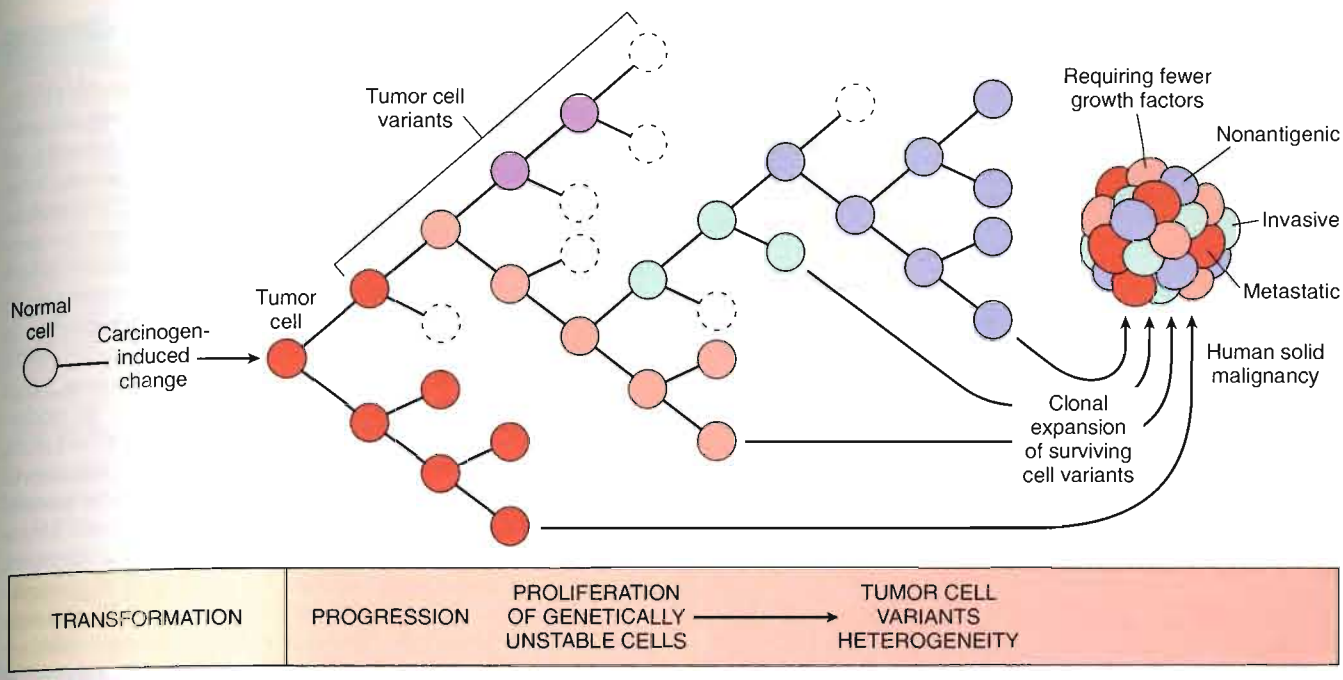


FIGURE 7–24 Tumor progression and generation of heterogeneity. New subclones arise from the descendants of the original transformed cell by multiple mutations. With progression the tumor mass becomes enriched for variants that are more adept at evading host defenses and are likely to be more aggressive.

be provided (angiogenesis). Furthermore, epithelia are separated from the interstitial matrix by a basement membrane, composed of extracellular matrix molecules, that must be broken down by invasive carcinoma cells. These protective barriers, both intrinsic and extrinsic to the cell, must be breached, and feedback loops that normally prevent uncontrolled cell division must be disabled by mutations before a fully malignant tumor can emerge. The main principles of the molecular basis of cancer are summarized in a simplified form in Figure 7–25.

In the following sections we discuss the nature of the genes involved in each of the seven biologic alterations listed earlier. We end with a discussion of epigenetic changes and chromosomal abnormalities in cancer.

SELF-SUFFICIENCY IN GROWTH SIGNALS: ONCOGENES

Genes that promote autonomous cell growth in cancer cells are called *oncogenes*, and their unmutated cellular counterparts are called *proto-oncogenes*. Oncogenes are created by mutations in proto-oncogenes and are characterized by the ability to promote cell growth in the absence of normal growth-promoting signals. Their products, called *oncoproteins*, resemble the normal products of proto-oncogenes except that oncoproteins are often devoid of important internal regulatory elements, and their production in the transformed cells does not depend on growth factors or other external signals. In this way cell growth becomes autonomous, freed from checkpoints and dependence upon external signals. To aid in the understanding of the nature and functions of oncoproteins and their role in cancer, it is necessary to briefly mention the sequential steps that characterize normal cell proliferation.

Under physiologic conditions cell proliferation can be readily resolved into the following steps:

- The binding of a growth factor to its specific receptor
- Transient and limited activation of the growth factor receptor, which, in turn, activates several signal-transducing proteins on the inner leaflet of the plasma membrane
- Transmission of the transduced signal across the cytosol to the nucleus via second messengers or by a cascade of signal transduction molecules
- Induction and activation of nuclear regulatory factors that initiate DNA transcription
- Entry and progression of the cell into the cell cycle, ultimately resulting in cell division

With this background we can readily identify the strategies used by cancer cells to acquire self-sufficiency in growth signals. They can be grouped on the basis of their role in growth factor–mediated signal transduction cascades and cell cycle regulation.

Proto-oncogenes, Oncogenes, and Oncoproteins

Proto-oncogenes have multiple roles, participating in cellular functions related to growth and proliferation. Proteins encoded by proto-oncogenes may function as growth factors or their receptors, signal transducers, transcription factors, or cell cycle components. Oncoproteins encoded by oncogenes generally serve functions similar to their normal counterparts (Table 7–6). However, *mutations convert proto-oncogenes into constitutively active cellular oncogenes that are involved in tumor development because the oncoproteins they encode endow the cell with self-sufficiency in growth.*[34]

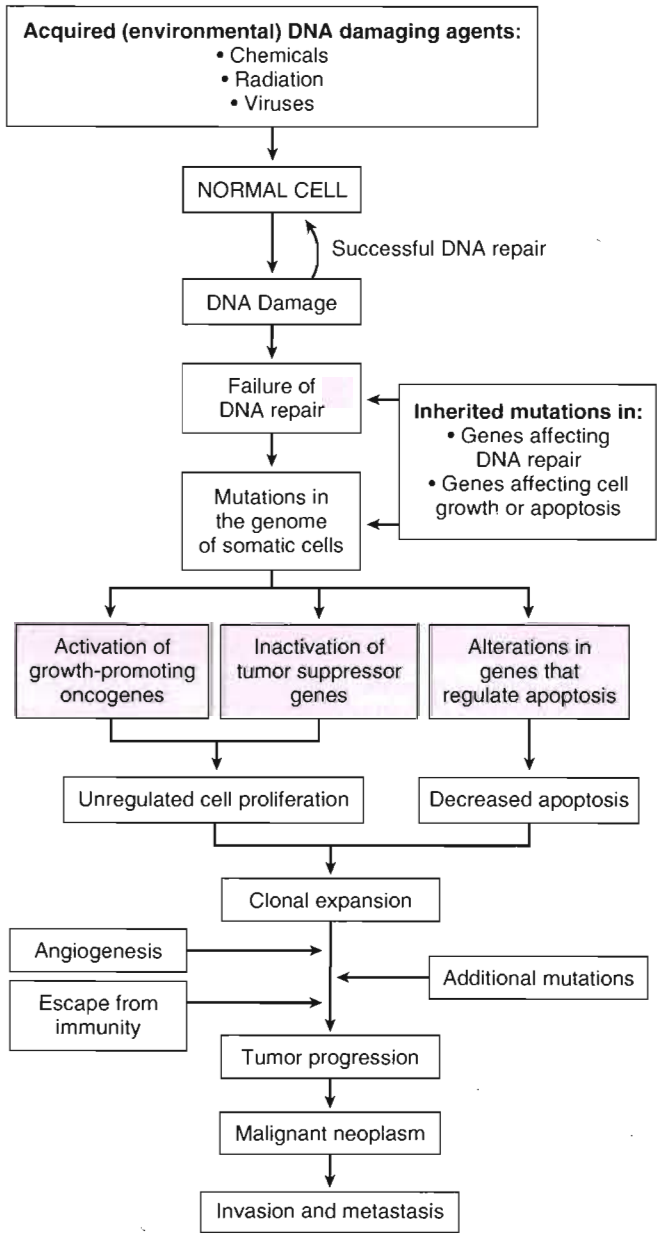


FIGURE 7–25 Flowchart depicting a simplified scheme of the molecular basis of cancer.

Two questions follow: (1) What are the functions of oncogene products, the oncoproteins? (2) How do the normally "civilized" proto-oncogenes turn into "enemies within"? These issues are discussed below.

Growth Factors. Normal cells require stimulation by growth factors to undergo proliferation. Most soluble growth factors are made by one cell type and act on a neighboring cell to stimulate proliferation (paracrine action). Many cancer cells, however, acquire the ability to synthesize the same growth factors to which they are responsive, generating an autocrine loop. For example, many glioblastomas secrete platelet-derived growth factor (PDGF) and express the PDGF receptor, and many sarcomas make both transforming growth factor α (TGF-α) and its receptor. Although an autocrine loop is considered to be an important element in the pathogenesis of several tumors, in most instances the growth factor gene itself is not altered or mutated. More commonly, products of other oncogenes that lie along many signal transduction pathways, such as *RAS*, cause overexpression of growth factor genes, thus forcing the cells to secrete large amounts of growth factors, such as TGF-α. Nevertheless, increased growth factor production is not sufficient for neoplastic transformation. In all likelihood growth factor driven proliferation contributes to the malignant phenotype by increasing the risk of spontaneous or induced mutations in the proliferating cell population.

Growth Factor Receptors. Several oncogenes that encode growth factor receptors have been found. To understand how mutations affect the function of these receptors, it should be recalled that one important class of growth factor receptors are transmembrane proteins with an external ligand-binding domain and a cytoplasmic tyrosine kinase domain (Chapter 3). In the normal forms of these receptors, the kinase is *transiently* activated by binding of the specific growth factors, followed rapidly by receptor dimerization and tyrosine phosphorylation of several substrates that are a part of the signaling cascade. *The oncogenic versions of these receptors are associated with constitutive dimerization and activation without binding to the growth factor.* Hence, the mutant receptors deliver continuous mitogenic signals to the cell, even in the absence of growth factor in the environment.

Growth factor receptors can be constitutively activated in tumors by multiple different mechanisms, including mutations, gene rearrangements, and overexpression. The *RET* proto-oncogene, a receptor tyrosine kinase, exemplifies oncogenic conversion via mutations and gene rearrangements.[33] The RET protein is a receptor for the glial cell line–derived neurotrophic factor and structurally related proteins that promote cell survival during neural development. RET is normally expressed in neuroendocrine cells, such as parafollicular C cells of the thyroid, adrenal medulla, and parathyroid cell precursors. Point mutations in the *RET* proto-oncogene are associated with dominantly inherited MEN types 2A and 2B and familial medullary thyroid carcinoma (Chapter 24). In MEN-2A, point mutations in the *RET* extracellular domain cause constitutive dimerization and activation, leading to medullary thyroid carcinomas and adrenal and parathyroid tumors. In MEN-2B, point mutations in the *RET* cytoplasmic catalytic domain alter the substrate specificity of the tyrosine kinase and lead to thyroid and adrenal tumors without involvement of the parathyroid. In all these familial conditions, the affected individuals inherit the *RET* mutation in the germline. Sporadic medullary carcinomas of the thyroid are associated with somatic rearrangements of the *RET* gene, generally similar to those found in MEN-2B.[35,36]

Oncogenic conversions by mutations and rearrangements have been found in other growth factor receptor genes. Point mutations in *FLT3*, the gene encoding the FMS-like tyrosine kinase 3 receptor, that lead to constitutive signaling have been detected in myeloid leukemias. In certain chronic myelomonocytic leukemias with the (5;12) translocation, the entire cytoplasmic domain of the PDGF receptor is fused with a segment of an ETS family transcription factor, resulting in permanent dimerization of the PDGF receptor. Greater than

TABLE 7–6 Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

Category	Proto-oncogene	Mode of Activation	Associated Human Tumor
GROWTH FACTORS			
PDGF-β chain	*SIS* (official name *PBGFB*)	Overexpression	Astrocytoma Osteosarcoma
Fibroblast growth factors	*HST1* *INT2* (official name *FGF3*)	Overexpression Amplification	Stomach cancer Bladder cancer Breast cancer Melanoma
TGF-α	*TGFA*	Overexpression	Astrocytomas Hepatocellular carcinomas
HGF	*HGF*	Overexpression	Thyroid cancer
GROWTH FACTOR RECEPTORS			
EGF-receptor family	*ERBB1* (*EGFR*), *ERRB2*	Overexpression	Squamous cell carcinoma of lung, gliomas
FMS-like tyrosine kinase 3	*FLT3*	Amplification	Breast and ovarian cancers
Receptor for neurotrophic factors	*RET*	Point mutation Point mutation	Leukemia Multiple endocrine neoplasia 2A and B, familial medullary thyroid carcinomas
PDGF receptor	*PDGFRB*	Overexpression, translocation	Gliomas, leukemias
Receptor for stem cell (steel) factor	*KIT*	Point mutation	Gastrointestinal stromal tumors, seminomas, leukemias
PROTEINS INVOLVED IN SIGNAL TRANSDUCTION			
GTP-binding	*KRAS* *HRAS* *NRAS*	Point mutation Point mutation Point mutation	Colon, lung, and pancreatic tumors Bladder and kidney tumors Melanomas, hematologic malignancies
Nonreceptor tyrosine kinase	*ABL*	Translocation	Chronic myeloid leukemia Acute lymphoblastic leukemia
RAS signal transduction	*BRAF*	Point mutation	Melanomas
WNT signal transduction	β-catenin	Point mutation Overexpression	Hepatoblastomas, hepatocellular carcinoma
NUCLEAR-REGULATORY PROTEINS			
Transcriptional activators	C-*MYC* N-*MYC*	Translocation Amplification	Burkitt lymphoma Neuroblastoma, small-cell carcinoma of lung
	L-*MYC*	Amplification	Small-cell carcinoma of lung
CELL CYCLE REGULATORS			
Cyclins	Cyclin D	Translocation Amplification	Mantle cell lymphoma Breast and esophageal cancers
	Cyclin E	Overexpression	Breast cancer
Cyclin-dependent kinase	*CDK4*	Amplification or point mutation	Glioblastoma, melanoma, sarcoma

90% of gastrointestinal stromal tumors have a constitutively activating mutation in the receptor tyrosine kinase c-KIT or PDGFR, which are the receptors for stem cell factor and PDGF, respectively. These mutations are amenable to specific inhibition by the tyrosine kinase inhibitor imatinib mesylate. This type of therapy, directed to a specific alteration in the cancer cell, is called *targeted therapy*.[37]

Far more common than mutations of these proto-oncogenes is overexpression of normal forms of growth factor receptors. In some tumors increased receptor expression results from gene amplification, but in many cases the molecular basis of increased receptor expression is not fully known. Two members of the epidermal growth factor (EGF) receptor family are the best described. The normal form of *ERBB1*, the EGF receptor gene, is overexpressed in up to 80% of squamous cell carcinomas of the lung, in 50% or more of *glioblastomas* (Chapter 28), and in 80% to 100% of head and neck tumors.[38,39] Likewise, the *ERBB2* gene (also called *HER-2/NEU*), the second member of the EGF receptor family, is amplified in approximately 25% of breast cancers and in human adenocarcinomas arising within the ovary, lung, stomach, and salivary glands.[36] Because the molecular alteration in *ERBB2* is specific for the cancer cells, new therapeutic agents consisting of monoclonal antibodies specific to *ERBB2* have been developed and are currently in use clinically, providing yet another example of targeted therapy.[38,39]

Signal-Transducing Proteins. Several examples of oncoproteins that mimic the function of normal cytoplasmic signal-transducing proteins have been found. Most such proteins are strategically located on the inner leaflet of the plasma membrane, where they receive signals from outside the cell (e.g., by activation of growth factor receptors) and transmit

them to the cell's nucleus. Biochemically, the signal-transducing proteins are heterogeneous. *The most well-studied example of a signal-transducing oncoprotein is the RAS family of guanine triphosphate (GTP)-binding proteins (G proteins).*

The RAS Oncogene. The *RAS* genes, of which there are three in the human genome (*HRAS*, *KRAS*, *NRAS*), were discovered initially in transforming retroviruses. *Point mutation of RAS family genes is the single most common abnormality of proto-oncogenes in human tumors.* Approximately 15% to 20% of all human tumors contain mutated versions of RAS proteins.[40] The frequency of such mutations varies with different tumors, but in some types of cancers it is very high. For example, 90% of pancreatic adenocarcinomas and cholangiocarcinomas contain a *RAS* point mutation, as do about 50% of colon, endometrial, and thyroid cancers and 30% of lung adenocarcinomas and myeloid leukemias.[41,42] In general, carcinomas (particularly from colon and pancreas) have mutations of *KRAS*, bladder tumors have *HRAS* mutations, and hematopoietic tumors bear *NRAS* mutations. *RAS* mutations are infrequent in certain other cancers, such as those arising in the uterine cervix or breast.

RAS plays an important role in signaling cascades downstream of growth factor receptors, resulting in mitogenesis. For example, abrogation of RAS function blocks the proliferative response to EGF, PDGF, and CSF-1. Normal RAS proteins are tethered to the cytoplasmic aspect of the plasma membrane, as well as the endoplasmic reticulum and Golgi membranes. They can be activated by growth factor binding to receptors at the plasma membrane.[40] RAS is a member of a family of small G proteins that bind guanosine nucleotides (guanosine triphosphate, GTP and guanosine diphosphate, GDP), similar to the larger trimolecular G proteins. Normally RAS proteins flip back and forth between an excited signal-transmitting state and a quiescent state. In the inactive state, RAS proteins bind GDP. Stimulation of cells by growth factors leads to exchange of GDP for GTP and subsequent conformational changes that generates active RAS (Fig. 7–26). The activated RAS stimulates downstream regulators of proliferation, such as the *mitogen-activated protein (MAP) kinase cascade*, which floods the nucleus with signals for cell proliferation.

The orderly cycling of the RAS protein depends on two reactions: (1) nucleotide exchange (GDP by GTP), which activates RAS protein, and (2) GTP hydrolysis, which converts the GTP-bound, active RAS to the GDP-bound, inactive form. Both these processes are extrinsically regulated by other proteins. The removal of GDP and its replacement by GTP during RAS activation are catalyzed by a family of guanine nucleo-

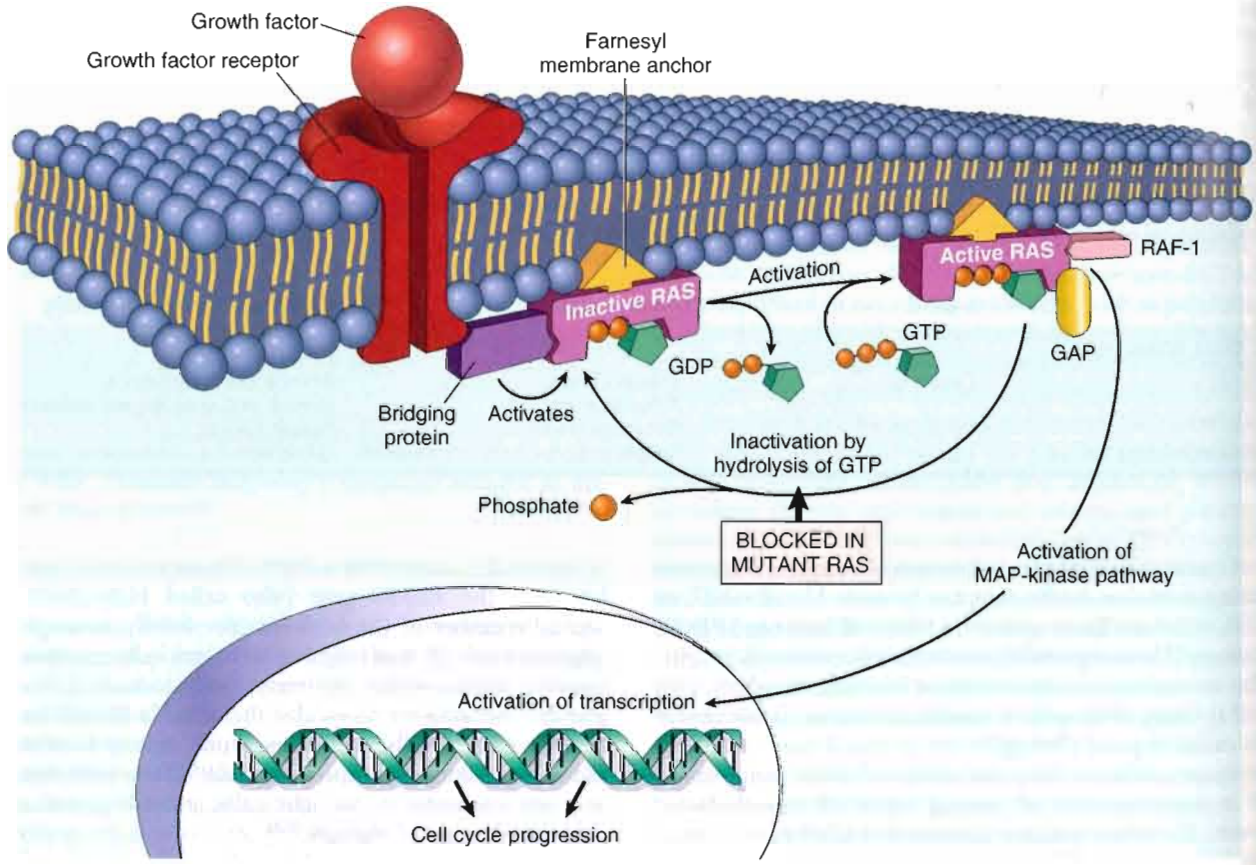


FIGURE 7–26 Model for action of *RAS* genes. When a normal cell is stimulated through a growth factor receptor, inactive (GDP-bound) RAS is activated to a GTP-bound state. Activated RAS recruits RAF and stimulates the MAP-kinase pathway to transmit growth-promoting signals to the nucleus. The mutated RAS protein is permanently activated because of inability to hydrolyze GTP, leading to continuous stimulation of cells without any external trigger. The anchoring of RAS to the cell membrane by the farnesyl moiety is essential for its action. See text for explanation of abbreviations.

tide–releasing proteins. Conversely, the GTPase activity intrinsic to normal RAS proteins is dramatically accelerated by *GTPase-activating proteins (GAPs)*. These widely distributed proteins bind to the active RAS and augment its GTPase activity by more than 1000-fold, leading to termination of signal transduction. Thus, GAPs function as "brakes" that prevent uncontrolled RAS activity.

Several distinct point mutations of *RAS* have been identified in cancer cells. The affected residues lie within either the GTP-binding pocket or the enzymatic region essential for GTP hydrolysis, and thus markedly reduce the GTPase activity of the RAS protein. Mutated RAS is trapped in its activated GTP-bound form, and the cell is forced into a continuously proliferating state. It follows from this scenario that the consequences of mutations in RAS protein would be mimicked by mutations in the GAPs that fail to activate the GTPase activity and thus restrain normal RAS proteins. Indeed, disabling mutation of neurofibromin 1, a GAP, is associated with the inherited cancer syndrome familial neurofibromatosis type 1 (Chapter 27).

In addition to RAS, downstream members of the RAS signaling cascade (RAS/RAF/MAP kinase) may also be altered in cancer cells, resulting in a similar phenotype. Thus, mutations in *BRAF*, one of the members of the *RAF* family, have been detected in more than 60% of melanomas and in more than 80% of benign nevi.[44,45] This suggests that dysregulation of the RAS/RAF/MAP kinase pathway may be one of the initiating events in the development of melanomas, although it is not sufficient by itself to cause tumorigenesis. Indeed, *BRAF* mutations alone lead to oncogene-induced senescence giving rise to benign nevi rather than malignant melanoma. Thus, oncogene-induced senescence is a barrier to carcinogenesis that must be overcome by mutation and disabling of key protective mechanisms, such as those provided by the *p53* gene (discussed later).[33]

Because *RAS* is so frequently mutated in human cancers, much effort has been spent to develop anti-RAS modalities of targeted therapy. Unfortunately, none of these strategies has so far proven to be successful for clinical use.

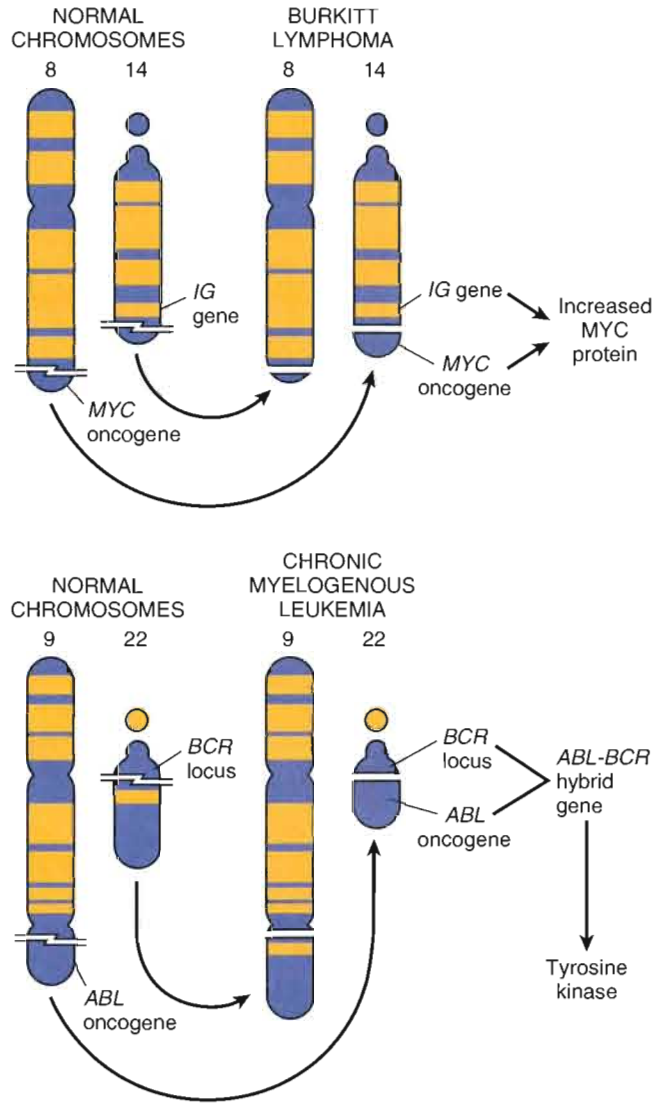


FIGURE 7–27 The chromosomal translocation and associated oncogenes in Burkitt lymphoma and chronic myelogenous leukemia.

Alterations in Nonreceptor Tyrosine Kinases

Mutations that unleash latent oncogenic activity occur in several non-receptor-associated tyrosine kinases, which normally function in signal transduction pathways that regulate cell growth (Chapter 3). As with receptor tyrosine kinases, in some instances the mutations take the form of chromosomal translocations or rearrangements that create fusion genes encoding constitutively active tyrosine kinases. An important example of this oncogenic mechanism involves the c-ABL tyrosine kinase. In CML and some acute lymphoblastic leukemias, the *ABL* gene is translocated from its normal abode on chromosome 9 to chromosome 22 (Fig. 7–27), where it fuses with the *BCR* gene (see discussion of chromosomal translocations, later in this chapter). The resultant chimeric gene encodes a constitutively active, oncogenic BCR-ABL tyrosine kinase. Several structural features of the BCR-ABL fusion protein contribute to the increased kinase activity, but the most important is that the BCR moiety promotes the self-association of BCR-ABL. This is a common theme, since many different oncogenic tyrosine kinases consist of fusion proteins in which the non–tyrosine kinase partner drives self-association.[46] Treatment of CML has been revolutionized by the development of imatinib mesylate, a "designer" drug with low toxicity and high therapeutic efficacy that inhibits the BCR-ABL kinase.[47–49] This is another example of rational drug design emerging from an understanding of the molecular basis of cancer. It is also an example of the concept of oncogene addiction.[50] Despite accumulation of numerous mutations throughout the genome, signaling through the BCR-ABL gene is required for the tumor to persist, hence inhibition of its activity is effective therapy. BCR-ABL translocation is an early, perhaps initiating event, during leukemogenesis. The remaining mutations are selected for, and built around, the constant signaling through BCR-ABL. BCR-ABL signaling can be seen as the central lodgepole around which the structure is

built. Remove the lodgepole by inhibition of the BCR-ABL kinase, and the structure collapses.

In other instances, nonreceptor tyrosine kinases are activated by point mutations that abrogate the function of negative regulatory domains that normally hold enzyme activity in check. For example, several myeloproliferative disorders, particularly polycythemia vera and primary myelofibrosis, are highly associated with activating point mutations in the tyrosine kinase JAK2 (Chapter 13).[51] The aberrant JAK2 kinase in turn activates transcription factors of the STAT family, which promote the growth factor–independent proliferation and survival of the tumor cells. Recognition of this molecular lesion has led to trials of JAK2 inhibitors in myeloproliferative disorders, and stimulated searches for activating mutations in other nonreceptor tyrosine kinases in a wide variety of human cancers.

Transcription Factors. Just as all roads lead to Rome, all signal transduction pathways converge to the nucleus, where a large bank of responder genes that orchestrate the cell's orderly advance through the mitotic cycle are activated. Indeed, the ultimate consequence of signaling through oncogenes like *RAS* or *ABL* is inappropriate and continuous stimulation of nuclear transcription factors that drive growth-promoting genes. Transcription factors contain specific amino acid sequences or motifs that allow them to bind DNA or to dimerize for DNA binding. Binding of these proteins to specific sequences in the genomic DNA activates transcription of genes. Growth autonomy may thus occur as a consequence of mutations affecting genes that regulate transcription. A host of oncoproteins, including products of the *MYC*, *MYB*, *JUN*, *FOS*, and *REL* oncogenes, are transcription factors that regulate the expression of growth-promoting genes, such as cyclins. *Of these, MYC is most commonly involved in human tumors,* and hence a brief overview of its function is warranted.

The MYC Oncogene. The *MYC* proto-oncogene is expressed in virtually all eukaryotic cells and belongs to the immediate early response genes, which are rapidly induced when quiescent cells receive a signal to divide (see discussion of liver regeneration in Chapter 3). After a transient increase of *MYC* messenger RNA, the expression declines to a basal level. The molecular basis of MYC function in cell replication is not entirely clear. As with many transcription factors, it is thought that MYC is involved in carcinogenesis by activating genes that are involved in proliferation. Indeed, some of its target genes, such as ornithine decarboxylase and cyclin D2, are known to be associated with cell proliferation. However, the range of activities modulated by MYC is very broad and includes histone acetylation, reduced cell adhesion, increased cell motility, increased telomerase activity, increased protein synthesis, decreased proteinase activity, and other changes in cellular matbolism that enable a high rate of cell division.[52] Genomic mapping of MYC binding sites has identified thousands of different sites and an equivalent number of genes that might be regulated.[53] However, there is little overlap in the MYC target genes in different cancers, preventing identification of a canonical MYC carcinogenesis program. Interestingly, it has been recently suggested that MYC interacts with components of the DNA-replication machinery, and plays a role in the selection of origins of replication.[54] Thus, overexpression of MYC may drive activation of more origins than needed for normal cell division, or bypass checkpoints involved in replication, leading to genomic damage and accumulation of mutations. Finally, MYC is one of a handful of transcription factors that can act in concert to reprogram somatic cells into pluripotent stem cells (Chapter 3); MYC may also enhance self-renewal, block differentiation, or both.

While on one hand *MYC* activation is linked to proliferation, on the other hand, cells in culture undergo apoptosis if *MYC* activation occurs in the absence of survival signals (growth factors).[55] The *MYC* proto-oncogene contains separate domains that encode the growth-promoting and apoptotic activities, but it is not clear whether MYC-induced apoptosis occurs in vivo.

In contrast to the regulated expression of *MYC* during normal cell proliferation, persistent expression, and in some cases overexpression, of the MYC protein are commonly found in tumors. Dysregulation of *MYC* expression resulting from translocation of the gene occurs in Burkitt lymphoma, a B-cell tumor (see Fig. 7–27). *MYC* is amplified in some cases of breast, colon, lung, and many other carcinomas. The related N-*MYC* and L-*MYC* genes are amplified in neuroblastomas (Fig. 7–28) and small-cell cancers of the lung, respectively.

Cyclins and Cyclin-Dependent Kinases. The ultimate outcome of all growth-promoting stimuli is the entry of quiescent cells into the cell cycle. Cancers may grow autonomously if the genes that drive the cell cycle become dysregulated by mutations or amplification. As described in Chapter 3, the

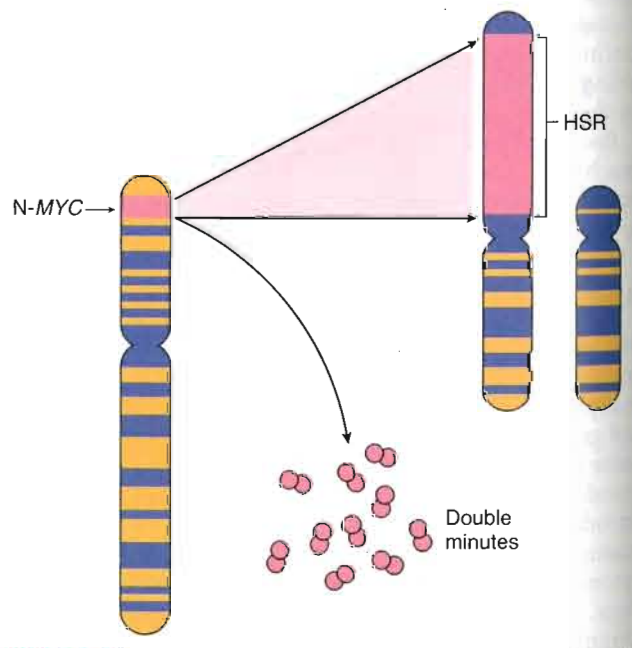


FIGURE 7–28 Amplification of the N-*MYC* gene in human neuroblastomas. The N-*MYC* gene, normally present on chromosome 2p, becomes amplified and is seen either as extra chromosomal double minutes or as a chromosomally integrated, homogeneous staining region (HSR). The integration involves other autosomes, such as 4, 9, or 13. (Modified from Brodeur GM: Molecular correlates of cytogenetic abnormalities in human cancer cells: implications for oncogene activation. In Brown EB (ed): Progress in Hematology, Vol 14. Orlando, FL, Grune & Stratton, 1986, p 229–256.)

orderly progression of cells through the various phases of the cell cycle is orchestrated by cyclin-dependent kinases (CDKs), which are activated by binding to *cyclins*, so called because of the cyclic nature of their production and degradation. The CDK-cyclin complexes phosphorylate crucial target proteins that drive the cell through the cell cycle. On completion of this task, cyclin levels decline rapidly. More than 15 cyclins have been identified; cyclins D, E, A, and B appear sequentially during the cell cycle and bind to one or more CDK. The cell cycle may thus be seen as a relay race in which each lap is regulated by a distinct set of cyclins, and as one set of cyclins leaves the track, the next set takes over (Fig. 7–29 and Table 7–7).

With this background it is easy to appreciate that mutations that dysregulate the activity of cyclins and CDKs favor cell proliferation. Mishaps affecting the expression of cyclin D or CDK4 seem to be a common event in neoplastic transformation. The cyclin D genes are overexpressed in many cancers, including those affecting the breast, esophagus, liver, and a subset of lymphomas. Amplification of the *CDK4* gene occurs in melanomas, sarcomas, and glioblastomas. Mutations affecting cyclin B and cyclin E and other CDKs also occur, but they are much less frequent.

While cyclins arouse the CDKs, their inhibitors (CDKIs), of which there are many, silence the CDKs and exert negative control over the cell cycle. The CIP/WAF family of CDKIs, composed of three proteins, called p21 (CDKN1A), p27 (CDKN1B), and p57 (CDKN1C), inhibits the CDKs broadly, whereas the INK4 family of CDK1s, made up of p15 (CDKN2B), p16 (CDKN2A), p18 (CDKN2C), and p19 (CDKN2D), has selective effects on cyclin D/CDK4 and cyclin D/CDK6. Expression of these inhibitors is down-regulated by mitogenic signaling pathways, thus promoting the progression of the cell cycle. For example, p27 (CDKN1B), a CDKI that inhibits cyclin E, is expressed throughout G_1. Mitogenic signals dampen the activity of p27 in a variety of ways, relieving

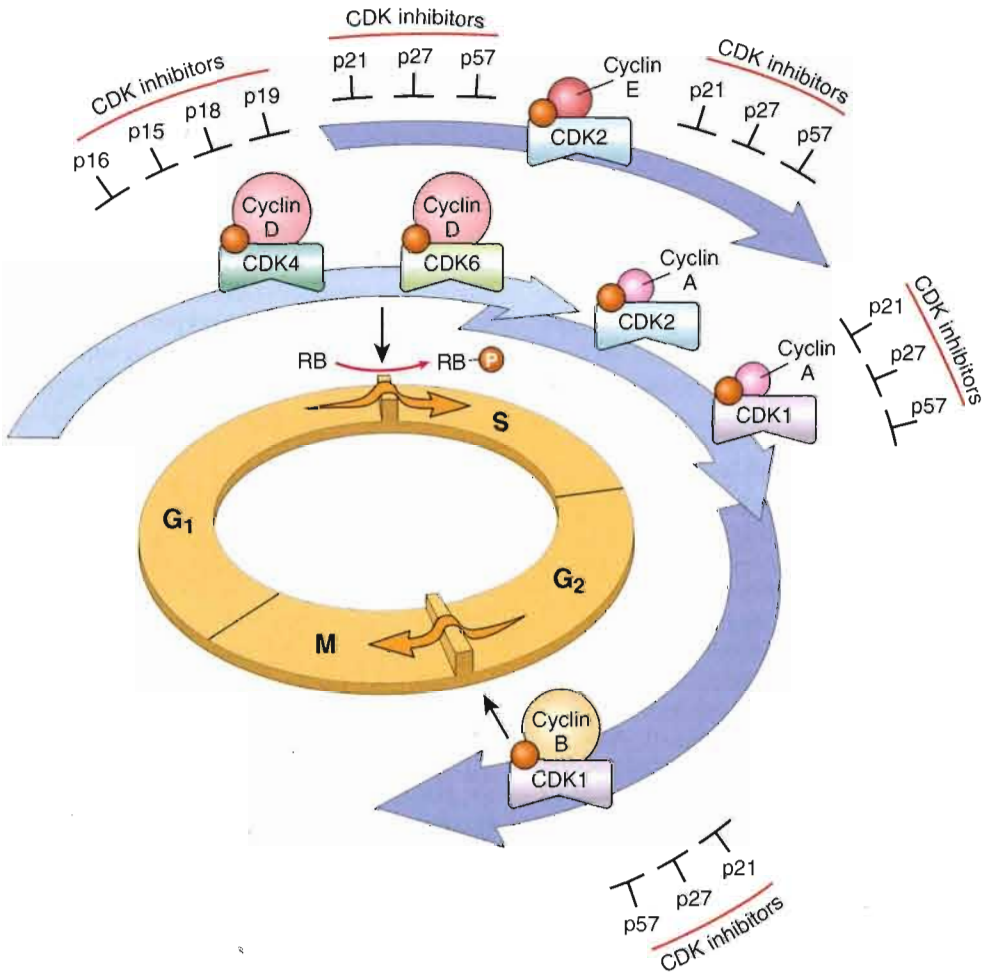


FIGURE 7–29 Schematic illustration of the role of cyclins, CDKs, and CDK inhibitors (CDKIs) in regulating the cell cycle. The shaded arrows represent the phases of the cell cycle during which specific cyclin-CDK complexes are active. As illustrated, cyclin D–CDK4, cyclin D–CDK6, and cyclin E–CDK2 regulate the G_1-to-S transition by phosphorylation of the RB protein (pRB). Cyclin A–CDK2 and cyclin A–CDK1 are active in the S phase. Cyclin B–CDK1 is essential for the G_2-to-M transition. Two families of CDKIs can block activity of CDKs and progression through the cell cycle. The so-called INK4 inhibitors, composed of p16, p15, p18, and p19, act on cyclin D–CDK4 and cyclin D–CDK6. The other family of three inhibitors, p21, p27, and p57, can inhibit all CDKs.

TABLE 7–7 Main Cell Cycle Components and Their Inhibitors

Cell Cycle Component	Main Function
CYCLIN-DEPENDENT KINASES	
CDK4	Forms a complex with cyclin D that phosphorylates RB, allowing the cell to progress through the G_1 restriction point.
CDK2	Forms a complex with cyclin E in late G_1, which is involved in G_1/S transition. Forms a complex with cyclin A at the S phase that facilitates G_2/M transition.
CDK1	Forms a complex with cyclin B that facilitates G_2/M transition.
INHIBITORS	
CIP/KIP family: p21, p27 (CDKN2A-C)	Block the cell cycle by binding to cyclin-CDK complexes; p21 is induced by the tumor suppressor p53; p27 responds to growth suppressors such as TGF-β.
INK4/ARF family (CDKN1A-D)	p16/INK4a binds to cyclin D–CDK4 and promotes the inhibitory effects of RB; p14/ARF increases p53 levels by inhibiting MDM2 activity.
CHECKPOINT COMPONENTS	
p53	Tumor suppressor gene altered in the majority of cancers; causes cell cycle arrest and apoptosis. Acts mainly through p21 to cause cell cycle arrest. Causes apoptosis by inducing the transcription of pro-apoptotic genes such as *BAX*. Levels of p53 are negatively regulated by MDM2 through a feedback loop. p53 is required for the G_1/S checkpoint and is a main component of the G_2/M checkpoint.
Ataxia-telangiectasia mutated	Activated by mechanisms that sense double-stranded DNA breaks. Transmits signals to arrest the cell cycle after DNA damage. Acts through p53 in the G_1/S checkpoint. At the G_2/M checkpoint, it acts both through p53-dependent mechanisms and through the inactivation of CDC25 phosphatase, which disrupts the cyclin B–CDK1 complex. Component of a network of genes that include *BRCA1* and *BRCA2*, which link DNA damage with cell cycle arrest and apoptosis.

inhibition of cyclin E-CDK2 and thus allowing the cell cycle to proceed.[56] The CDKIs are frequently mutated or otherwise silenced in many human malignancies. Germline mutations of *p16* (*CDKN2A*) are associated with 25% of melanoma-prone kindreds.[23] Somatically acquired deletion or inactivation of *p16* is seen in 75% of pancreatic carcinomas, 40% to 70% of glioblastomas, 50% of esophageal cancers, 20% to 70% of acute lymphoblastic leukemias, and 20% of non-small-cell lung carcinomas, soft-tissue sarcomas, and bladder cancers.[57]

Before closing this discussion of the cell cycle and its regulation, we should briefly discuss the internal controls of the cell cycle called *checkpoints*, since later discussion of tumor suppressor genes will illustrate the importance of cell cycle checkpoints in maintaining genomic integrity. There are two main cell cycle checkpoints, one at the G_1/S transition and the other at G_2/M. The S phase is the point of no return in the cell cycle. Before a cell makes the final commitment to replicate, the G_1/S checkpoint checks for DNA damage; if damage is present, the DNA-repair machinery and mechanisms that arrest the cell cycle are put in motion. The delay in cell cycle progression provides the time needed for DNA repair; if the damage is not repairable, apoptotic pathways are activated to kill the cell. Thus, the G_1/S checkpoint prevents the replication of cells that have defects in DNA, which would be perpetuated as mutations or chromosomal breaks in the progeny of the cell. DNA damaged after its replication can still be repaired as long as the chromatids have not separated. The G_2/M checkpoint monitors the completion of DNA replication and checks whether the cell can safely initiate mitosis and separate sister chromatids. This checkpoint is particularly important in cells exposed to ionizing radiation. Cells damaged by ionizing radiation activate the G_2/M checkpoint and arrest in G_2; defects in this checkpoint give rise to chromosomal abnormalities. To function properly, cell cycle checkpoints require sensors of DNA damage, signal transducers, and effector molecules.[58] The sensors and transducers of DNA damage seem to be similar for the G_1/S and G_2/M checkpoints. They include, as sensors, proteins of the RAD family and ataxia telangiectasia mutated (ATM) and as transducers, the CHK kinase families.[59] The checkpoint effector molecules differ, depending on the cell cycle stage at which they act. In the G_1/S checkpoint, cell cycle arrest is mostly mediated through p53, which induces the cell cycle inhibitor p21. Arrest of the cell cycle by the G_2/M checkpoint involves both p53-dependent and p53-independent mechanisms. *Defects in cell cycle checkpoint components are a major cause of genetic instability in cancer cells.*

INSENSITIVITY TO GROWTH INHIBITION AND ESCAPE FROM SENESCENCE: TUMOR SUPPRESSOR GENES

Failure of growth inhibition is one of the fundamental alterations in the process of carcinogenesis. Whereas oncogenes drive the proliferation of cells, the products of *tumor suppressor genes apply brakes to cell proliferation* (Table 7–8). It has become apparent that the tumor suppressor proteins form a network of checkpoints that prevent uncontrolled growth. Many tumor suppressors, such as RB and p53, are part of a regulatory network that recognizes genotoxic stress from any source, and responds by shutting down proliferation. Indeed, expression of an oncogene in an otherwise completely normal cell leads to quiescence, or to permanent cell cycle arrest (oncogene-induced senescence), rather than uncontrolled proliferation. Ultimately, the growth-inhibitory pathways may prod the cells into apoptosis. Another set of tumor suppressors seem to be involved in cell differentiation, causing cells

TABLE 7–8 Selected Tumor Suppressor Genes Involved in Human Neoplasms

Subcellular Locations	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Assocated with Inherited Mutations
Cell surface	TGF-β receptor	Growth inhibition	Carcinomas of colon	Unknown
	E-cadherin	Cell adhesion	Carcinoma of stomach	Familial gastric cancer
Inner aspect of plasma membrane	*NF1*	Inhibition of RAS signal transduction and of p21 cell cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	*NF2*	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromastosis type 2, acoustic schwannomas, and meningiomas
Cytosol	*APC*/β-catenin	Inhibition of signal transduction	Carcinomas of stomach, colon, pancreas; melanoma	Familial adenomatous polyposis coli/colon cancer
	PTEN	PI3 kinase signal transduction	Endometrial and prostate cancers	Cowden syndrome
	SMAD2 and *SMAD4*	TGF-β signal transduction	Colon, pancreas tumors	Unknown
Nucleus	*RB1*	Regulation of cell cycle	Retinoblastoma; osteosarcoma carcinomas of breast, colon, lung	Retinoblastomas, osteosarcoma
	p53	Cell cycle arrest and apoptosis in response to DNA damage	Most human cancers	Li-Fraumeni syndrome; multiple carcinomas and sarcomas
	WT1	Nuclear transcription	Wilms' tumor	Wilms' tumor
	P16/INK4a	Regulation of cell cycle by inhibition of cyclin-dependent kinases	Pancreatic, breast, and esophageal cancers	Malignant melanoma
	BRCA1 and *BRCA2*	DNA repair	Unknown	Carcinomas of female breast and ovary; carcinomas of male breast

PI3 kinase, phosphatidylinositol 3-kinase.

to enter a postmitotic, differentiated pool without replicative potential. Similar to mitogenic signals, growth-inhibitory, pro-differentiation signals originate outside the cell and use receptors, signal transducers, and nuclear transcription regulators to accomplish their effects; tumor suppressors form a portion of these networks.

In this section we describe tumor suppressor genes, their products, and possible mechanisms by which loss of their function contributes to unregulated cell growth. The protein products of tumor suppressor genes may function as transcription factors, cell cycle inhibitors, signal transduction molecules, cell surface receptors, and regulators of cellular responses to DNA damage. In the following section we discuss the functions of the most important tumor suppressor genes, and how their defects contribute to carcinogenesis.

We begin our discussion with *RB*, the first, and prototypic, tumor suppressor gene discovered. Like many discoveries in medicine, *RB* was discovered by studying a rare disease, in this case retinoblastoma. Approximately 60% of retinoblastomas are sporadic, and the remaining are familial, with the predisposition to develop the tumor being transmitted as an autosomal dominant trait. Patients with familial retinoblastoma are also at greatly increased risk of developing osteosarcoma and other soft-tissue sarcomas. To explain the inherited and sporadic occurrence of an apparently identical tumor, Knudson proposed his now canonical *"two-hit" hypothesis of oncogenesis.*[19,60] In molecular terms, Knudson's hypothesis can be stated as follows (Fig. 7–30):

- Two mutations (hits), involving both alleles of *RB* at chromosome locus 13q14, are required to produce retinoblastoma. In some cases, the genetic damage is large enough to be visible in the form of a deletion of 13q14.
- In familial cases, children inherit one defective copy of the *RB* gene in the germ line (one hit); the other copy is normal (Fig. 7–30). Retinoblastoma develops when the normal *RB* allele is mutated in retinoblasts as a result of spontaneous somatic mutation (second hit). Because only a single somatic mutation is required for loss of RB function in retinoblastoma families, familial retinoblastoma is inherited as an autosomal dominant trait.
- In sporadic cases both normal *RB* alleles must undergo somatic mutation in the same retinoblast (two hits). The end result is the same: a retinal cell that has completely lost *RB* function becomes cancerous.

At this point we should clarify some terminology. A child carrying an inherited mutant *RB* allele in all somatic cells is perfectly normal (except for the increased risk of developing cancer). Because such a child is heterozygous at the *RB* locus, this implies that heterozygosity for the *RB* gene does not affect cell behavior. *Cancer develops when the cell becomes*

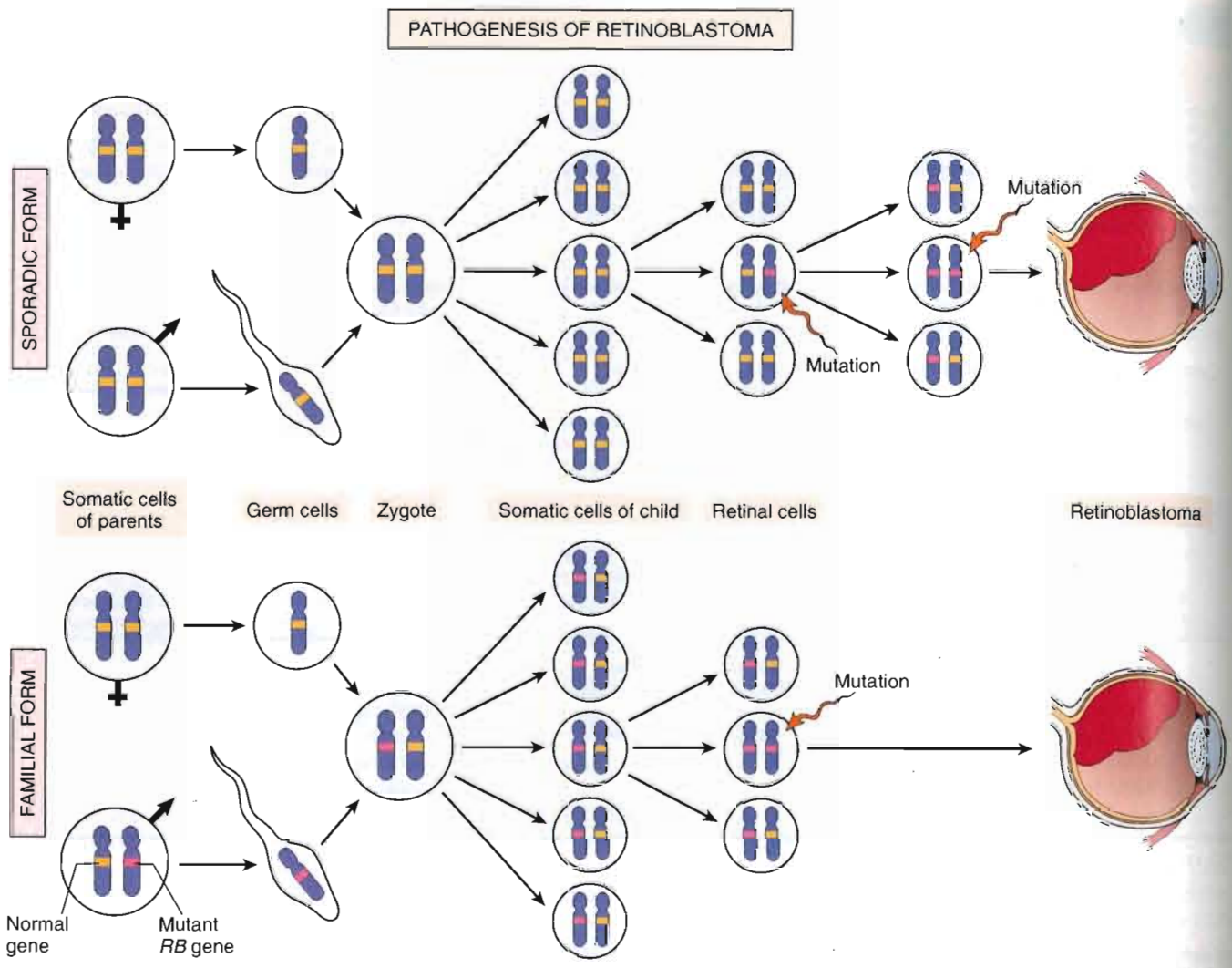


FIGURE 7–30 Pathogenesis of retinoblastoma. Two mutations of the *RB* locus on chromosome 13q14 lead to neoplastic proliferation of the retinal cells. In the sporadic form both mutations at the *RB* locus are acquired by the retinal cells after birth. In the familial form, all somatic cells inherit one mutant *RB* gene from a carrier parent. The second mutation affects the *RB* locus in one of the retinal cells after birth.

homozygous for the mutant allele or, put another way, when the cell loses heterozygosity for the normal RB gene (a condition known as LOH, for loss of heterozygosity). The *RB* gene stands as a paradigm for several other genes that act similarly. For example, one or more genes on the short arm of chromosome 11 play a role in the formation of Wilms' tumor, hepatoblastoma, and rhabdomyosarcoma. The von Hippel-Lindau (*VHL*) gene is a tumor suppressor gene that causes familial clear cell renal carcinomas and is also involved in sporadic forms of the same tumor.[61] *Consistent and nonrandom LOH has provided important clues to the location of several tumor suppressor genes.*

RB. RB protein, the product of the *RB* gene, is a ubiquitously expressed nuclear phosphoprotein that plays a key role in regulating the cell cycle. RB exists in an active hypophosphorylated state in quiescent cells and an inactive hyperphosphorylated state in the G_1/S cell cycle transition (Fig. 7–31). The importance of RB lies in its enforcement of G_1, or the gap between mitosis (M) and DNA replication (S). In embryos, cell divisions proceed at an amazing clip, with DNA replication beginning immediately after mitosis ends. However, as development proceeds, two gaps are incorporated into the cell cycle: Gap 1 (G_1) between mitosis (M) and DNA replication (S), and Gap 2 (G_2) between DNA replication (S) and mitosis (M) (see Fig. 7–29). Although each phase of the cell cycle circuitry is monitored carefully, the transition from G_1 to S is believed to be an extremely important checkpoint in the cell cycle clock. Once cells cross the G_1 checkpoint they can pause the cell cycle for a time, but they are obligated to complete mitosis. In G_1, however, cells can exit the cell cycle, either temporarily, called quiescence, or permanently, called senescence. In G_1, therefore, diverse signals are integrated to determine whether the cell should enter the cell cycle, exit the cell cycle and differentiate, or die. RB is a key node in this decision process. To understand why RB is such a crucial player, we must review the mechanisms that police the G_1 phase.[62]

The initiation of DNA replication requires the activity of cyclin E–CDK2 complexes, and expression of cyclin E is dependent on the E2F family of transcription factors. Early in

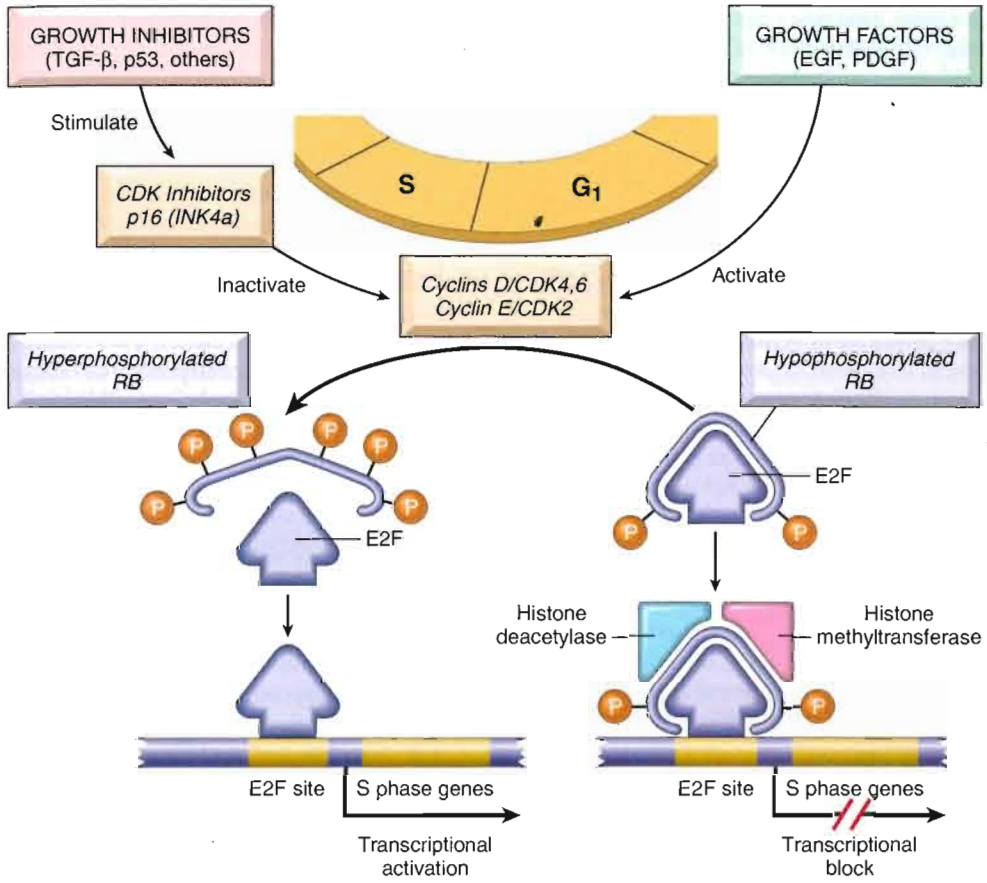


FIGURE 7–31 The role of RB in regulating the G_1-S checkpoint of the cell cycle. Hypophosphorylated RB in complex with the E2F transcription factors binds to DNA, recruits chromatin-remodeling factors (histone deacetylases and histone methyltransferases), and inhibits transcription of genes whose products are required for the S phase of the cell cycle. When RB is phosphorylated by the cyclin D-CDK4, cyclin D–CDK6, and cyclin E–CDK2 complexes, it releases E2F. The latter then activates transcription of S-phase genes. The phosphorylation of RB is inhibited by CDKIs, because they inactivate cyclin-CDK complexes. Virtually all cancer cells show dysregulation of the G_1-S checkpoint as a result of mutation in one of four genes that regulate the phosphorylation of RB; these genes are *RB1*, *CDK4*, the genes encoding cyclin D proteins, and *CDKN2A* (p16). EGF, epidermal growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-beta.

G_1, RB is in its hypophosphorylated active form, and it binds to and inhibits the E2F family of transcription factors, preventing transcription of cyclin E. Hypophosphorylated RB blocks E2F-mediated transcription in at least two ways (see Fig. 7–31). First, it sequesters E2F, preventing it from interacting with other transcriptional activators. Second, RB recruits chromatin-remodeling proteins, such as histone deacetylases and histone methyltransferases, which bind to the promoters of E2F-responsive genes such as cyclin E. These enzymes modify chromatin so as to make promoters insensitive to transcription factors. Mitogenic signaling leads to cyclin D expression and activation of cyclin D–CDK4/6 complexes. These complexes phosphorylate RB, inactivating the protein and releasing E2F to induce target genes such as cyclin E. Expression of cyclin E then stimulates DNA replication and progression through the cell cycle. When the cells enter S phase, they are committed to divide without additional growth factor stimulation. During the ensuing M phase the phosphate groups are removed from RB by cellular phosphatases, regenerating the hypophosphorylated form of RB. E2Fs are not the sole effectors of Rb-mediated G_1 arrest. Rb also controls the stability of the cell cycle inhibitor p27.[63,64]

If RB is absent (as a result of gene mutations) or its ability to regulate E2F transcription factors is derailed, the molecular brakes on the cell cycle are released, and the cells move through the cell cycle. The mutations of *RB* genes found in tumors are localized to a region of the RB protein, called the "RB pocket," that is involved in binding to E2F. However, the versatile RB protein has also been shown to bind to a variety of other transcription factors that regulate cell differentiation.[65] For example, RB stimulates myocyte-, adipocyte-, melanocyte-, and macrophage-specific transcription factors. Thus, the RB pathway couples control of cell cycle progression at G_1 with differentiation, which may explain how differentiation is associated with exit from the cell cycle. In addition to these dual activities, RB can also induce senescence, discussed below.

It was mentioned previously that germline loss or mutations of the *RB* gene predispose to occurrence of retinoblastomas and to a lesser extent osteosarcomas. Furthermore, somatically acquired RB mutations have been described in glioblastomas, small-cell carcinomas of lung, breast cancers, and bladder carcinomas. Given the presence of RB in every cell and its importance in cell cycle control, two questions

arise: (1) Why do patients with germline mutation of the *RB* locus develop mainly retinoblastomas? (2) Why are inactivating mutations of *RB* not much more common in human cancers? The reason for the occurrence of tumors restricted to the retina in persons who inherit one defective allele of *RB* is not fully understood, but some possible explanations have emerged from the study of mice with targeted disruption of the *rb* locus. For instance, RB family members may partially complement its function in cell types other than retinoblasts. Indeed, RB is a member of a small family of proteins, so-called pocket proteins, which also include p107 and p130.[66] All three proteins bind to E2F transcription factors. The complexity grows; there are seven E2F proteins (named E2F1 through E2F7), which function as either transcriptional activators or repressors. The pocket proteins are all thought to regulate progression through the cell cycle as well as differentiation in a manner similar to that described for RB above. However, each member of this protein family binds a different set of E2F proteins and is also expressed at different times in the cell cycle. Thus, although there is some redundancy in the network, their functions are not completely overlapping. The complexity of the pocket protein–E2F network is just now being unraveled. For example, in a mouse model of retinoblastoma, it has been shown that mutation of different members of the network in various combinations generates retinoblastomas not just from retinoblasts, but also from differentiated cells in the retina, such as horizontal interneurons.[67]

With respect to the second question (i.e., why the loss of *RB* is not more common in human tumors), the answer is much simpler: Mutations in other genes that control RB phosphorylation can mimic the effect of *RB* loss, and such genes are mutated in many cancers that may have normal *RB* genes. Thus, for example, mutational activation of cyclin D or CDK4 would favor cell proliferation by facilitating RB phosphorylation. As previously discussed, cyclin D is overexpressed in many tumors because of gene amplification or translocation. Mutational inactivation of CDKIs would also drive the cell cycle by unregulated activation of cyclins and CDKs. Thus, *the emerging paradigm is that loss of normal cell cycle control is central to malignant transformation and that at least one of four key regulators of the cell cycle (*p16/INK4a, cyclin D, CDK4, RB*) is dysregulated in the vast majority of human cancers.*[68] In cells that harbor mutations in any one of these other genes, the function of RB is disrupted even if the *RB* gene itself is not mutated.[34]

The transforming proteins of several oncogenic animal and human DNA viruses seem to act, in part, by neutralizing the growth-inhibitory activities of RB. In these cases, RB protein is functionally inactivated by the binding of a viral protein and no longer acts as a cell cycle inhibitor. Simian virus 40 and polyomavirus large T antigens, adenoviruses EIA protein, and HPV E7 protein all bind to the hypophosphorylated form of RB. The binding occurs in the same RB pocket that normally sequesters E2F transcription factors; in the case of HPV the binding is particularly strong for viral types, such as HPV type 16, that confer high risk for the development of cervical carcinomas. Thus, the RB protein, unable to bind the E2F transcription factors, is functionally inactivated, and the transcription factors are free to cause cell cycle progression.

Several other pathways of cell growth regulation, some to be discussed in more detail later, also converge on RB (see Fig. 7–31):

p53: Guardian of the Genome. The *p53* gene is located on chromosome 17p13.1, and it is the most common target for genetic alteration in human tumors.[69] (The official name of the gene is *TP53* and the protein is p53; for the sake of simplicity, we refer to both as "p53".) *A little over 50% of human tumors contain mutations in this gene.* Homozygous loss of *p53* occurs in virtually every type of cancer, including carcinomas of the lung, colon, and breast—the three leading causes of cancer death. In most cases, the inactivating mutations affect both *p53* alleles and are acquired in somatic cells (not inherited in the germ line). Less commonly, some individuals inherit one mutant *p53* allele. As with the *RB* gene, inheritance of one mutant allele predisposes individuals to develop malignant tumors because only one additional "hit" is needed to inactivate the second, normal allele. Such individuals, said to have the *Li-Fraumeni syndrome*, have a 25-fold greater chance of developing a malignant tumor by age 50 than the general population.[70] In contrast to individuals who inherit a mutant *RB* allele, the spectrum of tumors that develop in persons with the Li-Fraumeni syndrome is quite varied; the most common types of tumors are sarcomas, breast cancer, leukemia, brain tumors, and carcinomas of the adrenal cortex. As compared with sporadic tumors, those that afflict patients with the Li-Fraumeni syndrome occur at a younger age, and a given individual may develop multiple primary tumors.[71]

The fact that *p53* mutations are common in a variety of human tumors suggests that the p53 protein functions as a critical gatekeeper against the formation of cancer. Indeed, it is evident that p53 acts as a "*molecular policeman*" that prevents the propagation of genetically damaged cells. p53 is a transcription factor that is at the center of a large network of signals that sense cellular stress, such as DNA damage, shortened telomeres, and hypoxia. Many activities of the p53 protein are related to its function as a transcription factor. Several hundred genes have been shown to be regulated by p53 in numerous different contexts, but which genes are the key for the p53 response is not yet clear. Approximately 80% of the *p53* point mutations present in human cancers are located in the DNA-binding domain of the protein. However, the effects of different point mutations vary considerably; in some cases there is complete abrogation of transcriptional capabilities, whereas other mutants retain the ability to bind to and activate a subset of genes. In addition to somatic and inherited mutations, p53 functions can be inactivated by other mechanisms. As with RB, the transforming proteins of several DNA viruses, including the E6 protein of HPV, can bind to and promote the degradation of p53. Also, analagous to RB, it is thought that in the majority of tumors without a *p53* mutation, the function of the p53 pathway is blocked by mutation in another gene that regulates p53 function. For example, MDM2 and MDMX stimulate the degradation of p53; these proteins are frequently overexpressed in malignancies in which the gene encoding p53 is not mutated. Indeed, MDM2 is amplified in 33% of human sarcomas, thereby causing functional loss of p53 in these tumors.[72,73]

p53 thwarts neoplastic transformation by three interlocking mechanisms: activation of temporary cell cycle arrest (quiescence), induction of permanent cell cycle arrest (senescence), or triggering of programmed cell death (apoptosis).

In nonstressed, healthy cells, p53 has a short half-life (20 minutes), because of its association with MDM2, a protein that targets it for destruction. When the cell is stressed, for

example by an assault on its DNA, p53 undergoes post-transcriptional modifications that release it from MDM2 and increase its half-life. Unshackled from MDM2, p53 also becomes activated as a transcription factor. Hundreds of genes whose transcription is triggered by p53 have been found.[74,75] They can be grouped into two broad categories: those that cause cell cycle arrest and those that cause apoptosis. If DNA damage can be repaired during cell cycle arrest, the cell reverts to a normal state; if the repair fails, p53 induces apoptosis or senescence. Recently, however, the plot has thickened. It has been known that repression of a subset of pro-proliferative and anti-apoptotic genes is key to the p53 response, but it was not clear how p53 achieved repression, since in most assays it seemed to be an activator of transcription. At this point enter the recently famous miRNAs, the small guys with big clubs. It has been shown that p53 activates transcription of the mir34 family of miRNAs (mir34a–mir34c).[76] miRNAs, as discussed in Chapter 5, bind to cognate sequences in the 3′ untranslated region of mRNAs, preventing translation (Fig. 7–32B). Interestingly, blocking mir34 severely hampered the p53 response in cells, while ectopic expression of mir34 without p53 activation is sufficient to induce growth arrest and apoptosis. Thus, mir34 microRNAs are able to recapitulate many of the functions of p53 and are necessary for these functions, demonstrating the importance of mir34 to the p53 response. Targets of mir34s include pro-proliferative genes such as cyclins, and anti-apoptotic genes such as *BCL2*. p53 regulation of mir34 explains, at least in part, how p53 is able to repress gene expression, and it seems that regulation of this miRNA is crucial for the p53 response.

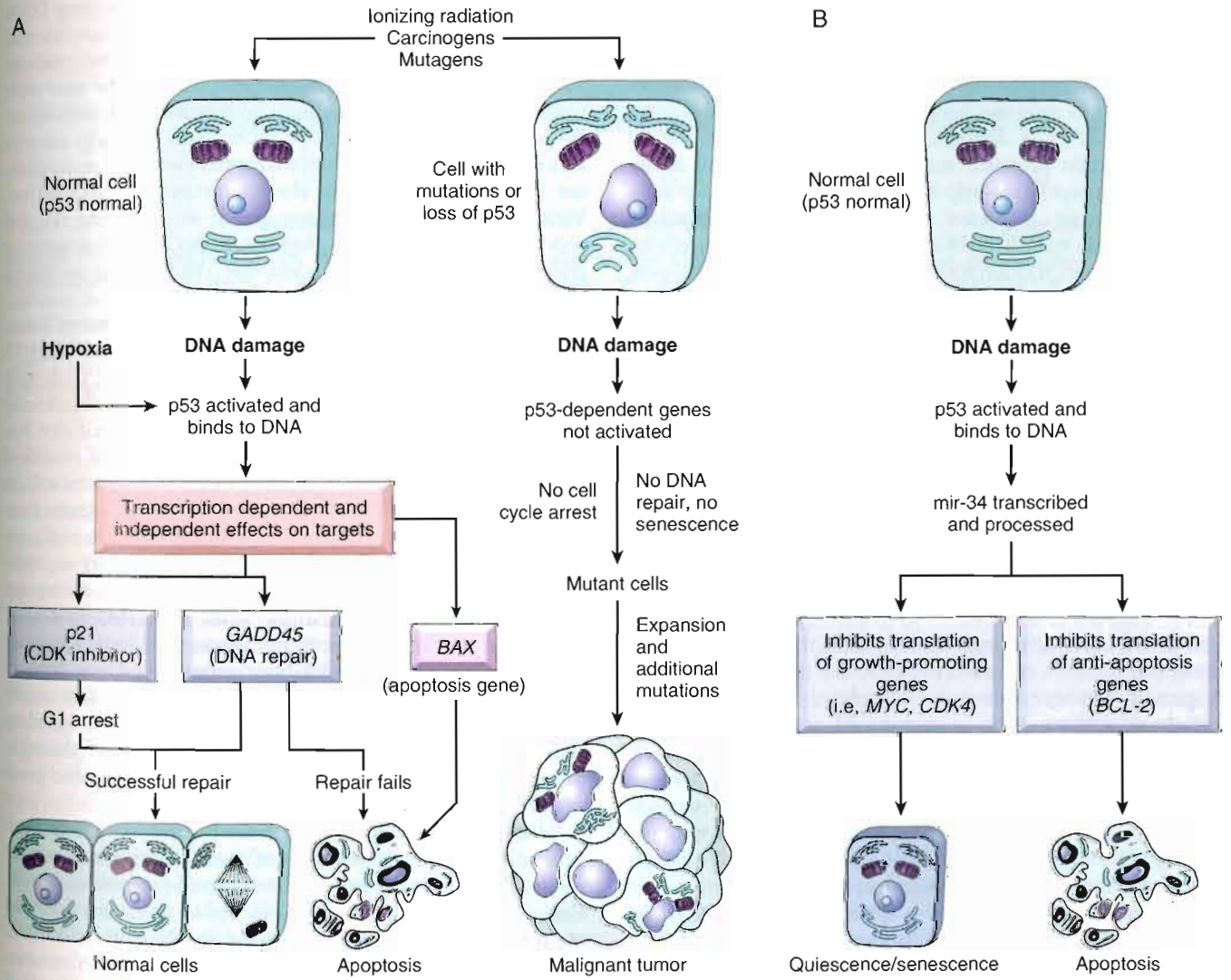


FIGURE 7–32 **A,** The role of *p53* in maintaining the integrity of the genome. Activation of normal *p53* by DNA-damaging agents or by hypoxia leads to cell cycle arrest in G_1 and induction of DNA repair, by transcriptional up-regulation of the cyclin-dependent kinase inhibitor *CDKN1A* (p21) and the *GADD45* genes. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, *p53* triggers either apoptosis or senescence. In cells with loss or mutations of *p53,* DNA damage does not induce cell cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms. **B,** p53 mediates gene repression by activating transcription of miRNAs. p53 activates transcription of the mir34 family of miRNAs. mir34s repress translation of both proliferative genes, such as cyclins, and anti-apoptotic genes, such as *BCL2*. Repression of these genes can promote either quiescence or senescence as well as apoptosis.

The manner in which p53 senses DNA damage and determines the adequacy of DNA repair is beginning to be understood. The key initiators of the DNA-damage pathway are two related protein kinases: *ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3 related (ATR).*[77,78] As the name implies, the *ATM* gene was originally identified as the germ-line mutation in individuals with ataxia-telangiectasia. Persons with this disease, which is characterized by an inability to repair certain kinds of DNA damage, suffer from an increased incidence of cancer. The types of damage sensed by ATM and ATR are different, but the downstream pathways they activate are similar. Once triggered, both ATM and ATR phosphorylate a variety of targets, including p53 and DNA-repair proteins. Phosphorylation of these two targets leads to a pause in the cell cycle and stimulation of DNA-repair pathways, respectively.

p53-*mediated cell cycle arrest may be considered the primordial response to DNA damage* (Fig. 7–32). It occurs late in the G_1 phase and is caused mainly by p53-dependent transcription of the CDK inhibitor *CDKN1A* (*p21*). As discussed, p21 inhibits cyclin-CDK complexes and phosphorylation of RB, thereby preventing cells from entering G_1 phase. Such a pause in cell cycling is welcome, because it gives the cells "breathing time" to repair DNA damage. p53 also helps the process by inducing certain proteins, such as GADD45 (growth arrest and DNA damage), that help in DNA repair.[75] p53 can stimulate DNA-repair pathways by transcription-independent mechanisms as well. If DNA damage is repaired successfully, p53 up-regulates transcription of MDM2, leading to its own destruction and thus releasing the cell cycle block. If the damage cannot be repaired, the cell may enter p53-induced senescence or undergo p53-directed apoptosis.

p53-induced senescence is a permanent cell cycle arrest characterized by specific changes in morphology and gene expression that differentiate it from quiescence or reversible cell cycle arrest. Senescence requires activation of p53 and/or RB and expression of their mediators, such as the CDK inhibitors, and is generally irreversible, although it may require the continued expression of p53. The mechanisms of senescence are unclear but involve epigenetic changes that result in the formation of heterochromatin at different loci throughout the genome.[80] These senescence-associated heterochromatin foci include pro-proliferative genes regulated by E2F; this drastically and permanently alters expression of these E2F targets. Like all p53 responses, senescence may be stimulated in response to a variety of stresses, such as unopposed oncogene signaling, hypoxia, and shortened telomeres.

p53-induced apoptosis of cells with irreversible DNA damage is the ultimate protective mechanism against neoplastic transformation. p53 directs the transcription of several pro-apoptotic genes such as *BAX* and *PUMA* (approved name *BBC3*; described later). Exactly how a cell decides whether to repair its DNA or to enter apoptosis is unclear. It appears that the affinity of p53 for the promoters and enhancers of DNA-repair genes is stronger than its affinity for pro-apoptotic genes.[80] Thus, the DNA-repair pathway is stimulated first, while p53 continues to accumulate. Eventually, if the DNA damage is not repaired, enough p53 accumulates to stimulate transcription of the pro-apoptotic genes and the cell dies. While this scheme is generally correct, there seem to be important cell type–specific responses as well, with some cell types succumbing to apoptosis early, while others opt for senescence.[80] Such differential responses may be related to the functions of other p53 family members expressed in different cell types (see below).

To summarize, p53 links cell damage with DNA repair, cell cycle arrest, and apoptosis. In response to DNA damage, p53 is phosphorylated by genes that sense the damage and are involved in DNA repair. p53 assists in DNA repair by causing G_1 arrest and inducing DNA-repair genes. A cell with damaged DNA that cannot be repaired is directed by p53 to undergo apoptosis (see Fig. 7–32). In view of these activities, p53 has been rightfully called a "guardian of the genome." With loss of function of p53, DNA damage goes unrepaired, mutations accumulate in dividing cells, and the cell marches along a one-way street leading to malignant transformation.

The ability of p53 to control apoptosis in response to DNA damage has important practical therapeutic implications. Irradiation and chemotherapy, the two common modalities of cancer treatment, mediate their effects by inducing DNA damage and subsequent apoptosis. Tumors that retain normal p53 are more likely to respond to such therapy than tumors that carry mutated alleles of the gene. Such is the case with testicular teratocarcinomas and childhood acute lymphoblastic leukemias. By contrast, tumors such as lung cancers and colorectal cancers, which frequently carry *p53* mutations, are relatively resistant to chemotherapy and irradiation. Various therapeutic modalities aimed at increasing normal p53 activity in tumor cells that retain this type of activity or selectively killing cells with defective p53 function are being investigated.

The discovery of p53 family members p63 and p73 has revealed that p53 has collaborators. Indeed, p53, p63, and p73 are players in a complex network with significant cross-talk that is only beginning to be unraveled.[81,82] p53 is ubiquitously expressed, while p63 and p73 show more tissue specificity. For example, p63 is essential for the differentiation of stratified squamous epithelia, while p73 has strong pro-apoptotic effects after DNA damage induced by chemotheraputic agents. Furthermore, both p63 and p73, and probably p53 as well, are expressed as different isoforms, some of which act as transcriptional activators and others that function as dominant negatives. An illustrative example of the concerted actions of these three musketeers is seen in the so-called basal subset of breast cancers, which have a poor prognosis. These tumors have been shown to have mutations in *p53* and additionally express a dominant-negative version of p63 that antagonizes the apoptotic activity of p73. This perturbation of the p53-p63-p73 network contributes to the chemoresistance and poor prognosis of these tumors.[83]

APC/β-Catenin Pathway. Adenomatous polyposis coli genes (APC) represents a class of tumor suppressors whose main function is to down-regulate growth-promoting signals. Germ-line mutations at the *APC* (5q21) loci are associated with familial adenomatous polyposis, in which all individuals born with one mutant allele develop thousands of adenomatous polyps in the colon during their teens or 20s (familial adenomatous polyposis; Chapter 17). Almost invariably, one or more of these polyps undergoes malignant transformation, giving rise to colon cancer. As with other tumor suppressor genes, both copies of the *APC* gene must be lost for a tumor to arise. This conclusion is supported by the development of colon adenomas in mice with targeted disruption of *apc* genes

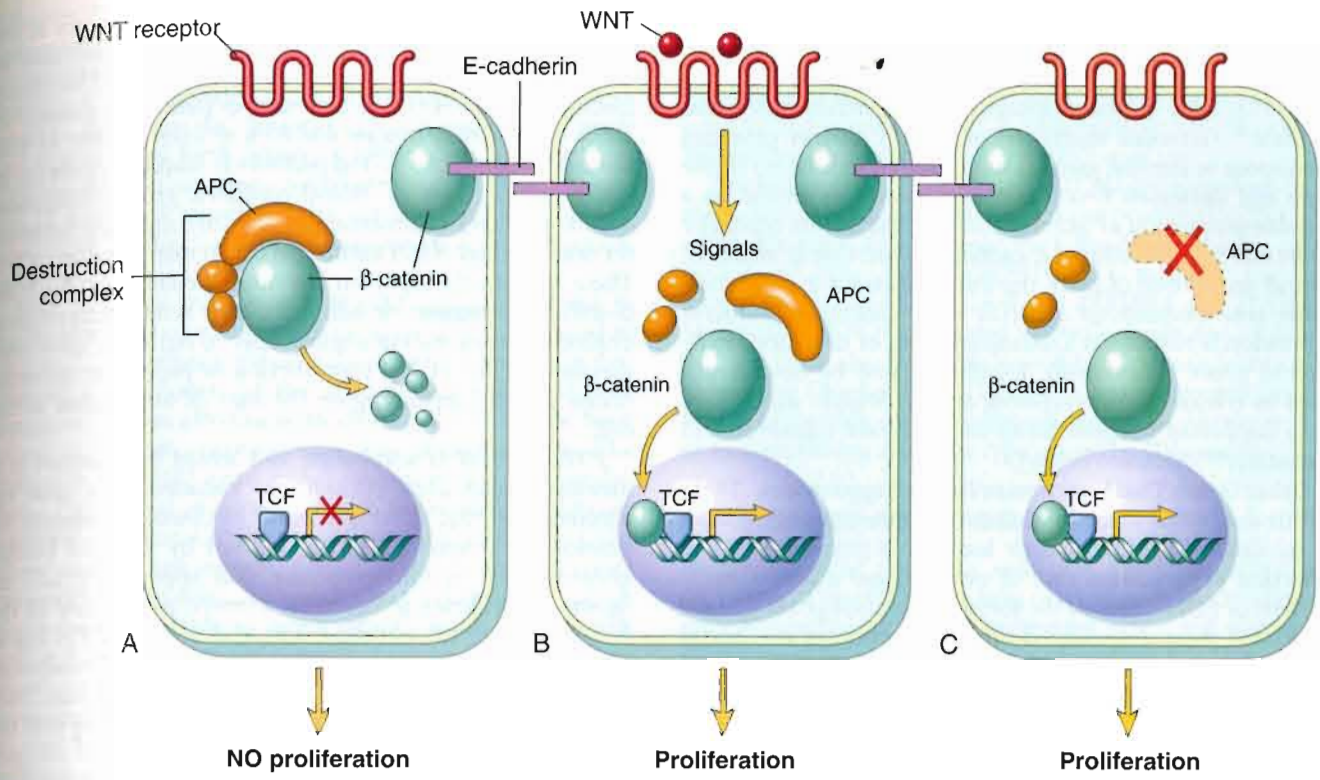


FIGURE 7–33 **A,** The role of APC in regulating the stability and function of β-catenin. APC and β-catenin are components of the WNT signaling pathway. In resting cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low. **B,** When cells are stimulated by WNT molecules, the *destruction complex* is deactivated, β-catenin degradation does not occur, and cytoplasmic levels increase. β-catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates genes involved in cell cycle progression. **C,** When *APC* is mutated or absent, the destruction of β-catenin cannot occur. β-catenin translocates to the nucleus and coactivates genes that promote entry into the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

in the colonic mucosa.[84] As discussed later, several additional mutations must occur if cancers are to develop in the adenomas. In addition to these tumors, which have a strong hereditary predisposition, 70% to 80% of nonfamilial colorectal carcinomas and sporadic adenomas also show homozygous loss of the *APC* gene, thus firmly implicating *APC* loss in the pathogenesis of colonic tumors.[85]

APC is a component of the WNT signaling pathway, which has a major role in controlling cell fate, adhesion, and cell polarity during embryonic development (Fig. 7–33). WNT signaling is also required for self-renewal of hematopoietic stem cells. WNT signals through a family of cell surface receptors called frizzled (FRZ), and stimulates several pathways, the central one involving β-catenin and APC.

An important function of the APC protein is to down-regulate β-catenin. In the absence of WNT signaling APC causes degradation of β-catenin, preventing its accumulation in the cytoplasm.[85] It does so by forming a macromolecular complex with β-catenin, axin, and GSK3β, which leads to the phosphorylation and eventually ubiquitination of β-catenin and destruction by the proteasome. Signaling by WNT blocks the APC-AXIN-GSK3β destruction complex, allowing β-catenin to translocate from the cytoplasm to the nucleus. In the cell nucleus, β-catenin forms a complex with TCF, a transcription factor that up-regulates cellular proliferation by increasing the transcription of c-*MYC*, *cyclin D1*, and other genes. Since inactivation of the *APC* gene disrupts the destruction complex, β-catenin survives and translocates to the nucleus, where it can activate transcription in cooperation with TCF.[85] Thus, cells with loss of APC behave as if they are under continuous WNT signaling. The importance of the APC/β-catenin signaling pathway in tumorigenesis is attested to by the fact that colon tumors that have normal *APC* genes harbor mutations in β-catenin that prevent its destruction by APC, allowing the mutant protein to accumulate in the nucleus. Dysregulation of the APC/β-catenin pathway is not restricted to colon cancers; mutations in the β-catenin gene are present in more than 50% of hepatoblastomas and in approximately 20% of hepatocellular carcinomas.[86] As mentioned in Chapter 3, β-catenin binds to the cytoplasmic tail of E-cadherin, a cell surface protein that maintains intercellular adhesiveness. Loss of cell-cell contact, such as in a wound or injury to the epithelium, disrupts the interaction between E-cadherin and β-catenin, and allows β-catenin to travel to the nucleus and stimulate proliferation; this is an appropriate response to injury that can help repair the wound. Re-establishment of these E-cadherin contacts as the wound heals leads to β-catenin again being sequestered at the membrane and reduction in the proliferative signal; these cells are said to be "contact-inhibited." Loss of contact inhibition, by mutation of the E-cadherin/β-catenin axis, or by other methods, is a key characteristic of carcinomas. Furthermore, loss of cadherins can favor the malignant phenotype by

allowing easy disaggregation of cells, which can then invade locally or metastasize. Reduced cell surface expression of E-cadherin has been noted in many types of cancers, including those that arise in the esophagus, colon, breast, ovary, and prostate.[87] Germline mutations of the E-cadherin gene can predispose to familial gastric carcinoma, and mutation of the gene and decreased E-cadherin expression are present in a variable proportion of gastric cancers of the diffuse type. The molecular basis of reduced E-cadherin expression is varied. In a small proportion of cases, there are mutations in the E-cadherin gene (located on 16q); in other cancers, E-cadherin expression is reduced as a secondary effect of mutations in β-catenin genes. Additionally, E-cadherin may be down-regulated by transcription repressors, such as SNAIL, which have been implicated in epithelial-to-mesenchymal transition and metastasis[88] (discussed below).

Other Genes That Function as Tumor Suppressors. There is little doubt that many more tumor suppressor genes remain to be discovered. Often, their location is suspected by the detection of consistent sites of *chromosomal deletions* or by analysis of *LOH*. Some of the tumor suppressor genes that are associated with well-defined clinical syndromes are briefly described below (see Table 7–8):

INK4a/ARF. Also called the *CDKN2A* gene locus, the *INK4a/ARF* locus encodes two protein products; the p16/INK4a CDKI, which blocks cyclin D/CDK2-mediated phosphorylation of RB, keeping the RB checkpoint in place. The second gene product, p14/ARF, activates the p53 pathway by inhibiting MDM2 and preventing destruction of p53. Both protein products function as tumor suppressors, and thus mutation or silencing of this locus impacts both the RB and p53 pathways. p16 in particular is crucial for the induction of senescence. Mutations at this locus have been detected in bladder, head and neck tumors, acute lymphoblastic leukemias, and cholangiocarcinomas. In some tumors, such as cervical cancer, p16/INK4a is frequently silenced by hypermethylation of the gene, without the presence of a mutation (see discussion of epigenetic changes). The other CDKIs also function as tumor suppressors and are frequently mutated or otherwise silenced in many human malignancies, including 20% of familial melanomas, 50% of sporadic pancreatic adenocarcinomas, and squamous cell carcinomas of the esophagus.

The TGF-β Pathway. In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of proliferation. It regulates cellular processes by binding to a serine-threonine kinase complex composed of TGF-β receptors I and II. Dimerization of the receptor upon ligand binding leads to activation of the kinase and phosphorylation of receptor SMADs (R-SMADs). Upon phosphorylation, R-SMADs can enter the nucleus, bind to SMAD-4, and activate transcription of genes, including the CDKIs p21 and p15/INK4b. In addition, TGF-β signaling leads to repression of c-MYC, CDK2, CDK4, and cyclins A and E. As can be inferred from our earlier discussion, these changes result in decreased phosphorylation of RB and cell cycle arrest.

In many forms of cancer the growth-inhibiting effects of TGF-β pathways are impaired by mutations in the TGF-β signaling pathway. These mutations may affect the type II TGF-β receptor or interfere with SMAD molecules that serve to transduce antiproliferative signals from the receptor to the nucleus. Mutations affecting the type II receptor are seen in cancers of the colon, stomach, and endometrium. Mutational inactivation of SMAD4 is common in pancreatic cancers. *In 100% of pancreatic cancers and 83% of colon cancers, at least one component of the TGF-β pathway is mutated.* However, in many cancers, loss of TGF-β-mediated growth inhibition occurs at a level downstream of the core signaling pathway, for example, loss of p21 and/or persistent expression of c-Myc. These tumor cells can then use other elements of the TGF-β–induced program, including immune system suppression/evasion or promotion of angiogenesis, to facilitate tumor progression.[89] Thus TGF-β can function to prevent or promote tumor growth, depending on the state of other genes in the cell.

PTEN. *PTEN* (*P*hosphatase and *ten*sin homologue) is a membrane-associated phosphatase encoded by a gene on chromosome 10q23 that is mutated in Cowden syndrome, an autosomal dominant disorder marked by frequent benign growths, such as tumors of the skin appendages, and an increased incidence of epithelial cancers, particularly of the breast (Chapter 23), endometrium, and thyroid. PTEN acts as a tumor suppressor by serving as a brake on the pro-survival/pro-growth PI3K/AKT pathway.[90,91] As you will recall from Chapter 3, this pathway is normally stimulated (along with the RAS and JAK/STAT pathways) when ligands bind to receptor tyrosine kinases and involves a cascade of phosphorylation events. First, PI3K (phosphoinositide 3-kinase) phosphorylates the lipid inositide-3-phosphate to give rise to inositide-3,4,5-triphosphate, which binds and activates the kinase PDK1. PDK1 and other factors in turn phosphorylate and activate the serine/threonine kinase AKT, which is a major node in the pathway with several important functions. By phosphorylating a number of substrates, including BAD and MDM2, AKT enhances cell survival. AKT also inactivates the TSC1/TSC2 complex. TSC1 and TSC2 are the products of two tumor suppressor genes that are mutated in tuberous sclerosis (Chapter 28), an autosomal dominant disorder associated with developmental malformations and unusual benign neoplasms such as cardiac rhabdomyomas (Chapter 12), renal angiomyolipomas, and giant cell astrocytomas. Inactivation of TSC1/TSC2 unleashes the activity of yet another kinase called mTOR (mammalian target of rapamycin, a potent immunosuppressive drug), which stimulates the uptake of nutrients such as glucose and amino acids that are needed for growth and augments the activity of several factors that are required for protein synthesis. Although acquired loss of PTEN function is one of the most common ways that PI3K/AKT signaling is upregulated in various cancers, many other components of the pathway, including PI3K itself, may also be mutated so as to increase signaling. Considering all of these molecular lesions collectively, it is said that this may be the most commonly mutated pathway in human cancer. As a result there is great interest in targeting the PI3K/AKT pathway with inhibitors of mTOR, AKT, and other kinases in the pathway.

NF1. Individuals who inherit one mutant allele of the *NF1* gene develop numerous benign neurofibromas and optic nerve gliomas as a result of inactivation of the second copy of the gene.[92] This condition is called *neurofibromatosis type 1*

(Chapter 27). Some of the neurofibromas later develop into malignant peripheral nerve sheath tumors. *Neurofibromin*, the protein product of the *NF1* gene, contains a GTPase-activating domain, which regulates signal transduction through RAS proteins. Recall that RAS transmits growth-promoting signals and flips back and forth between GDP-binding (inactive) and GTP-binding (active) states. Neurofibromin facilitates conversion of RAS from an active to an inactive state. With loss of neurofibromin function, RAS is trapped in an active, signal-emitting state.

NF2. Germline mutations in the *NF2* gene predispose to the development of *neurofibromatosis type 2*.[93] As discussed in Chapter 27, individuals with mutations in *NF2* develop benign bilateral schwannomas of the acoustic nerve. In addition, somatic mutations affecting both alleles of *NF2* have also been found in sporadic meningiomas and ependymomas. The product of the *NF2* gene, called *neurofibromin 2* or *merlin*, shows a great deal of homology with the red cell membrane cytoskeletal protein 4.1 (Chapter 14), and is related to the ERM (ezrin, radixin, and moesin) family of membrane cytoskeleton-associated proteins. Although the mechanism by which *merlin* deficiency leads to carcinogenesis is not known, cells lacking this protein are not capable of establishing stable cell-to-cell junctions and are insensitive to normal growth arrest signals generated by cell-to-cell contact. Merlin is a key member of the Salvador-Warts-Hippo (SWH) tumor suppressor pathway, originally described in *Drosophila*. The signaling pathway controls organ size by modulating cell growth, proliferation, and apoptosis. Many human homologues of genes in the SWH pathway have been implicated in human cancers.[94]

VHL. Germline mutations of the von Hippel-Lindau (*VHL*) gene on chromosome 3p are associated with hereditary renal cell cancers, pheochromocytomas, hemangioblastomas of the central nervous system, retinal angiomas, and renal cysts.[60] Mutations of the *VHL* gene have also been noted in sporadic renal cell cancers (Chapter 20). The VHL protein is part of a ubiquitin ligase complex. A critical substrate for this activity is HIF1α (hypoxia-inducible transcription factor 1α). In the presence of oxygen, HIF1α is hydroxylated and binds to the VHL protein, leading to ubiquitination and proteasomal degradation. This hydroxylation reaction requires oxygen; in hypoxic environments the reaction cannot occur, and HIF1α escapes recognition by VHL and subsequent degradation. HIF1α can then translocate to the nucleus and turn on many genes, such as the growth/angiogenic factors vascular endothelial growth factor (VEGF) and PDGF. Lack of VHL activity prevents ubiquitination and degradation of HIF1α and is associated with increased levels of angiogenic growth factors.

WT1. The *WT1* gene, located on chromosome 11p13, is associated with the development of Wilms' tumor, a pediatric kidney cancer.[95] Both inherited and sporadic forms of Wilms' tumor occur, and mutational inactivation of the *WT1* locus has been seen in both forms. The WT1 protein is a transcriptional activator of genes involved in renal and gonadal differentiation. It regulates the mesenchymal-to-epithelial transition that occurs in kidney development. Though not precisely known, it is likely that the tumorigenic effect of WT1 deficiency is intimately connected with the role of the gene in the differentiation of genitourinary tissues. Interestingly, although WT1 is a tumor suppressor in Wilms' tumor, a variety of adult cancers, including leukemias and breast carcinomas, have also been shown to overexpress WT1. Since these tissues do not normally express WT1 at all, it has been suggested that WT1 may function as an oncogene in these cancers. Another Wilms' gene, *WT2*, located on 11p15, is associated with the Beckwith-Wiedemann syndrome (Chapter 10).

Patched (PTCH). *PTCH1* and *PTCH2* are tumor suppressor genes that encode a cell membrane protein (PATCHED), which functions as a receptor for a family of proteins called *Hedgehog*.[96] The Hedgehog/PATCHED pathway regulates several genes, including *TGF-β* and *PDGFRA* and *PDGFRB*. Mutations in *PTCH* are related to Gorlin syndrome, an inherited condition also known as nevoid basal cell carcinoma syndrome (see Chapter 26). *PTCH* mutations are present in 20% to 50% of sporadic cases of basal cell carcinoma. About one half of such mutations are of the type caused by UV exposure.

EVASION OF APOPTOSIS

Accumulation of neoplastic cells may result not only from activation of growth-promoting oncogenes or inactivation of growth-suppressing tumor suppressor genes, but also from mutations in the genes that regulate apoptosis.[97–99] Thus, apoptosis represents a barrier that must be surmounted for cancer to occur. In the adult, cell death by apoptosis is a physiologic response to several pathologic conditions that might contribute to malignancy if the cells remained viable. A cell with genomic injury can be induced to die, preventing the accumulation of cells with mutations. A variety of signals, ranging from DNA damage to loss of adhesion to the basement membrane (termed *anoikis*), can trigger apoptosis. A large family of genes that regulate apoptosis has been identified. Before we can understand how tumor cells evade apoptosis, it is essential to review briefly the biochemical pathways to apoptosis.

As discussed in Chapter 1, there are two distinct programs that activate apoptosis, the extrinsic and intrinsic pathways. Figure 7–34 shows, in simplified form, the sequence of events that lead to apoptosis by signaling through the death receptor CD95/Fas (extrinsic pathway) and by DNA damage (intrinsic pathway). The extrinsic pathway is initiated when CD95/Fas binds to its ligand, CD95L/FasL, leading to trimerization of the receptor and its cytoplasmic *death domains*, which attract the intracellular adaptor protein FADD. This protein recruits procaspase 8 to form the death-inducing signaling complex. Procaspase 8 is activated by cleavage into smaller subunits, generating caspase 8. Caspase 8 then activates downstream caspases such as caspase 3, a typical *executioner caspase* that cleaves DNA and other substrates to cause cell death. Additionally, caspase 8 can cleave and activate the BH3-only protein BID, activating the intrinsic pathway as well. The intrinsic pathway of apoptosis is triggered by a variety of stimuli, including withdrawal of survival factors, stress, and injury. Activation of this pathway leads to permeabilization of the mitochondrial outer membrane, with resultant release of molecules, such as cytochrome *c*, that initiate apoptosis. The integrity of the mitochondrial outer membrane is regulated by pro-apoptotic and anti-apoptotic members of the BCL2 family of proteins.[100] The pro-apoptotic proteins BAX and BAK are required for apoptosis and directly promote mitochondrial permeabilization. Their action is inhibited by the

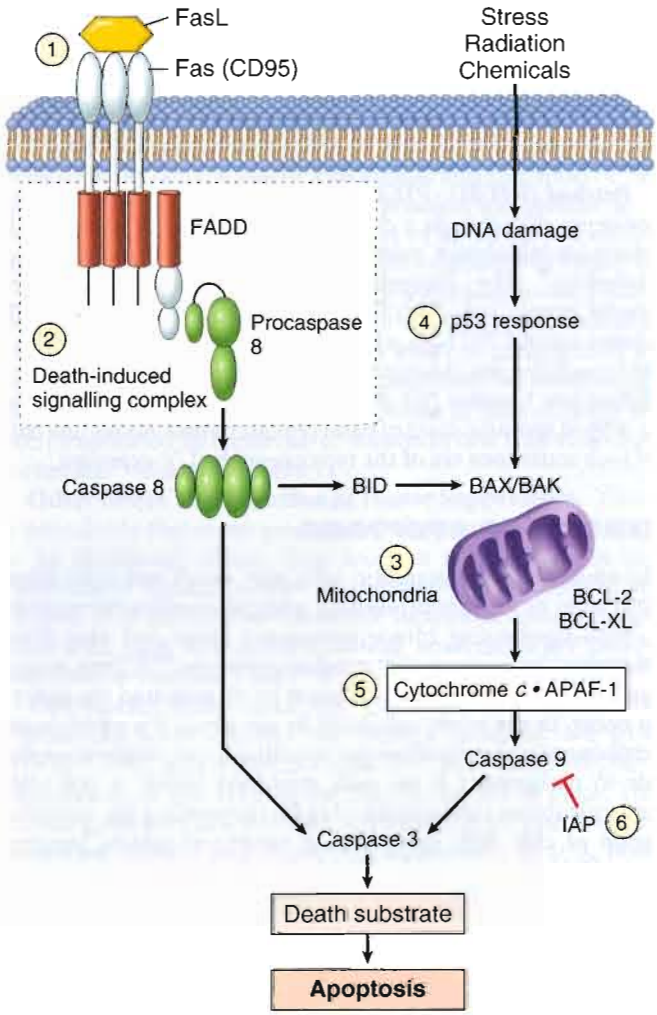


FIGURE 7–34 CD95 receptor–induced and DNA damage–triggered pathways of apoptosis and mechanisms used by tumor cells to evade cell death. (1) Reduced CD95 level. (2) Inactivation of death-induced signaling complex by FLICE protein (caspase 8; apoptosis-related cysteine peptidase). (3) Reduced egress of cytochrome *c* from mitochondrion as a result of up-regulation of BCL2. (4) Reduced levels of pro-apoptotic BAX resulting from loss of p53. (5) Loss of apoptotic peptidase activating factor 1 (APAF1). (6) Up-regulation of inhibitors of apoptosis (IAP). FADD, Fas-associated via death domain.

anti-apoptotic members of this family exemplified by BCL2 and BCL-XL. A third set of proteins (so-called BH3-only proteins), including BAD, BID, and PUMA, regulate the balance between the pro- and anti-apoptotic members of the BCL2 family. The BH3-only proteins sense death-inducing stimuli and promote apoptosis by neutralizing the actions of anti-apoptotic proteins like BCL2 and BCL-XL. When the sum total of all BH3 proteins expressed "overwhelms" the anti-apoptotic BCL2/BCL-XL protein barrier, BAX and BAK are activated and form pores in the mitochondrial membrane. Cytochrome *c* leaks into the cytosol, where it binds to APAF1, activating caspase 9. Like caspase 8 of the extrinsic pathway, caspase 9 can cleave and activate the executioner caspases. The caspases can be inhibited by a family of proteins called Inhibitors of Apoptosis Proteins (IAPs). Some tumors avoid apoptosis by upregulating these proteins, and there is interest in developing drugs that can block the interaction between IAPs and caspases. Because of the pro-apoptotic effect of BH3-only proteins, efforts are underway to develop BH3 mimetic drugs.

Within this framework it is possible to illustrate the multiple sites at which apoptosis is frustrated by cancer cells[101] (see Fig. 7–34). Starting from the surface, reduced levels of CD95/Fas may render the tumor cells less susceptible to apoptosis by CD95L/FasL. Some tumors have high levels of FLIP, a protein that can bind death-inducing signaling complex and prevent activation of caspase 8. Of all these genes, perhaps *best established is the role of BCL2 in protecting tumor cells from apoptosis.* As discussed later, approximately 85% of B-cell lymphomas of the follicular type (Chapter 13) carry a characteristic t(14;18)(q32;q21) translocation. Recall that 14q32, the site where immunoglobulin heavy-chain (IgH) genes are found, is also involved in the pathogenesis of Burkitt lymphoma. Juxtaposition of this transcriptionally active locus with *BCL2* (located at 18q21) causes overexpression of the BCL2 protein. This in turn increases the BCL2/BCL-XL buffer, protecting lymphocytes from apoptosis and allowing them to survive for long periods; there is therefore a steady accumulation of B lymphocytes, resulting in lymphadenopathy and marrow infiltration. Because BCL2-overexpressing lymphomas arise in large part from reduced cell death rather than explosive cell proliferation, they tend to be indolent (slow growing) compared with many other lymphomas.

As mentioned before, *p53 is an important pro-apoptotic gene that induces apoptosis in cells that are unable to repair DNA damage.* The actions of p53 are mediated in part by transcriptional activation of BAX, but there are other connections as well between p53 and the apoptotic machinery. Thus, the apoptotic machinery in cancer may be thwarted by mutations affecting the component proteins directly, as well as by loss of sensors of genomic integrity such as p53.

LIMITLESS REPLICATIVE POTENTIAL: TELOMERASE

As was discussed in the section on cellular aging (Chapter 1), most normal human cells have a capacity of 60 to 70 doublings. After this, the cells lose their ability to divide and become senescent. This phenomenon has been ascribed to progressive shortening of *telomeres* at the ends of chromosomes. Indeed, short telomeres seem to be recognized by the DNA-repair machinery as double-stranded DNA breaks, and this leads to cell cycle arrest mediated by p53 and RB.[102] In cells in which the checkpoints are disabled by *p53* or *RB1* mutations, the nonhomologous end-joining pathway is activated as a last-ditch effort to save the cell, joining the shortened ends of two chromosomes.[103] This inappropriately activated repair system results in dicentric chromosomes that are pulled apart at anaphase, resulting in new double-stranded DNA breaks. The resulting genomic instability from the repeated bridge-fusion-breakage cycles eventually produces mitotic catastrophe, characterized by massive cell death. *It follows that for tumors to grow indefinitely, as they often do, loss of growth restraints is not enough. Tumor cells must also develop ways to avoid both cellular senescence and mitotic catastrophe* (Fig. 7–35). If during crisis a cell manages to reactivate telom-

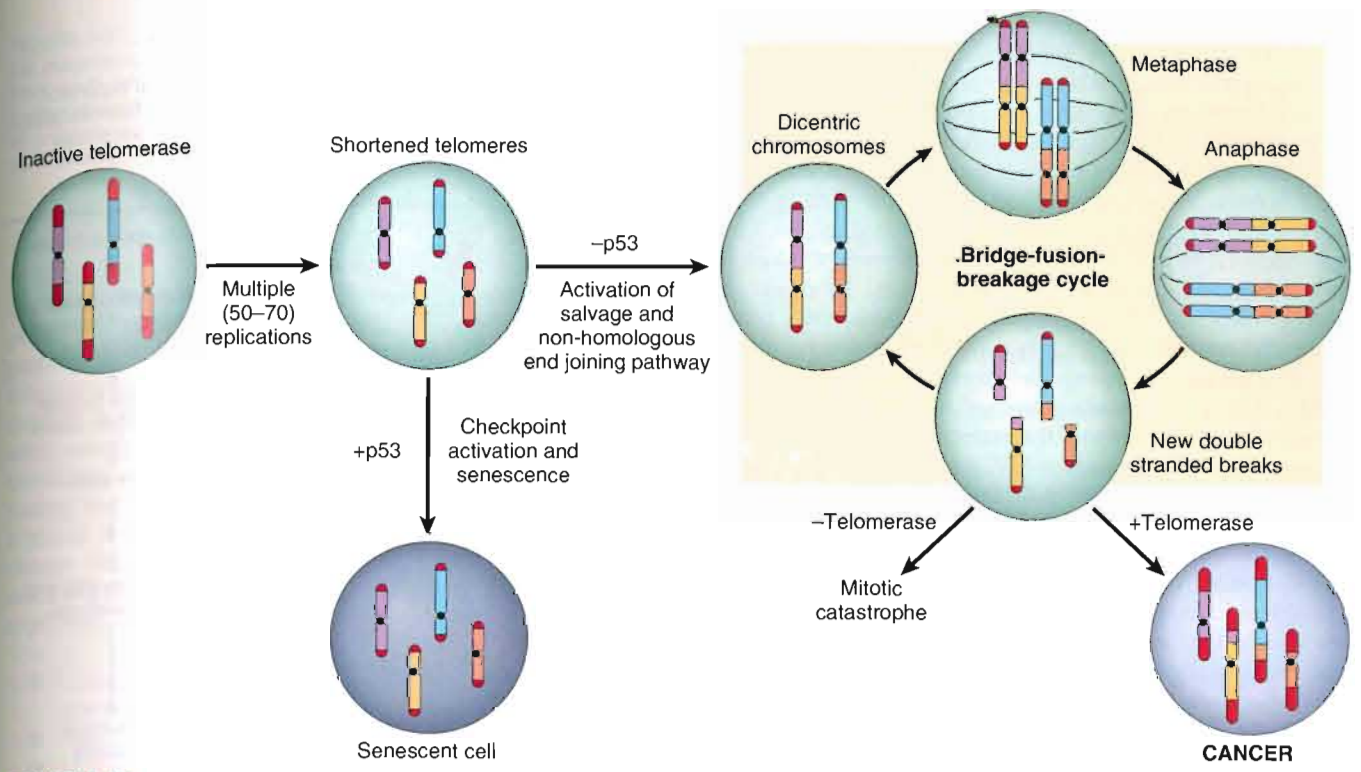


FIGURE 7–35 Sequence of events in the development of limitless replicative potential. Replication of somatic cells, which do not express telomerase, leads to shortened telomeres. In the presence of competent checkpoints, cells undergo arrest and enter nonreplicative senescence. In the absence of checkpoints, DNA-repair pathways are inappropriately activated, leading to the formation of dicentric chromosomes. At mitosis the dicentric chromosomes are pulled apart, generating random double-stranded breaks, which then activate DNA-repair pathways, leading to the random association of double-stranded ends and the formation, again, of dicentric chromosomes. Cells undergo numerous rounds of this bridge-fusion-breakage cycle, which generates massive chromosomal instability and numerous mutations. If cells fail to re-express telomerase, they eventually undergo mitotic catastrophe and death. Re-expression of telomerase allows the cells to escape the bridge-fusion-breakage cycle, thus promoting their survival and tumorigenesis.

erase, the bridge-fusion-breakage cycles cease and the cell is able to avoid death. However, during the period of genomic instability that precedes telomerase activation, numerous mutations could accumulate, helping the cell march toward malignancy. Passage through a period of genomic instability may explain the complex karyotypes frequently seen in human carcinomas. Telomerase, active in normal stem cells, is normally absent, or expressed at very low levels in most somatic cells. By contrast, telomere maintenance is seen in virtually all types of cancers. In 85% to 95% of cancers, this is due to up-regulation of the enzyme telomerase. A few tumors use other mechanisms, termed *alternative lengthening of telomeres*, which probably depend on DNA recombination. Interestingly, in the progression from colonic adenoma to colonic adenocarcinoma, early lesions had a high degree of genomic instability with low telomerase expression, whereas malignant lesions had complex karyotypes with high levels of telomerase activity, consistent with a model of telomere-driven tumorigenesis in human cancer. Several other mechanisms of genomic instability are discussed later.

ANGIOGENESIS

Even with all the genetic abnormalities discussed above, solid tumors cannot enlarge beyond 1 to 2 mm in diameter unless they are vascularized. Like normal tissues, tumors require delivery of oxygen and nutrients and removal of waste products; presumably the 1- to 2-mm zone represents the maximal distance across which oxygen, nutrients, and waste can diffuse from blood vessels. Cancer cells can stimulate neoangiogenesis, during which new vessels sprout from previously existing capillaries, or, in some cases, vasculogenesis, in which endothelial cells are recruited from the bone marrow (Chapter 3). Tumor vasculature is abnormal, however. The vessels are leaky and dilated, and have a haphazard pattern of connection. Neovascularization has a dual effect on tumor growth: perfusion supplies needed nutrients and oxygen, and newly formed endothelial cells stimulate the growth of adjacent tumor cells by secreting growth factors, such as insulin-like growth factors (IGFs), PDGF, and granulocyte-macrophage colony-stimulating factor. Angiogenesis is required not only for continued tumor growth but also for access to the vasculature and hence for metastasis. *Angiogenesis is thus a necessary biologic correlate of malignancy.*[104]

How do growing tumors develop a blood supply? The emerging paradigm is that tumor angiogenesis is controlled by the balance between angiogenesis promoters and inhibitors. Early in their growth, most human tumors do not induce angiogenesis. They remain small or in situ, possibly for years, until the *angiogenic switch* terminates this stage of vascular quiescence.[105] The molecular basis of the angiogenic switch involves increased production of angiogenic factors and/or

loss of angiogenic inhibitors. These factors may be produced directly by the tumor cells themselves or by inflammatory cells (e.g., macrophages) or other stromal cells associated with the tumors. Proteases, either elaborated by the tumor cells directly or from stromal cells in response to the tumor, are also involved in regulating the balance between angiogenic and anti-angiogenic factors. Many proteases can release the proangiogenic basic fibroblast growth factors (bFGF) stored in the ECM; conversely, three potent angiogenesis inhibitors—angiostatin, endostatin, and vasculostatin—are produced by proteolytic cleavage of plasminogen, collagen, and transthyretin, respectively. The angiogenic switch is controlled by several physiologic stimuli, such as hypoxia. Relative lack of oxygen stimulates HIF1α, an oxygen-sensitive transcription factor mentioned above, which then activates transcription of a variety of pro-angiogenic cytokines, such as VEGF and bFGF. These factors create an angiogenic gradient that stimulates the proliferation of endothelial cells and guides the growth of new vessels toward the tumor. VEGF also increases the expression of ligands that activate the Notch signaling pathway, which plays a crucial role in regulating the branching and density of the new vessels (Chapter 3). Both pro- and anti-angiogenic factors are regulated by many other genes frequently mutated in cancer. For example, in normal cells, p53 can stimulate expression of anti-angiogenic molecules such as thrombospondin-1, and repress expression of pro-angiogenic molecules such as VEGF. Thus, loss of p53 in tumor cells not only removes the cell cycle checkpoints listed above but also provides a more permissive environment for angiogenesis. The transcription of VEGF is also influenced by signals from the RAS-MAP kinase pathway, and mutations of *RAS* or *MYC* up-regulate the production of VEGF. The mechanisms whereby bFGF, VEGF, and the Notch pathway work together to coordinate angiogenesis were discussed in Chapter 3. bFGF and VEGF are commonly expressed in a wide variety of tumor cells, and elevated levels can be detected in the serum and urine of a significant fraction of cancer patients. Indeed, an anti-VEGF monoclonal antibody, bevacizumab, has recently been approved for use in the treatment of multiple cancers.[106] Another emerging strategy involves the use of antibodies that inhibit Notch activation. These antibodies cause new vessels to be so malformed that they cannot deliver blood to the tumor effectively.[107,108]

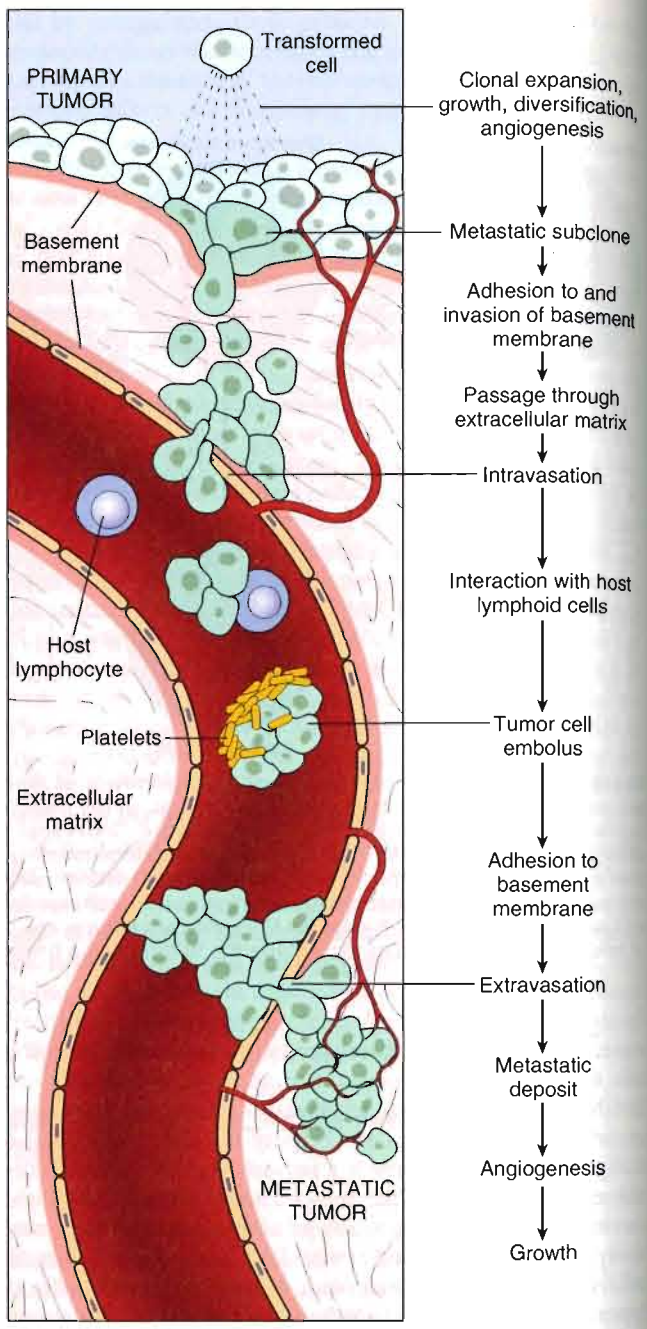


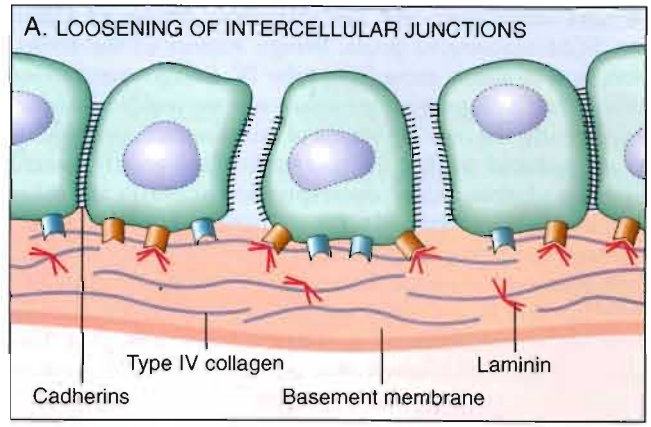
FIGURE 7–36 The metastatic cascade. Sequential steps involved in the hematogenous spread of a tumor.

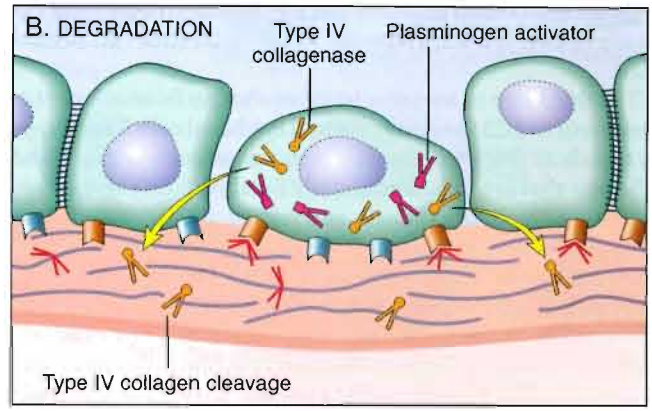
INVASION AND METASTASIS

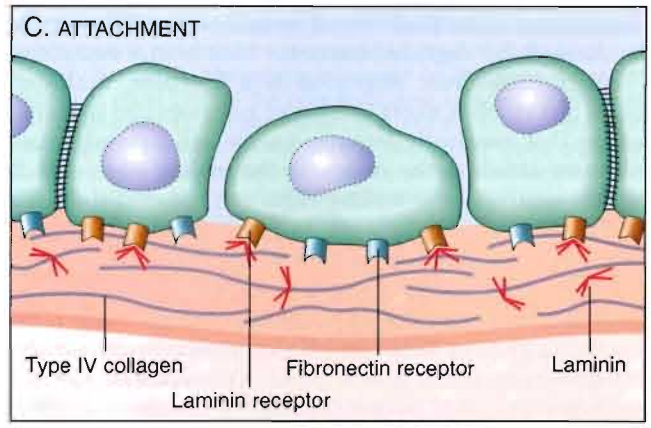
Invasion and metastasis are biologic hallmarks of malignant tumors. They are the major cause of cancer-related morbidity and mortality and hence are the subjects of intense scrutiny. Studies in mice and humans reveal that although millions of cells are released into the circulation each day from a primary tumor, only a few metastases are produced. Indeed, tumor cells can be frequently detected in the blood and marrow of patients with breast cancer who have not, and do not ever, develop gross metastatic disease. Why is the metastatic process so inefficient? Each step in the process is subject to a multitude of controls; hence, at any point in the sequence the breakaway cell may not survive.[109] For tumor cells to break loose from a primary mass, enter blood vessels or lymphatics, and produce a secondary growth at a distant site, they must go through a series of steps (summarized in Fig. 7–36). For the purpose of this discussion, the metastatic cascade will be divided into two phases: (1) invasion of the extracellular matrix (ECM); (2) vascular dissemination, homing of tumor cells, and colonization. Subsequently, the molecular genetics of the metastatic cascade, as currently understood, will be presented.

Invasion of Extracellular Matrix

The structural organization and function of normal tissues is to a great extent determined by interactions between cells and the ECM.[110] As we discussed in Chapter 3, tissues are organized into compartments separated from each other by two types of ECM: basement membrane and interstitial connec-







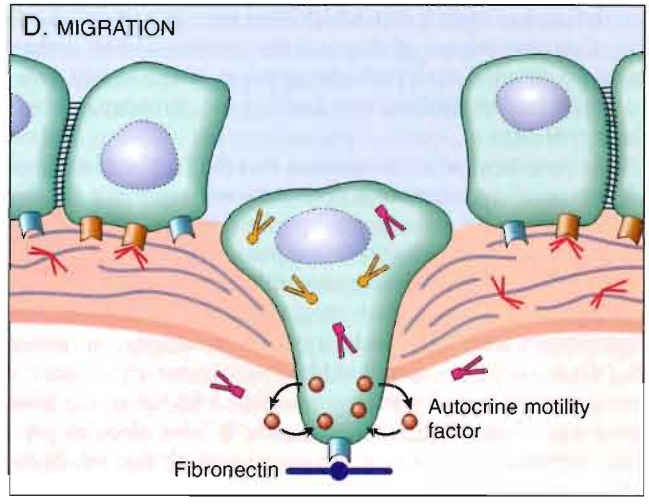


FIGURE 7–37 **A–D,** Sequence of events in the invasion of epithelial basement membranes by tumor cells. Tumor cells detach from each other because of reduced adhesiveness, then secrete proteolytic enzymes, degrading the basement membrane. Binding to proteolytically generated binding sites and tumor cell migration follow.

tive tissue. Though organized differently, each of these components of ECM is made up of collagens, glycoproteins, and proteoglycans. As shown in Figure 7–36, tumor cells must interact with the ECM at several stages in the metastatic cascade. A carcinoma must first breach the underlying basement membrane, then traverse the interstitial connective tissue, and ultimately gain access to the circulation by penetrating the vascular basement membrane. This process is repeated in reverse when tumor cell emboli extravasate at a distant site. Invasion of the ECM initiates the metastatic cascade and *is an active process that can be resolved into several steps* (Fig. 7–37):

- Changes ("loosening up") of tumor cell-cell interactions
- Degradation of ECM
- Attachment to novel ECM components
- Migration of tumor cells

Dissociation of cells from one another is often the result of alterations in intercellular adhesion molecules. Normal cells are neatly glued to each other and their surroundings by a variety of adhesion molecules.[111] Cell-cell interactions are mediated by the cadherin family of transmembrane glycoproteins. E-cadherins mediate homotypic adhesions in epithelial tissue, thus serving to keep the epithelial cells together and to relay signals between the cells; intracellularly the E-cadherins are connected to β-catenin and the actin cytoskeleton. In several epithelial tumors, including adenocarcinomas of the colon and breast, there is a down-regulation of E-cadherin expression. Presumably, this down-regulation reduces the ability of cells to adhere to each other and facilitates their detachment from the primary tumor and their advance into the surrounding tissues. E-cadherins are linked to the cytoskeleton by the *catenins*, proteins that lie under the plasma membrane (see Fig. 7–33). The normal function of E-cadherin is dependent on its linkage to catenins. In some tumors E-cadherin is normal, but its expression is reduced because of mutations in the gene for α catenin.

The second step in invasion is local *degradation of the basement membrane and interstitial connective tissue.* Tumor cells may either secrete proteolytic enzymes themselves or induce stromal cells (e.g., fibroblasts and inflammatory cells) to elaborate proteases. Many different families of proteases, such as matrix metalloproteinases (MMPs), cathepsin D, and urokinase plasminogen activator, have been implicated in tumor cell invasion. MMPs regulate tumor invasion not only by remodeling insoluble components of the basement membrane and interstitial matrix but also by releasing ECM-sequestered growth factors. Indeed, cleavage products of collagen and proteoglycans also have chemotactic, angiogenic, and growth-promoting effects.[112] For example, MMP9 is a gelatinase that cleaves type IV collagen of the epithelial and vascular

basement membrane and also stimulates release of VEGF from ECM-sequestered pools. Benign tumors of the breast, colon, and stomach show little type IV collagenase activity, whereas their malignant counterparts overexpress this enzyme. Concurrently, the concentrations of metalloproteinase inhibitors are reduced so that the balance is tilted greatly toward tissue degradation. Indeed, overexpression of MMPs and other proteases has been reported for many tumors. However, recent in vivo imaging experiments have shown that tumor cells can adopt a second mode of invasion, termed *ameboid migration.*[113] In this type of migration the cell squeezes through spaces in the matrix instead of cutting its way through it. This ameboid migration is much quicker, and tumor cells seem to be able to use collagen fibers as high-speed railways in their travels. Tumor cells, in vitro at least, seem to be able to switch between the two forms of migration, perhaps explaining the disappointing performance of MMP inhibitors in clinical trials.

The third step in invasion involves *changes in attachment of tumor cells to ECM proteins.* Normal epithelial cells have receptors, such as integrins, for basement membrane laminin and collagens that are polarized at their basal surface; these receptors help to maintain the cells in a resting, differentiated state. Loss of adhesion in normal cells leads to induction of apoptosis, while, not surprisingly, tumor cells are resistant to this form of cell death. Additionally, the matrix itself is modified in ways that promote invasion and metastasis. For example, cleavage of the basement membrane proteins collagen IV and laminin by MMP2 or MMP9 generates novel sites that bind to receptors on tumor cells and stimulate migration.

Locomotion is the final step of invasion, propelling tumor cells through the degraded basement membranes and zones of matrix proteolysis. Migration is a complex, multistep process that involves many families of receptors and signaling proteins that eventually impinge on the actin cytoskeleton. Cells must attach to the matrix at the leading edge, detach from the matrix at the trailing edge, and contract the actin cytoskeleton to ratchet forward. Such movement seems to be potentiated and directed by tumor cell–derived cytokines, such as autocrine motility factors. In addition, cleavage products of matrix components (e.g., collagen, laminin) and some growth factors (e.g., IGFs I and II) have chemotactic activity for tumor cells. Furthermore, proteolytic cleavage liberates growth factors bound to matrix molecules. Stromal cells also produce paracrine effectors of cell motility, such as hepatocyte growth factor–scatter factor, which bind to receptors on tumor cells. Concentrations of hepatocyte growth factor–scatter factor are elevated at the advancing edges of the highly invasive brain tumor glioblastoma multiforme, supporting their role in motility.

It has become clear in recent years that the ECM and stromal cells surrounding tumor cells do not merely represent a static barrier for tumor cells to traverse but instead represent a varied environment in which reciprocal signaling between tumor cells and stromal cells may either promote or prevent tumorigenesis and/or tumor progression.[24] Stromal cells that interact with tumors include innate and adaptive immune cells (discussed later), as well as fibroblasts. A variety of studies have demonstrated that tumor-associated fibroblasts exhibit altered expression of genes that encode ECM molecules, proteases, protease inhibitors, and various growth factors. Thus, tumor cells live in a complex and ever-changing milieu composed of ECM, growth factors, fibroblasts, and immune cells, with significant cross-talk among all the components. The most successful tumors may be those that can co-opt and adapt this environment to their own nefarious ends.

Vascular Dissemination and Homing of Tumor Cells

Once in the circulation, tumor cells are vulnerable to destruction by a variety of mechanisms, including mechanical shear stress, apoptosis stimulated by loss of adhesion, (which has been termed *anoikis*), and innate and adaptive immune defenses. The details of tumor immunity are considered later.

Within the circulation, tumor cells tend to aggregate in clumps. This is favored by homotypic adhesions among tumor cells as well as heterotypic adhesion between tumor cells and blood cells, particularly platelets (see Fig. 7–36). Formation of platelet-tumor aggregates may enhance tumor cell survival and implantability. Tumor cells may also bind and activate coagulation factors, resulting in the formation of emboli. Arrest and extravasation of tumor emboli at distant sites involves adhesion to the endothelium, followed by egress through the basement membrane. Involved in these processes are adhesion molecules (integrins, laminin receptors) and proteolytic enzymes, discussed earlier. Of particular interest is the CD44 adhesion molecule, which is expressed on normal T lymphocytes and is used by these cells to migrate to selective sites in the lymphoid tissue. Such migration is accomplished by the binding of CD44 to hyaluronate on high endothelial venules, and overexpression of CD44 may favor metastatic spread. At the new site, tumor cells must proliferate, develop a vascular supply, and evade the host defenses.[109]

The site at which circulating tumor cells leave the capillaries to form secondary deposits is related, in part, to the anatomic location of the primary tumor, with most metastases occurring in the first capillary bed available to the tumor. Many observations, however, suggest that natural pathways of drainage do not wholly explain the distribution of metastases. For example, prostatic carcinoma preferentially spreads to bone, bronchogenic carcinomas tend to involve the adrenals and the brain, and neuroblastomas spread to the liver and bones. Such organ tropism may be related to the following mechanisms:

- Because the first step in extravasation is adhesion to the endothelium, tumor cells may have adhesion molecules whose ligands are expressed preferentially on the endothelial cells of the target organ. Indeed, it has been shown that the endothelial cells of the vascular beds of various tissues differ in their expression of ligands for adhesion molecules.
- Chemokines have an important role in determining the target tissues for metastasis. For instance, some breast cancer cells express the chemokine receptors CXCR4 and CCR7.[114] The chemokines that bind to these receptors are highly expressed in tissues to which breast cancers commonly metastasize. Blockage of the interaction between CXCR4 and its receptor decreases breast cancer metastasis to lymph nodes and lungs. Some target organs may liberate chemoattractants that recruit tumor cells to the site. Examples include IGFs I and II.

- In some cases, the target tissue may be a nonpermissive environment—unfavorable soil, so to speak, for the growth of tumor seedlings. For example, though well vascularized, skeletal muscles are rarely the site of metastases.

Despite their "cleverness" in escaping their sites of origin, tumor cells are quite inefficient in colonizing distant organs. Millions of tumors cells are shed daily from even small tumors. These cells can be detected in the bloodstream and in small foci in the bone marrow, even in patients that never develop gross metastatic lesions. Indeed, the concept of dormancy, referring to the prolonged survival of micrometastases without progression, is well described in melanoma and in breast and prostate cancer. Although the molecular mechanisms of colonization are just beginning to be unraveled in mouse models, a constant pattern seems to be that tumor cells secrete cytokines, growth factors, and ECM molecules that act on the resident stromal cells, which in turn make the metastatic site habitable for the cancer cell.[115] For example, breast cancer metastases to bone are osteolytic because of the activation of osteoclasts in the metastatic site. Breast cancer cells secrete parathyroid hormone–related protein (PTHRP), which stimulates osteoblasts to make RANK ligand (RANKL). RANKL then activates osteoclasts, which degrade the bone matrix and release growth factors embedded within it, like IGF and TGF-β. With a better molecular understanding of the mechanisms of metastasis our ability to target them therapeutically will be greatly enhanced.

Molecular Genetics of Metastasis Development

Why do only some tumors metastasize? What are the genetic changes that allow metastases? Why is the metastatic process so inefficient? Several competing theories have been proposed to explain how the metastatic phenotype arises. The clonal evolution model suggest that, as mutations accumulate in genetically unstable cancer cells and the tumor become heterogeneous (Fig. 7–38A), a subset of tumor cell subclones develop the right combination of gene products to complete all the steps involved in metastasis. Thus, metastatic subclones result from clonal evolution, and it is only the rare cell that acquires all the necessary genetic alterations and can complete all the steps. However, recent experiments, in which gene expression profiles of primary tumors and metastatic deposits have been compared, challenge this hypothesis. For example, a subset of breast cancers has a gene expression signature similar to that found in metastases, although no clinical evidence for metastasis is apparent. In these tumors it seems that most if not all cells develop a predilection for metastatic spread during early stages of carcinogenesis. Metastases, according to this view, are not dependent on the stochastic generation of metastatic subclones postulated above. The alternative hypothesis suggested by these data is that metastasis is the result of multiple abnormalities that occur in many, perhaps most, cells of a primary tumor, and perhaps early in the development of the tumor (Fig. 7–38B and C). Such abnormalities give most cells within the tumor a general predisposition for metastasis, often called the "metastasis signature."[116] This signature may involve not only properties intrinsic to the cancer cells but also the characteristics of their

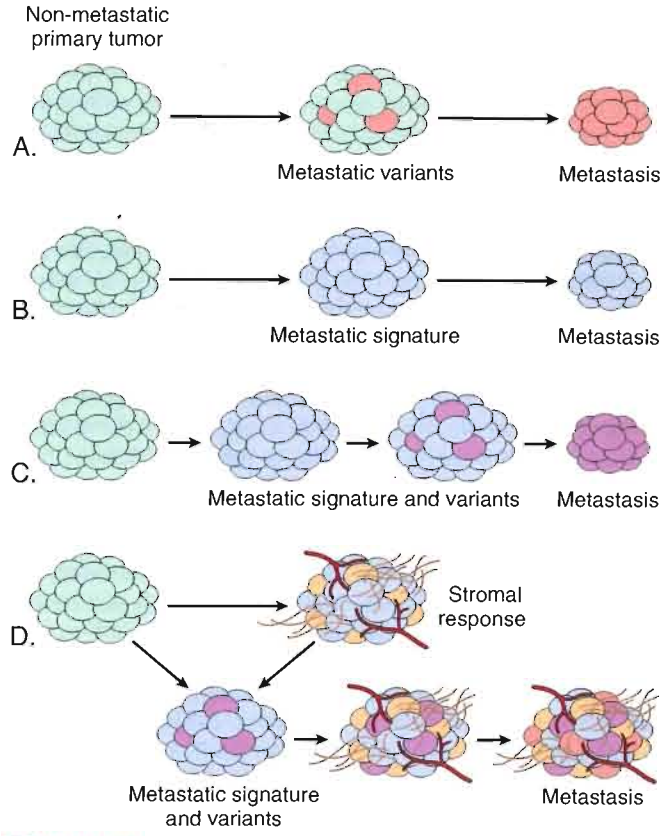


FIGURE 7–38 Mechanisms of metastasis development within a primary tumor. A nonmetastatic primary tumor is shown (light blue) on the left side of all diagrams. Four models are presented: **A,** Metastasis is caused by rare variant clones that develop in the primary tumor; **B,** Metastasis is caused by the gene expression pattern of most cells of the primary tumor, referred to as a metastatic signature; **C,** A combination of **A** and **B**, in which metastatic variants appear in a tumor with a metastatic gene signature; **D,** Metastasis development is greatly influenced by the tumor stroma, which may regulate angiogenesis, local invasiveness, and resistance to immune elimination, allowing cells of the primary tumor, as in **C**, to become metastatic.

microenvironment, such as the components of the stroma, the presence of infiltrating immune cells, and angiogenesis (Fig. 7–38D). It should be noted, however, that gene expression analyses like those described above would not detect a small subset of metastatic subclones within a large tumor. Perhaps both mechanisms are operative, with aggressive tumors acquiring a metastases-permissive gene expression pattern early in tumorigenesis that requires some additional random mutations to complete the metastatic phenotype. A third hypothesis suggests that background genetic variation, and the resulting variation in gene expression, in the human population contributes to the generation of metastases. In mouse models, cancers induced with the same oncogenic mutations can have very different metastatic outcomes depending on the strain (i.e., background genetics) of the mouse used. Even very strong oncogenes can be significantly affected by background genetics. The fourth hypothesis is a corollary of the tumor stem cell hypothesis, which suggests that if tumors derive from rare tumor stem cells, metastases require the spread of the tumor stem cells themselves.

One open question in the field is, are there genes whose principal or sole contribution to tumorigenesis is to control metastasis? This question is of more than academic interest, because if altered forms of certain genes promote or suppress the metastatic phenotype, their detection in a primary tumor would have both prognostic and therapeutic implications. Since metastasis is a complex phenomenon involving a variety of steps and pathways described above, it is thought that, unlike transformation, in which a subset of proteins like p53 and RB seem to play a key role, genes that function as "metastasis oncogenes" or "metastatic suppressors" are rare. A metastasis suppressor gene is defined as a gene whose loss promotes the development of metastasis without an effect on the primary tumor. Accordingly, expression of a metastasis oncogene favors the development of metastasis without effect upon the primary tumor. At least a dozen genes lost in metastatic lesions have been confirmed to function as "metastasis suppressors".[117,118] Their molecular functions are varied and not yet completely clear; however, most appear to affect various signaling pathways. Interestingly, recent work has suggested that two miRNAs, mir335 and mir126, suppress the metastasis of breast cancer, while a second set (mir10b) promotes metastasis.[119,120]

Among candidates for metastasis oncogenes are SNAIL and TWIST, which encode transcription factors whose primary function is to promote a process called epithelial-to-mesenchymal transition (EMT).[88] In EMT, carcinoma cells down-regulate certain epithelial markers (e.g., E-cadherin) and up-regulate certain mesenchymal markers (e.g., vimentin and smooth muscle actin). These changes are believed to favor the development of a promigratory phenotype that is essential for metastasis. Loss of E-cadherin expression seems to be a key event in EMT, and SNAIL and TWIST are transcriptional repressors that down-regulate E-cadherin expression.[121] EMT has been documented mainly in breast cancers; whether this is a general phenomenon remains to be established.

GENOMIC INSTABILITY—ENABLER OF MALIGNANCY

Although humans literally swim in environmental agents that are mutagenic (e.g., chemicals, radiation, sunlight), cancers are relatively rare outcomes of these encounters. This state of affairs results from the ability of normal cells to repair DNA damage, the death of cells with unrepairable damage[122] (see "Evasion of Apoptosis" above), and other mechanisms, such as oncogene-induced senescence and immune surveillance (discussed later). The importance of DNA repair in maintaining the integrity of the genome is highlighted by several inherited disorders in which genes that encode proteins involved in DNA repair are defective. *Individuals born with such inherited defects in DNA-repair proteins are at a greatly increased risk of developing cancer.* Moreover, defects in repair mechanisms are present in sporadic human cancers. *DNA-repair genes themselves are not oncogenic, but their abnormalities allow mutations in other genes during the process of normal cell division.* Typically, genomic instability occurs when both copies of the DNA repair gene are lost; however, recent work has suggested that at least a subset of these genes may promote cancer in a haploinsufficient manner. Defects in three types of DNA-repair systems—mismatch repair, nucleotide excision repair, and recombination repair—contribute to different types of cancers.

Hereditary Nonpolyposis Colon Cancer Syndrome. HNPCC syndrome, characterized by familial carcinomas of the colon affecting predominantly the cecum and proximal colon (Chapter 17), results from defects in genes involved in DNA mismatch repair.[123] When a strand of DNA is being replicated, these genes act as "spell checkers." For example, if there is an erroneous pairing of G with T rather than the normal A with T, the mismatch-repair genes correct the defect. Without these "proofreaders," errors gradually accumulate randomly in the genome, and some of these errors may involve proto-oncogenes and tumor suppressor genes. One of the hallmarks of patients with mismatch-repair defects is microsatellite instability.[21] Microsatellites are tandem repeats of one to six nucleotides found throughout the genome. In normal people the length of these microsatellites remains constant. However, in people with HNPCC, these satellites are unstable and increase or decrease in length in tumor cells, creating alleles not found in normal cells of the same patient. Of the various DNA mismatch-repair genes, at least four are involved in the pathogenesis of HNPCC, but germline mutations in the *MSH2* (2p16) and *MLH1* (3p21) genes each account for approximately 30% of cases. The remaining cases have mutations in other mismatch repair genes. Each affected individual inherits one defective copy of a DNA mismatch-repair gene and acquires the second hit in colonic epithelial cells. Thus, DNA-repair genes behave like tumor suppressor genes in their mode of inheritance, but in contrast to tumor suppressor genes (and oncogenes), they affect cell growth only indirectly—by allowing mutations in other genes during the process of normal cell division. Although HNPCC accounts only for 2% to 4% of all colonic cancers, microsatellite instability can be detected in about 15% of sporadic colon cancers. The growth-regulating genes that are mutated in HNPCC tumors have not yet been fully characterized but include the genes encoding TGF-β receptor II, the TCF component of the β-catenin pathway, *BAX*, and other oncogenes and tumor suppressor genes.[124]

Xeroderma Pigmentosum. Individuals with another inherited disorder of defective DNA repair, xeroderma pigmentosum, are at increased risk for the development of cancers of the skin particularly following exposure to the UV light contained in sun rays.[125] UV radiation causes cross-linking of pyrimidine residues, preventing normal DNA replication. Such DNA damage is repaired by the nucleotide excision repair system. Several proteins are involved in nucleotide excision repair, and an inherited loss of any one can give rise to xeroderma pigmentosum.

Diseases with Defects in DNA Repair by Homologous Recombination. A group of autosomal recessive disorders comprising Bloom syndrome, ataxia-telangiectasia, and Fanconi anemia is characterized by hypersensitivity to other DNA-damaging agents, such as ionizing radiation (Bloom syndrome and ataxia-telangiectasia), or DNA cross-linking agents, such as many chemotherapeutic agents (Fanconi anemia).[126,127] Their phenotype is complex and includes, in addition to predisposition to cancer, features such as neural symptoms (ataxia-telangiectasia), bone marrow aplasia (Fanconi anemia), and developmental defects (Bloom syndrome). As mentioned earlier, the gene mutated in ataxia-telangiectasia, *ATM*, is important in recognizing and responding to DNA damage caused by ionizing radiation. Persons with Bloom syndrome have a predisposition to a very broad spectrum of tumors. The defective gene is located on chromosome 15 and encodes a

helicase that participates in DNA repair by homologous recombination. There are 13 genes that make up the Fanconi anemia complex; mutation of any one of these genes can result in the phenotype.[126,128] Interestingly, *BRCA2*, which is mutated in some individuals with familial breast cancer, is also mutated in a subset of persons with Fanconi anemia. Evidence for the role of DNA-repair genes in the origin of cancer also comes from the study of hereditary breast cancer. Mutations in two genes, *BRCA1* (chromosome 17q21) and *BRCA2* (chromosome 13q12–13), account for 25% of cases of familial breast cancer. In addition to breast cancer, women with *BRCA1* mutations have a substantially higher risk of epithelial ovarian cancers, and men have a slightly higher risk of prostate cancer. Likewise, mutations in the *BRCA2* gene increase the risk of breast cancer in both men and women as well as cancer of the ovary, prostate, pancreas, bile ducts, stomach, and melanocytes. Although the functions of these genes have not been elucidated fully, cells that lack these genes develop chromosomal breaks and severe aneuploidy. Indeed, both *BRCA1* and *BRCA2* have been shown to associate with a variety of proteins involved in the homologous recombination repair pathway. The Fanconi anemia proteins and the BRCA proteins form a DNA-damage response network whose purpose is to resolve and repair intrastrand and interstrand DNA cross-links induced by chemical cross-linking agents. Failure to resolve these cross-links before separation of the two strands would lead to chromosome breakage and exposed chromosome ends. Generation of such ends would, as with short telomeres (see above), lead to the activation of the salvage nonhomologous end joining pathway, formation of dicentric chromosomes, bridge-fusion-breakage cycles, and massive aneuploidy. Similar to other tumor suppressor genes, both copies of *BRCA1* and *BRCA2* must be inactivated for cancer to develop. Although linkage of *BRCA1* and *BRCA2* to familial breast cancers is established, these genes are rarely inactivated in sporadic cases of breast cancer. In this regard, *BRCA1* and *BRCA2* are different from other tumor suppressor genes, such as *APC* and *p53*, which are inactivated in both familial and sporadic cancers.

STROMAL MICROENVIRONMENT AND CARCINOGENESIS

Although we have mostly focused on the neoplastic parenchymal cells in our discussion, tumors are not composed of a single cell type. Indeed, tumors are comprised of a complex mixture of cells of numerous lineages, including the tumor cells themselves, innate and adaptive immune cells, fibroblasts, endothelial cells, and others. Additionally, numerous examples of cross-talk between the ECM and tumor cells have been described. For example, cleavage of matrix components such as type IV collagen releases angiogenic factors (VEGF), and enzymatic degradation of laminin-5 by MMP2 reveals a cryptic proteolytic fragment that favors cancer cell motility.[112] The ECM also stores growth factors in inactive forms, which are released by active matrix proteases. Such factors include PDGF, TGF-β, and bFGF, which in turn affect the growth of tumor cells in a paracrine manner. Successful tumor cells must co-opt these and other interactions and use them to promote their growth and invasion. More interesting is whether tumor cells are dependent upon these interactions for proliferation, survival, or metastases. If so, these interactions, and the stromal cells themselves, become potential therapeutic targets.

Both the inflammatory cells and fibroblasts within the tumor have been shown to have a complex relationship to the cancer cells and to each other. The role of chronic inflammation in the development of cancer has already been described (see above). Various mechanisms, such as the expression of pro-survival and pro-proliferation cytokines by immune cells, not only promote the development of cancer, but also promote survival and progression of tumor cells. Additionally, it has been suggested that macrophages infiltrating the tumor can be induced by the tumor cells to secrete factors that promote metastasis.[129] In a mouse model of breast cancer, genetic deletion of macrophages prevented metastases. Furthermore, in vivo imaging of tumors in animal models has shown that macrophages surrounding blood vessels secrete EGF, resulting in chemotactic migration of tumor cells toward the vasculature.[113] Fibroblasts play an important role in tumors as well. Fibroblasts secrete the matrix that results in the desmoplastic response to tumors. Interestingly, in vitro experiments that altered the stiffness of the matrix alone could change the aggressiveness of a cancer cell line. Thus, the desmoplastic response to cancer may be stimulated by the cancer cells and may promote their growth. On the other hand, in a prostate cancer model, injection of immortalized, but nontumorigenic cells, together with fibroblasts derived from a tumor (cancer-associated fibroblasts) led to the development of poorly differentiated tumors in athymic mice.[130] These carcinomas had multiple genetic abnormalities that were not present in the parent cell line, suggesting that the stroma can drive genetic changes that promote carcinogenesis. Indeed, some of the predictions of tumor behavior based on gene expression profiling are turning out to be based on genes highly expressed in stromal cells, rather than tumor cells. How such changes come about remains mysterious, as does their relevance to carcinogenesis in vivo. However, the results are sufficiently intriguing to merit attention, since they suggest a novel form of cancer therapy that could be targeted to stromal cells. The role of stromal cells in tumor growth and progression is highlighted by recent studies in which gene expression profiles of stroma cells predicted clinical outcome in human breast cancer.[131]

METABOLIC ALTERATIONS: THE WARBURG EFFECT

Even in the presence of ample oxygen, cancer cells shift their glucose metabolism away from the oxygen hungry, but efficient, mitochondria to glycolysis.[132–134] This phenomenon, called the Warburg effect and also known as aerobic glycolysis, has been recognized for many years (indeed, Otto Warburg received the Nobel Prize for discovery of the effect that bears his name in 1931), but was largely neglected until recently. This metabolic alteration is so common to tumors that some would call it the eighth hallmark of cancer. Indeed, clinically, the "glucose-hunger" of tumors is used to visualize tumors via positron emission tomography (PET) scanning, in which patients are injected with 18F-fluorodeoxyglucose, a non-metabolizable derivative of glucose that is preferentially taken up into tumor cells (as well as normal, actively dividing tissues such as the bone marrow). Most tumors are PET-positive, and rapidly growing ones are markedly so. However, the causal relationship between aerobic glycolysis and tumor progression is not entirely clear, nor is the initial insult that drives these metabolic changes.

Indeed, as is well known, glycolysis generates 2 ATP molecules per molecule of glucose, while oxidative phosphorylation in the mitochondria generates more than 20. How does a switch to the less efficient glycolysis lead to a growth advantage for a tumor? Several mutually non-exclusive hypotheses have been offered. One attractive hypothesis to explain the Warburg effect is that altered metabolism confers a growth advantage in the hypoxic tumor microenvironment.[133–134] Although angiogenesis generates increased vasculature, the vessels are poorly formed, and tumors are still relatively hypoxic compared to normal tissues. Indeed, the activation of HIF1α by hypoxia not only stimulates angiogenesis, but also increases the expression of numerous metabolic enzymes in the glycolytic pathway as well as downregulates genes involved in oxidative phosphorylation. So the simplest explanation is basic economics: supply and demand. Decreased demand by individual tumor cells increases the oxygen supply, thus increasing the number of tumor cells that can be supported by the vasculature and increasing the size of the tumor.

However, the Warburg effect refers to aerobic glycolysis; glycolysis that occurs in the face of adequate oxygen for oxidative phosphorylation. Thus, the changes that promote the switch in metabolism during hypoxia must become fixed in the tumor cell. It may be that continuous rounds of hypoxia followed by normoxia, as is frequently seen in tumors, select for tumor cells that constitutively upregulate glycolysis. Additionally, or perhaps alternatively, mutations in oncogenes and tumor suppressors that favor growth, such as *RAS*, *p53* and *PTEN*, also stimulate metabolic changes in the cell. Which brings us to the second part of the supply and demand equation that may help explain why tumor cells opt for a less efficient energy production pipeline.

In addition to doubling its DNA content prior to division, an actively dividing cell (whether normal or transformed) must also double all of its other components, including membranes, proteins, and organelles. This task requires increased uptake of nutrients, particularly glucose (which produces the energy needed for biosynthesis of these components) and amino acids (which provide the building blocks used for protein synthesis) as well as increased synthesis of the necessary building blocks. Halting the breakdown of glucose at pyruvate allows these carbons to be shunted to anabolic pathways, such as lipid and nucleotide production; additionally, tumor cells are able to shunt glutamine into both the glycolytic as well as anabolic pathways.[134–135] Thus, the metabolic changes that tumor cells undergo increase their ability to synthesize the building blocks they need for cell division. Indeed, alterations in signaling pathways involved in cancer also stimulate the uptake of glucose and other nutrients, favor glycolysis over oxidative phosphorylation, and increase anabolic pathways in the cell. Normally, growth factors stimulate glucose and amino acid uptake through the PI3K/AKT/mTOR pathway, which is downstream of receptor tyrosine kinases and other growth factor receptors; in tumors, these signals are cell autonomous. Thus, mutation of tumor suppressors and oncogenes not only leads to constitutive activation of pathways that favor survival and proliferation, but they also make glycolysis and anabolic biosynthesis a permanent fixture of the tumor cell.[135–136]

Now that the Warburg effect has been "rediscovered," other fascinating connections between metabolism and neoplasia are emerging that involve both tumor suppressors and oncoproteins. One example of the former involves *LKB1*, a tumor suppressor gene encoding a threonine kinase that is mutated in Peutz-Jegher syndrome (Chapter 17), which is associated with benign and malignant epithelial proliferations of the gastrointestinal tract. At least one aspect of LKB1's tumor suppressive activity is mediated through its ability to activate AMP-dependent protein kinase (AMPK), a conserved sensor of cellular energy status that is an important negative regulator of mTOR. Thus LKB1 suppresses tumor formation, at least in part, by putting the brakes on anabolic metabolism. Of note, two other tumor suppressors that are mutated in tuberous sclerosis, TSC1 and TSC2 (Chapter 28), also negatively regulate mTOR. On the other side of the ledger, it has been claimed that the transforming effects of many oncoproteins, including mutated receptor tyrosine kinases and the notorious oncogenic transcription factor c-MYC, are mediated in part through induction of the "Warburg effect." These kinds of insights have spurred many recent attempts to target signaling pathways that drive anabolic metabolism in cancer cells, such as the PI3K/AKT/mTOR pathway.

As we have discussed earlier, cells have many regulatory barriers to prevent inappropriate growth. One adaptive response of normal cells to oxygen and glucose deprivation is *autophagy*, a state in which cells arrest their growth and cannibalize their own organelles, proteins, and membranes as carbon sources for energy production (Chapter 1). If this adaptation fails, the cells die. Tumor cells often seem to be able to grow under marginal environmental conditions without triggering autophagy, suggesting that the pathways that induce autophagy are deranged. In keeping with this, several genes that promote autophagy are tumor suppressors, most notable PTEN (a negative regulator of the PI3K/AKT pathway), which is mutated or epigenetically silenced in a wide variety of human cancers. Whether autophagy is always bad from the vantage point of the tumor, however, is a matter of active investigation and debate. For example, under conditions of severe nutrient deprivation tumor cells may use autophagy to become "dormant," a state of metabolic hibernation that allows cells to survive hard times for long periods. Such cells are believed to be resistant to therapies that kill actively dividing cells, and could therefore be responsible for therapeutic failures. Thus, autophagy may be a tumor's friend or foe depending on how the signaling pathways that regulate it are "wired" in a given tumor.

DYSREGULATION OF CANCER-ASSOCIATED GENES

The genetic damage that activates oncogenes or inactivates tumor suppressor genes may be subtle (e.g., point mutations) or may involve segments of chromosomes large enough to be detected in a routine karyotype. Activation of oncogenes and loss of function of tumor suppressor genes by mutations were discussed earlier in this chapter. Here we discuss chromosomal abnormalities. We end this section by discussing the epigenetic changes that contribute to carcinogenesis.

Chromosomal Changes

In certain neoplasms, karyotypic abnormalities are nonrandom and common. Specific chromosomal abnormalities have been identified in most leukemias and lymphomas, many sar-

TABLE 7-9 Selected Examples of Oncogenes Activated by Translocation

Malignancy	Translocation	Affected Genes*
Chronic myeloid leukemia	(9;22)(q34;q11)	**ABL** 9q34 *BCR* 22q11
Acute leukemias (AML and ALL)	(8;21)(q22;q22)	*AML* 8q22 **ETO** 21q22
	(15;17)(q22;q21)	**PML** 15q22 **RARA** 17q21
Burkitt lymphoma	(8;14)(q24;q32)	c-*MYC* 8q24 **IGH** 14q32
Mantle cell lymphoma	(11;14)(q13;q32)	*CCND1* 11q13 **IGH** 14q32
Follicular lymphoma	(14;18)(q32;q21)	**IGH** 14q32 *BCL2* 18q21
T-cell ALL	(10;14)(q24;q11)	*HOX 11* 10q24 **TCRA** 14q11
Ewing sarcoma	(11;22)(q24;q12)	*FLI1* 11q24 **EWSR1** 22q12
Prostatic adenocarcinoma	(21;21)(q22;q22)	*TMPRSS2* (21q22.3)
	(7:21)(p22;q22)	*ERG* (21q22.2)
	(17:21)(p21;q22)	*ETV1* (7p21.2) *ETV4* (17q21)

*Genes in boldface are involved in multiple translocations.
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia.

comas, and an increasing number of carcinomas. In addition, whole chromosomes may be gained or lost. Although changes in chromosome number (aneuploidy) and structure are generally considered to be late phenomena in cancer progression, it has been suggested that aneuploidy and chromosomal instability may be the initiating events in tumor growth.

The study of chromosomal changes in tumor cells is important on two accounts. First, molecular cloning of genes in the vicinity of chromosomal breakpoints or deletions has been extremely useful in identification of oncogenes (e.g., *BCL2*, *ABL*) and tumor suppressor genes (e.g., *APC*, *RB*). Second, certain karyotypic abnormalities are specific enough to be of diagnostic value, and in some cases they are predictive of clinical course. The translocations associated with the *ABL* oncogene in CML and with c-*MYC* in Burkitt lymphoma have been mentioned earlier, in the context of molecular defects in cancer cells (see Fig. 7–27). Several other karyotype alterations in cancer cells are presented in the discussion of specific tumors in later chapters.

Two types of chromosomal rearrangements can activate proto-oncogenes—translocations and inversions. Chromosomal translocations are much more common (Table 7–9) and are discussed here. Translocations can activate proto-oncogenes in two ways:

- In lymphoid tumors specific translocations result in overexpression of proto-oncogenes by swapping their regulatory elements with those of another gene.
- In many hematopoietic tumors, sarcomas, and certain carcinomas, the translocations allow normally unrelated sequences from two different chromosomes to recombine and form hybrid fusion genes that encode chimeric proteins that variously promote growth and survival, or enhance self-renewal and block differentiation.

Overexpression of a proto-oncogene caused by translocation is best exemplified by Burkitt lymphoma. All such tumors carry one of three translocations, each involving chromosome 8q24, where the *MYC* gene has been mapped, as well as one of the three immunoglobulin gene–carrying chromosomes. At its normal locus, *MYC* is tightly controlled, and is most highly expressed in actively dividing cells. In Burkitt lymphoma the most common form of translocation results in the movement of the *MYC*-containing segment of chromosome 8 to chromosome 14q32 (see Fig. 7–27), placing it close to the *IGH* gene. The genetic notation for the translocation is t(8:14)(q24;q32). The molecular mechanisms of the translocation-associated activation of *MYC* are variable, as are the precise breakpoints within the gene. In most cases the translocation causes mutation or loss of the regulatory sequences of the *MYC* gene, replacing them with the control regions of the *IGH* locus, which is highly expressed in B-cell precursors. As the coding sequences remain intact, the gene is constitutively expressed at high levels. The invariable presence of the translocated *MYC* gene in Burkitt lymphomas attests to the importance of *MYC* overactivity in the pathogenesis of this tumor.

There are other examples of oncogenes translocated to antigen receptor loci in lymphoid tumors. As mentioned earlier, in mantle cell lymphoma the cyclin D1 gene (*CCND1*) on chromosome 11q13 is overexpressed by juxtaposition to the *IGH* locus on 14q32. In follicular lymphomas, a t(14;18)(q32;q21) translocation, the most common translocation in lymphoid malignancies, causes activation of the *BCL2* gene. Not unexpectedly, all these tumors in which the immunoglobulin gene is involved are of B-cell origin. In an analogous situation, overexpression of several proto-oncogenes in T-cell tumors results from translocations of oncogenes into the T-cell antigen receptor locus. The affected oncogenes are diverse, but in most cases, as with *MYC*, they encode nuclear transcription factors.

The Philadelphia chromosome, characteristic of CML and a subset of acute lymphoblastic leukemias, provides the prototypic example of an oncogene formed by *fusion of two separate genes*. In these cases, a reciprocal translocation between chromosomes 9 and 22 relocates a truncated portion of the proto-oncogene c-*ABL* (from chromosome 9) to the *BCR* (breakpoint cluster region) on chromosome 22 (see Fig. 7–27). The hybrid fusion gene *BCR-ABL* encodes a chimeric protein that has constitutive tyrosine kinase activity. As mentioned, BCR-ABL tyrosine kinase has served as a target for leukemia therapy, with remarkable success so far. Although the translocations are cytogenetically identical in CML and acute lymphoblastic leukemias, they usually differ at the molecular level. In most cases of CML the chimeric protein has a molecular weight of 210 kD, whereas in the more aggressive acute leukemias a 190-kD BCR-ABL fusion protein is typically formed.[48,49]

Transcription factors are often the partners in gene fusions occurring in cancer cells. For instance, the *MLL* (myeloid, lymphoid leukemia) gene on 11q23, which itself is a component of a chromatin-remodeling complex, is known to be involved in 50 different translocations with several different partner genes, some of which encode transcription factors (see

Table 7–9). Ewing sarcoma/primitive neuroectodermal tumor (PNET) is defined by translocation of the Ewing sarcoma (*EWSR1*) gene at 22q12, which is involved in numerous translocations, and all of its partner genes analyzed so far also encode a transcription factor. In Ewing sarcoma/PNET, for example, the *EWSR1* gene fuses with the *FLI1* gene, also a member of the ETS transcription factor family; the resultant chimeric EWS-FLI1 protein has transforming ability. One might ask, why are particular translocations so strongly associated with specific tumors? This is incompletely understood, but one recurrent theme is that at least one of the affected genes often encodes a transcription factor that is required for the development and differentiation of normal cells of the same lineage as the tumor. For example, in acute leukemias many genes involved by recurrent translocations (such as *MLL*) play essential roles in regulating the self-renewal of hematopoietic stem cells and the normal differentiation of lymphoid and myeloid cells. The fusion proteins resulting from translocations most often inhibit, but occasionally increase, transcriptional function. Until recently, most known translocations were discovered in leukemias/lymphomas and sarcomas; few common translocations had been identified in carcinomas, even though carcinomas are more common. The complex karyotypes of most carcinomas have made identifying translocations difficult. Recently, however, a translocation involving an androgen-regulated gene, *TMPRSS2* (21q22), and one of three ETS family transcription factors (*ERG* [21q22], *ETV1* [7p22.2], or *ETV4* [17q21]) was found to be present in 50% or more prostate adenocarcinomas.[137,138] Development of this translocation seems to occur early in carcinogenesis, in that it is also present in high-grade prostatic intraepithelial neoplasia, a precursor lesion. Although the mechanism by which this translocation causes cancer is not completely understood, it removes the ETS family gene from its normal control region and fuses it to the androgen-regulated *TMPRSS2*. Thus, the ETS family transcription factor is inappropriately expressed in prostate cells, and as noted above with Ewing sarcoma, when ETS proteins are inappropriately expressed they have transforming ability. There is significant interest in identifying additional fusion genes in other carcinomas. Many fusion genes are thought to be initiators in carcinogenesis, and it is postulated that many cancers may be "addicted" to their properties, similar to the oncogene addiction seen in CML with the BCR-ABL fusion. Thus, inhibition of these genes may provide an avenue for targeted therapy.

Deletions. Chromosomal deletions are the second most prevalent structural abnormality in tumor cells. *Compared with translocations, deletions are more common in nonhematopoietic solid tumors.* Deletion of specific regions of chromosomes is associated with the loss of particular tumor suppressor genes. As discussed, deletions involving chromosome 13q14, the site of the *RB* gene, are associated with retinoblastoma. Deletions of 17p, 5q, and 18q have all been noted in colorectal cancers; these regions harbor three tumor suppressor genes. Deletion of 3p, noted in several tumors, is extremely common in small-cell lung carcinomas, and the hunt is on for one or more cancer suppressor genes at this locale.

Gene Amplification

Activation of proto-oncogenes associated with overexpression of their products may result from reduplication and *amplification* of their DNA sequences.[139] Such amplification may produce several hundred copies of the proto-oncogene in the tumor cell. The amplified genes can be readily detected by molecular hybridization with appropriate DNA probes. In some cases the amplified genes produce chromosomal changes that can be identified microscopically. Two mutually exclusive patterns are seen: multiple small, centric structures called *double minutes* and *homogeneous staining regions*. The latter derive from the insertion of the amplified genes into new chromosomal locations, which may be distant from the normal location of the involved genes; because regions containing amplified genes lack a normal banding pattern, they appear homogeneous in a G-banded karyotype (see Fig. 7–28). The most interesting cases of amplification involve N-*MYC* in neuroblastoma and *ERBB2* in breast cancers. N-*MYC* is amplified in 25% to 30% of neuroblastomas, and the amplification is associated with poor prognosis. In neuroblastomas with N-*MYC* amplification, the gene is present both in double minutes and homogeneous staining regions. *ERBB2* amplification occurs in about 20% of breast cancers, and antibody therapy directed against this receptor has proven effective in this subset of tumors. Amplification of C-*MYC*, L-*MYC*, or N-*MYC* correlates with disease progression in small-cell cancer of the lung.

Epigenetic Changes

Epigenetics refers to reversible, heritable changes in gene expression that occur without mutation. Such changes involve post-translational modifications of histones and DNA methylation, both of which affect gene expression. In normal, differentiated cells, the majority of the genome is not expressed. Some portions of the genome are silenced by DNA methylation and histone modifications that lead to the compaction of DNA into heterochromatin. On the other hand, cancer cells are characterized by a global DNA hypomethylation and selective promoter-localized hypermethylation.[140] Indeed, it has become evident during the past few years that tumor suppressor genes are sometimes silenced by hypermethylation of promoter sequences rather than mutation. One example is CDKN2A, a complex locus that encodes two tumor suppressors, p14/ARF and p16/INK4a from two different reading frames; p14/ARF is epigenetically silenced in colon and gastric cancers, while p16/INK4a is silenced in a wide variety of cancers. Since this locus produces two tumor suppressors which affect the p53 and Rb pathways, silencing this locus has the pleasing effect (from the cancer's point of view) of removing two checkpoints with a single alteration. Other tumor suppressor genes subject to silencing by methylation include *BRCA1* in breast cancer, *VHL* in renal cell carcinomas, and the *MLH1* mismatch-repair gene in colorectal cancer.[140] You will recall from Chapter 5 that methylation also participates in the phenomenon called *genomic imprinting*, in which the maternal or paternal allele of a gene or chromosome is modified by methylation and is inactivated. The reverse phenomenon—that is, demethylation of an imprinted gene leading to its biallelic expression (loss of imprinting)—can also occur in tumor cells.[141] There has been great interest in developing potential therapeutic agents that act to demethylate DNA sequences in tumor suppressor genes. Recent data demonstrating that genomic hypomethylation causes chromosomal instability and induces tumors in mice greatly strengthen the notion that epigenetic changes may directly contribute to tumor development.[141]

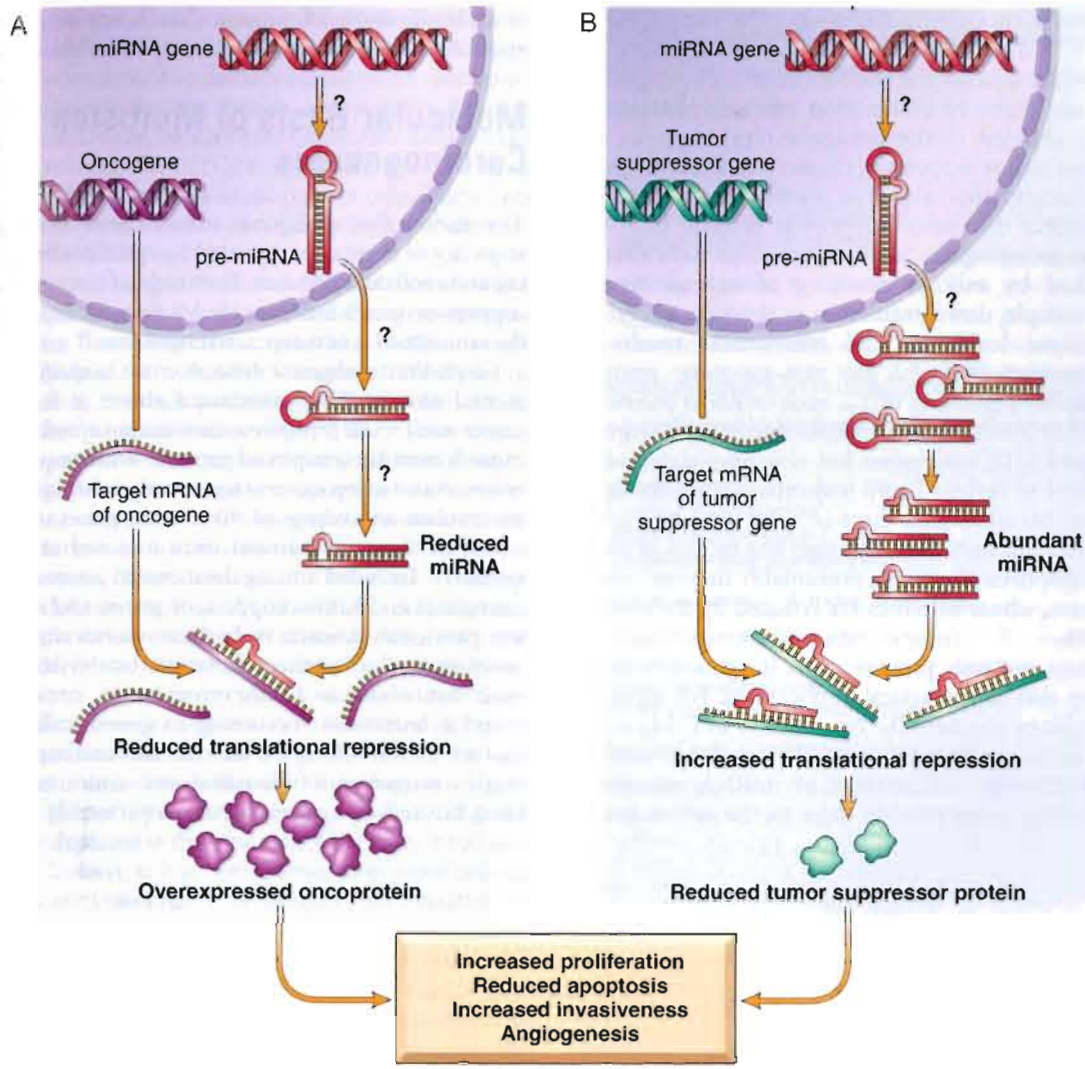


FIGURE 7–39 Role of miRNAs in tumorigenesis. **A,** Reduced activity of a miRNA that inhibits translation of an oncogene gives rise to an excess of oncoproteins. **B,** Overactivity of a miRNA that targets a tumor suppression gene reduces the production of the tumor suppressor protein. Question marks in **A** and **B** indicate that the mechanisms by which changes in the level or activity of miRNA are not entirely known.

The chromatin changes that contribute to carcinogenesis are less well understood. The current paradigm is that there is a histone code in which various modifications to the tails of histones, such as acetylation and methylation, lead to activation or repression of transcription. Several chromatin-modifying enzymes, such as EZH2, have been shown to be overexpressed in breast and prostate carcinomas.[141] EZH2 is the enzymatic component of the multiprotein polycomb repressive complex 2, which places repressive chromatin marks at the promoter of genes. Although its targets in cancer in vivo have not yet been defined, in cell lines overexpression of EZH2 leads to the repression of tumor suppressors, such as p21. Interestingly, in flies and mammals the polycomb repressive complexes are required for the maintenance of stem cells, as well as to silence lineage-specific transcription factors until the proper cues signal differentiation. Inappropriate repression or expression of such genes could give cancer cells a stem cell–like, undifferentiated quality. There is, of course, significant cross-talk between the chromatin-remodeling enzymes and the DNA-methylation machinery. For example, the placement of repressive chromatin marks by enzymes like EZH2 in cancer cells results in the recruitment of DNA methylases, methylation of promoters, and durable repression of gene expression.

miRNAs and Cancer

As discussed in Chapter 5, miRNAs are small noncoding, single-stranded RNAs, approximately 22 nucleotides in length, that are incorporated into the RNA-induced silencing complex. The miRNAs mediate sequence-specific recognition of mRNAs and, through the action of the RNA-induced silencing complex, mediate post-transcriptional gene silencing. Given that miRNAs control cell growth, differentiation, and cell survival, it is not surprising that they play a role in carcinogenesis.[142] miRNAs have been shown to undergo changes in expression in cancer cells, and frequent amplifications and deletions of miRNA loci have been identified in many cancers. As illustrated in Figure 7–39, miRNAs can participate in neo-

plastic transformation either by increasing the expression of oncogenes or by reducing the expression of tumor suppressor genes. If a miRNA inhibits the translation of an oncogene, a reduction in the quantity or function of that miRNA will lead to overproduction of the oncogene product; thus, the miRNA acts as a tumor suppressor. Conversely, if the target of a miRNA is a tumor suppressor gene, then overactivity of the miRNA can reduce the tumor suppressor protein; thus, the miRNA acts as an oncogene. Such relationships have already been established by miRNA profiling of several human tumors. For example, down-regulation or deletion of certain miRNAs in some leukemias and lymphomas results in increased expression of BCL2, the anti-apoptotic protein. Thus, by negatively regulating BCL2, such miRNAs behave as tumor suppressor genes. Similar miRNA-mediated upregulation of *RAS* and *MYC* oncogenes has also been detected in lung tumors and in certain B-cell leukemias, respectively. In some brain and breast tumors there is 5- to 100-fold greater expression of certain miRNAs. Although the targets of these miRNAs have not been identified, presumably they are tumor suppressor genes, whose activities are reduced by the overexpressed miRNA.

These findings not only provide novel insights into carcinogenesis, they also have practical implications. For instance, drugs that inhibit or augment the functions of miRNAs could be useful in chemotherapy. Since miRNAs regulate normal cellular differentiation, the patterns of miRNA expression ("miRNA profiling") can provide clues to the cell of origin and classification of tumors. Much remains to be learned about these oncogenic miRNAs, or so called "oncomirs."

Molecular Basis of Multistep Carcinogenesis

The notion that malignant tumors arise from a protracted sequence of events is supported by epidemiologic, experimental, and molecular studies. The study of oncogenes and tumor suppressor genes has provided a firm molecular footing for the concept of multistep carcinogenesis.[143]

Given that malignant tumors must acquire several fundamental abnormalities, discussed above, it follows that *each cancer must result from the accumulation of multiple mutations.* Indeed, recently completed genome-wide sequencing analysis of breast and colon cancers has revealed that individual tumors accumulate an average of 90 mutant genes. A much smaller subset of these (11/tumor) were mutated at significant frequency.[144] Included among the mutated genes are some known oncogenes and tumor suppressor genes, and others that were not previously known to be tumor-associated. It is not yet established which of these mutations establish the transformed state, contribute to tumor progression, or are "passengers" (neutral mutations) occurring in genomically unstable cells that are merely "along for the ride". More directly, however, no single oncogene can fully transform nonimmortalized cells in vitro, but *cells can generally be transformed by combinations of*

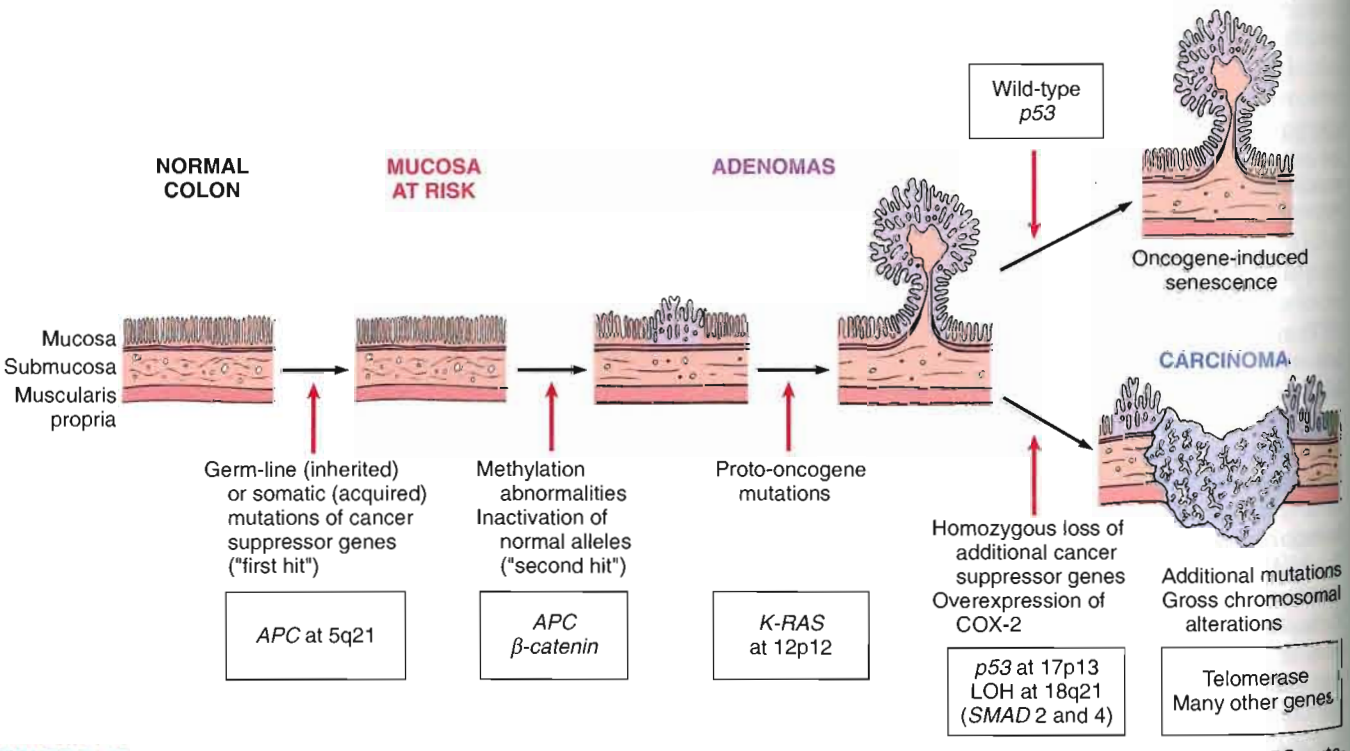


FIGURE 7–40 Molecular model for the evolution of colorectal cancers through the adenoma-carcinoma sequence. Although *APC* mutation is an early event and loss of *p53* occurs late in the process of tumorigenesis, the timing for the other changes may be variable. Note also that individual tumors may not have all of the changes listed. *Top right,* cells that gain oncogene signaling without loss of p53 eventually enter oncogene-induced senescence.

oncogenes. Such cooperation is required because each oncogene is specialized to induce part of the phenotype necessary for full transformation. For instance, the *RAS* oncogene induces cells to secrete growth factors and permits them to grow without anchorage to a normal substrate (anchorage independence), whereas the *MYC* oncogene renders cells more sensitive to growth factors and immortalizes cells. These two genes, acting in conjunction, can cause neoplastic transformation of mouse fibroblasts in culture.

Furthermore, it seems that evolution has installed a variety of "intrinsic tumor-suppressive mechanisms" such as apoptosis and senescence that thwart the actions of growth-promoting mutations. Indeed, in cells with competent checkpoints, oncogenic signaling through proteins like RAS leads not to transformation but to senescence or apoptosis.[33] Thus, emergence of malignant tumors requires mutational loss of many genes, including those that regulate apoptosis and senescence.[145] A classic example of incremental acquisition of the malignant phenotype is documented by the study of colon carcinoma. Many of these cancers are believed to evolve through a series of morphologically identifiable stages: colon epithelial hyperplasia followed by formation of adenomas that progressively enlarge and ultimately undergo malignant transformation (Chapter 17). The proposed molecular correlates of this adenoma-carcinoma sequence are illustrated in Figure 7–40. According to this scheme, inactivation of the *APC* tumor suppressor gene occurs first, followed by activation of *RAS* and, ultimately, loss of a tumor suppressor gene on 18q and loss of *p53*. Also depicted is the senescence pathway if *p53* loss does not occur. Indeed, it has been shown that most cells in most adenomas are senescent. It is thought that mutation of a proto-oncogene such as *RAS* drives a cell into senescence instead of proliferation[33] by activating the DNA-damage checkpoint, as discussed previously. The loss of p53 in adenomas prevents oncogene-induced senescence, allowing the adenomatous cells to continue to proliferate, generating a carcinoma. While multiple mutations, including gain of oncogenes and loss of tumor suppressors, are required for carcinogenesis, the precise temporal sequence of mutations may be different in each organ and tumor type.

Carcinogenic Agents and Their Cellular Interactions

More than 200 years ago the London surgeon Sir Percival Pott correctly attributed scrotal skin cancer in chimney sweeps to chronic exposure to soot. Based on this observation, the Danish Chimney Sweeps Guild ruled that its members must bathe daily. No public health measure since that time has achieved so much in the control of a form of cancer. Subsequently, hundreds of chemicals have been shown to be carcinogenic in animals.

Some of the major agents are presented in Table 7–10. A few comments are offered on a handful of these.

Steps Involved in Chemical Carcinogenesis

As discussed earlier, carcinogenesis is a multistep process. This is most readily demonstrated in experimental models of chemical carcinogenesis, in which the stages of initiation and progression during cancer development were first described.[146] The classic experiments that allowed the distinction between initiation and promotion were performed on mouse skin and are outlined in Figure 7–41. The following concepts relating to the initiation-promotion sequence have emerged from these experiments:

- *Initiation* results from exposure of cells to a sufficient dose of a carcinogenic agent (initiator); an initiated cell is altered, making it potentially capable of giving rise to a tumor (groups 2 and 3). *Initiation alone, however, is not sufficient for tumor formation* (group 1).
- *Initiation causes permanent DNA damage (mutations). It is therefore rapid and irreversible and has "memory."* This is illustrated by group 3, in which tumors were produced even if the application of the promoting agent was delayed for several months after a single application of the initiator.
- *Promoters can induce tumors in initiated cells, but they are nontumorigenic by themselves* (group 5). Furthermore, tumors do not result when the promoting agent is applied

TABLE 7–10 Major Chemical Carcinogens

DIRECT-ACTING CARCINOGENS

Alkylating Agents

β-Propiolactone
Dimethyl sulfate
Diepoxybutane
Anticancer drugs (cyclophosphamide, chlorambucil, nitrosoureas, and others)

Acylating Agents

1-Acetyl-imidazole
Dimethylcarbamyl chloride

PROCARCINOGENS THAT REQUIRE METABOLIC ACTIVATION

Polycyclic and Heterocyclic Aromatic Hydrocarbons

Benz[*a*]anthracene
Benzo[*a*]pyrene
Dibenz[*a,h*]anthracene
3-Methylcholanthrene
7,12-Dimethylbenz[*a*]anthracene

Aromatic Amines, Amides, Azo Dyes

2-Naphthylamine (β-naphthylamine)
Benzidine
2-Acetylaminofluorene
Dimethylaminoazobenzene (butter yellow)

Natural Plant and Microbial Products

Aflatoxin B_1
Griseofulvin
Cycasin
Safrole
Betel nuts

Others

Nitrosamine and amides
Vinyl chloride, nickel, chromium
Insecticides, fungicides
Polychlorinated biphenyls

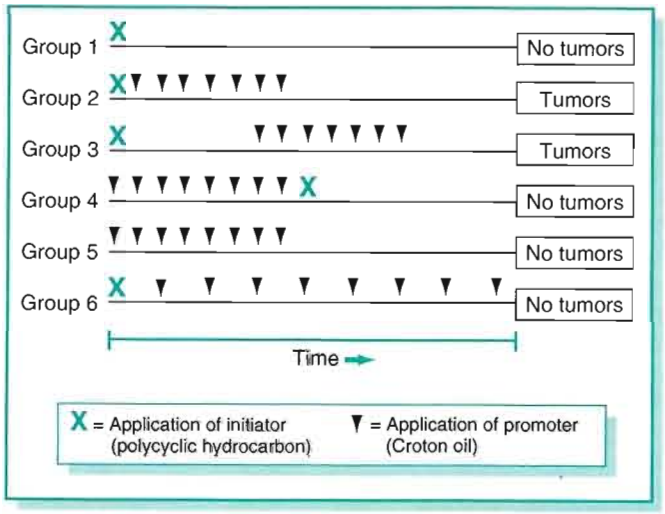


FIGURE 7–41 Experiments demonstrating the initiation and promotion phases of carcinogenesis in mice. Group 2: application of promoter repeated at twice-weekly intervals for several months. Group 3: application of promoter delayed for several months and then applied twice weekly. Group 6: promoter applied at monthly intervals.

before, rather than after, the initiating agent (group 4). This indicates that, *in contrast to the effects of initiators, the cellular changes resulting from the application of promoters do not affect DNA directly and are reversible.* As discussed later, promoters enhance the proliferation of initiated cells, an effect that may contribute to the development of additional mutations in these cells. That the effects of promoters are reversible is further documented in group 6, in which tumors failed to develop in initiated cells if the time between multiple applications of the promoter was sufficiently extended.

Although the concepts of initiation and promotion have been derived largely from experiments involving induction of skin cancer in mice, these stages are also discernible in the development of cancers of the liver, urinary bladder, breast, colon, and respiratory tract. With this brief overview of two major steps in carcinogenesis, we can examine initiation and promotion in more detail (Fig. 7–42). All initiating chemical carcinogens are highly reactive electrophiles (have electron-deficient atoms) that can react with nucleophilic (electron-rich) sites in the cell. Their targets are DNA, RNA, and proteins, and in some cases these interactions cause cell death. Initiation, obviously, inflicts nonlethal damage on the DNA that cannot be repaired. The mutated cell then passes on the DNA lesions to its daughter cells. Chemicals that can cause initiation of carcinogenesis can be classified into two categories: direct acting and indirect acting.

Direct-Acting Agents

Direct-acting agents require no metabolic conversion to become carcinogenic. Most of them are weak carcinogens but are important because some are cancer chemotherapeutic drugs (e.g., alkylating agents) that have successfully cured, controlled, or delayed recurrence of certain types of cancer (e.g., leukemia, lymphoma, and ovarian carcinoma), only to evoke later a second form of cancer, usually acute myeloid leukemia. The risk of induced cancer is low, but its existence dictates judicious use of such agents.

Indirect-Acting Agents

The designation *indirect-acting agent* refers to chemicals that require metabolic conversion to an *ultimate carcinogen* before they become active. Some of the most potent indirect chemical carcinogens—the polycyclic hydrocarbons—are present in fossil fuels. Others, for example, benzo[*a*]pyrene and other carcinogens, are formed in the high-temperature combustion of tobacco in cigarette smoking. *These products are implicated in the causation of lung cancer in cigarette smokers.* Polycyclic hydrocarbons may also be produced from animal fats during the process of broiling meats and are present in smoked meats and fish. The principal active products in many hydrocarbons are epoxides, which form covalent adducts (addition products) with molecules in the cell, principally DNA, but also with RNA and proteins.

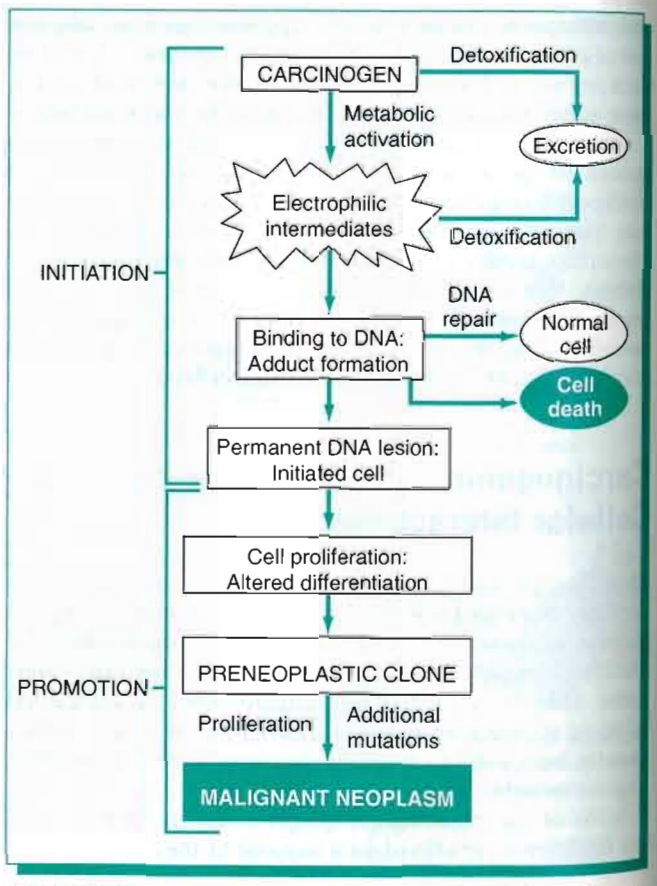


FIGURE 7–42 General schema of events in chemical carcinogenesis. Note that promoters cause clonal expansion of the initiated cell, thus producing a preneoplastic clone. Further proliferation induced by the promoter or other factors causes accumulation of additional mutations and emergence of a malignant tumor.

The aromatic amines and azo dyes are another class of indirect-acting carcinogens that were widely used in the past in the aniline dye and rubber industries.[147] Many other occupational carcinogens are listed in Table 7–3.

Most chemical carcinogens require metabolic activation for conversion into ultimate carcinogens (see Fig. 7–42). Other metabolic pathways may lead to the inactivation (detoxification) of the procarcinogen or its derivatives. Thus, *the carcinogenic potency of a chemical is determined not only by the inherent reactivity of its electrophilic derivative but also by the balance between metabolic activation and inactivation reactions.*

Most of the known carcinogens are metabolized by cytochrome P-450–dependent mono-oxygenases. The genes that encode these enzymes are quite polymorphic, and the activity and inducibility of these enzymes have been shown to vary among different individuals. Because these enzymes are essential for the activation of procarcinogens, the susceptibility to carcinogenesis is regulated in part by polymorphisms in the genes that encode these enzymes. Thus, it may be possible to assess cancer risk in a given individual by genetic analysis of such enzyme polymorphisms.[147]

The metabolism of polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene by the product of the P-450 gene, *CYP1A1*, provides an instructive example. Approximately 10% of the white population has a highly inducible form of this enzyme that is associated with an increased risk of lung cancer in smokers.[148,149] Light smokers with the susceptible genotype *CYP1A1* have a sevenfold higher risk of developing lung cancer, compared with smokers without the permissive genotype. Not all variations in the activation or detoxification of carcinogens are genetically determined. Age, sex, and nutritional status also determine the internal dose of toxicants produced and hence influence the risk of cancer development in a particular individual.[150]

Molecular Targets of Chemical Carcinogens. Because malignant transformation results from mutations, it comes as no surprise that the majority of initiating chemicals are mutagenic. Thus, DNA is the primary target for chemical carcinogens, but there is no single or unique alteration associated with initiation of chemical carcinogenesis. Although any gene may be the target of chemical carcinogens, the commonly mutated oncogenes and tumor suppressors, such as *RAS* and *p53*, are particularly important targets. An illustrative example of a chemical carcinogenesis is aflatoxin B_1, a naturally occurring agent produced by some strains of *Aspergillus*, a mold that grows on improperly stored grains and nuts. There is a *strong correlation between the dietary level of this food contaminant and the incidence of hepatocellular carcinoma in parts of Africa and the Far East.* Interestingly, aflatoxin B_1 produces mutations in the *p53* gene; 90% or more of these mutations are a characteristic G:C→T:A transversion in codon 249 (called *249(ser) p53* mutation).[151] By contrast, *p53* mutations are much less frequent in liver tumors from areas where aflatoxin contamination of food is not a risk factor, and the *249(ser)* mutation is uncommon. Thus, the detection of the "*signature mutation*" within the *p53* gene establishes aflatoxin as the causative agent. These associations are proving to be useful tools in epidemiologic studies of chemical carcinogenesis.

Additionally, vinyl chloride, arsenic, nickel, chromium, insecticides, fungicides, and polychlorinated biphenyls are potential carcinogens in the workplace and at home. Finally, nitrites used as food preservatives have caused concern, since they cause nitrosylation of amines contained in the food. The nitrosoamines so formed are suspected to be carcinogenic.

Initiation and Promotion of Chemical Carcinogenesis

Unrepaired alterations in the DNA are essential first steps in the process of initiation. *For the change to be heritable, the damaged DNA template must be replicated. Thus, for initiation to occur, carcinogen-altered cells must undergo at least one cycle of proliferation so that the change in DNA becomes fixed.* In the liver, many chemicals are activated to reactive electrophiles, yet most of them do not produce cancers unless the liver cells proliferate within a few days of the formation of DNA adducts. In tissues that are normally quiescent, the mitogenic stimulus may be provided by the carcinogen itself, because many cells die as a result of toxic effects of the carcinogenic chemical, thereby stimulating regeneration in the surviving cells. Alternatively, cell proliferation may be induced by concurrent exposure to biologic agents such as viruses and parasites, dietary factors, or hormonal influences. Agents that do not cause mutation but instead stimulate the division of mutated cells are known as *promoters.*

The carcinogenicity of some initiators is augmented by subsequent administration of *promoters* (such as phorbol esters, hormones, phenols, and drugs) that by themselves are nontumorigenic. Application of promoters leads to proliferation and clonal expansion of initiated (mutated) cells. Such cells have reduced growth factor requirements and may also be less responsive to growth-inhibitory signals in their extracellular milieu. Driven to proliferate, the initiated clone of cells suffers additional mutations, developing eventually into a malignant tumor. Thus, the process of tumor promotion includes multiple steps: proliferation of preneoplastic cells, malignant conversion, and eventually tumor progression, which depends on changes in tumor cells and the tumor stroma—the process of multistep carcinogenesis highlighted above.

RADIATION CARCINOGENESIS

Radiant energy, whether in the form of the UV rays of sunlight or as ionizing electromagnetic and particulate radiation, is a well-established carcinogen. UV light is clearly implicated in the causation of skin cancers, and ionizing radiation exposure from medical or occupational exposure, nuclear plant accidents, and atomic bomb detonations has produced a variety of cancers. Although the contribution of radiation to the total human burden of cancer is probably small, the well-known latency of damage caused by radiant energy and its cumulative effect require extremely long periods of observation and make it difficult to ascertain its full significance. An increased incidence of breast cancer has become apparent decades later among women exposed during childhood to atomic bomb tests. The incidence peaked during 1988–1992 and then declined.[152] Moreover, radiation's possible additive or synergistic effects with other potential carcinogenic influences add another dimension to the picture.

Ultraviolet Rays

There is ample evidence from epidemiologic studies that *UV rays* derived from the sun cause an increased incidence of squamous cell carcinoma, basal cell carcinoma, and possibly melanoma of the skin.[153] The degree of risk depends on the type of UV rays, the intensity of exposure, and the quantity of the light-absorbing "protective mantle" of melanin in the skin. Persons of European origin who have fair skin that repeatedly becomes sunburned but stalwartly refuses to tan and who live in locales receiving a great deal of sunlight (e.g., Queensland, Australia, close to the equator) have among the highest incidence of skin cancers (melanomas, squamous cell carcinomas, and basal cell carcinomas) in the world. Nonmelanoma skin cancers are associated with total cumulative exposure to UV radiation, whereas melanomas are associated with intense intermittent exposure—as occurs with sunbathing. The UV portion of the solar spectrum can be divided into three wavelength ranges: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). Of these, UVB is believed to be responsible for the induction of cutaneous cancers. UVC, although a potent mutagen, is not considered significant because it is filtered out by the ozone shield around the earth (hence the concern about ozone depletion).

The carcinogenicity of UVB light is attributed to its formation of pyrimidine dimers in DNA. This type of DNA damage is repaired by the nucleotide excision repair pathway. There are five steps in nucleotide excision repair, and in mammalian cells the process may involve 30 or more proteins. It is postulated that with excessive sun exposure, the capacity of the nucleotide excision repair pathway is overwhelmed, and error-prone nontemplated DNA-repair mechanisms become operative that provide for the survival of the cell at the cost of genomic mutations that in some instances, lead to cancer. The importance of the nucleotide excision repair pathway of DNA repair is most graphically illustrated by the high frequency of cancers in individuals with the hereditary disorder *xeroderma pigmentosum* (discussed previously).[126]

Ionizing Radiation

Electromagnetic (x-rays, γ rays) and particulate (α particles, β particles, protons, neutrons) radiations are all carcinogenic. The evidence is so voluminous that a few examples suffice.[152,154] Many individuals pioneering the use of x-rays developed skin cancers. Miners of radioactive elements in central Europe and the Rocky Mountain region of the United States have a tenfold increased incidence of lung cancers compared to the rest of the population. Most telling is the follow-up of survivors of the atomic bombs dropped on Hiroshima and Nagasaki. Initially there was a marked increase in the incidence of leukemias—principally acute and chronic myelogenous leukemia—after an average latent period of about 7 years. Subsequently the incidence of many solid tumors with longer latent periods (e.g., breast, colon, thyroid, and lung) increased.

In humans there is a hierarchy of vulnerability of different tissues to radiation-induced cancers. Most frequent are the acute and chronic myeloid leukemia. Cancer of the thyroid follows closely but only in the young. In the intermediate category are cancers of the breast, lungs, and salivary glands. In contrast, skin, bone, and the gastrointestinal tract are relatively resistant to radiation-induced neoplasia, even though the gastrointestinal epithelial cells are vulnerable to the acute cell-killing effects of radiation, and the skin is in the pathway of all external radiation. Nonetheless, the physician dare not forget: practically *any* cell can be transformed into a cancer cell by sufficient exposure to radiant energy.

MICROBIAL CARCINOGENESIS

Many RNA and DNA viruses have proved to be oncogenic in animals as disparate as frogs and primates. Despite intense scrutiny, however, only a few viruses have been linked with human cancer. Our discussion focuses on human oncogenic viruses as well as the emerging role of the bacterium *Helicobacter pylori* in gastric cancer.

Oncogenic RNA Viruses

Human T-Cell Leukemia Virus Type 1. Although the study of animal retroviruses has provided spectacular insights into the molecular basis of cancer, only one human retrovirus, human T-cell leukemia virus type 1 (HTLV-1), is firmly implicated in the causation of cancer in humans.

HTLV-1 causes a form of T-cell leukemia/lymphoma that is endemic in certain parts of Japan and the Caribbean basin but is found sporadically elsewhere, including the United States.[155] Similar to the human immunodeficiency virus, which causes acquired immunodeficiency syndrome (AIDS), HTLV-1 has tropism for CD4+ T cells, and hence this subset of T cells is the major target for neoplastic transformation. Human infection requires transmission of infected T cells via sexual intercourse, blood products, or breastfeeding. Leukemia develops in only 3% to 5% of the infected individuals after a long latent period of 40 to 60 years.

There is little doubt that HTLV-1 infection of T lymphocytes is necessary for leukemogenesis, but the molecular mechanisms of transformation are not entirely clear. In contrast to several murine retroviruses, HTLV-1 does not contain an oncogene, and no consistent integration next to a proto-oncogene has been discovered. In leukemic cells, however, viral integration shows a clonal pattern. In other words, although the site of viral integration in host chromosomes is random (the viral DNA is found at different locations in different cancers), the site of integration is identical within all cells of a given cancer. This would not occur if HTLV-1 were merely a passenger that infects cells after transformation. The HTLV-1 genome contains the *gag*, *pol*, *env*, and long-terminal-repeat regions typical of other retroviruses, but, in contrast to other leukemia viruses, it contains another region, referred to as *tax*. It seems that the secrets of its transforming activity are locked in the *tax* gene.[156] The product of this gene is essential for viral replication, because it stimulates transcription of viral mRNA by acting on the 5′ long terminal repeat. It is now established that the Tax protein can also activate the transcription of several host cell genes involved in proliferation and differentiation of T cells. These include the immediate early gene *FOS*, genes encoding interleukin-2 (IL-2) and its receptor, and the gene for the myeloid growth factor granulocyte-macrophage colony-stimulating factor. In addition, Tax

protein inactivates the cell cycle inhibitor p16/INK4a and enhances cyclin D activation, thus dysregulating the cell cycle. Tax also activates NF-κb, a transcription factor that regulates a host of genes, including pro-survival/anti-apoptotic genes. Another mechanism by which Tax may contribute to malignant transformation is through genomic instability. Recent data show that Tax interferes with DNA-repair functions and inhibits ATM-mediated cell cycle checkpoints activated by DNA damage.[156]

The main steps that lead to the development of adult T-cell leukemia/lymphoma may be summarized as follows. Infection by HTLV-1 causes the expansion of a nonmalignant polyclonal cell population through stimulatory effects of Tax on cell proliferation. The proliferating T cells are at increased risk of mutations and genomic instability induced by Tax. This instability allows the accumulation of mutations and chromosomal abnormalities, and eventually a monoclonal neoplastic T-cell population emerges. The malignant cells replicate independently of IL-2 and contain molecular and chromosomal abnormalities.

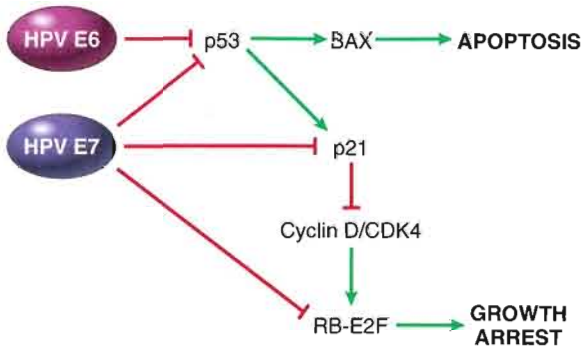


FIGURE 7–43 Effect of HPV proteins E6 and E7 on the cell cycle. E6 and E7 enhance p53 degradation, causing a block in apoptosis and decreased activity of the p21 cell cycle inhibitor. E7 associates with p21 and prevents its inhibition of the cyclin-CDK4 complex; E7 can bind to RB, removing cell cycle restriction. The net effect of HPV E6 and E7 proteins is to block apoptosis and remove the restraints to cell proliferation (see Fig. 7–29). (Modified from Münger K, Howley PM: Human papillomavirus immortalization and transformation functions. Virus Res 89:213–228, 2002.)

Oncogenic DNA Viruses

As with RNA viruses, several oncogenic DNA viruses that cause tumors in animals have been identified. Of the various human DNA viruses, four—HPV, Epstein-Barr virus (EBV), hepatitis B virus (HBV), and Kaposi sarcoma herpesvirus, also called human herpesvirus 8—have been implicated in the causation of human cancer. A fifth virus, Merkel cell polyomavirus, has been identified in Merkel cell carcinomas and may soon join the rogue's gallery; it is described in Chapter 25. Kaposi sarcoma herpesvirus is discussed in Chapters 6 and 11. Though not a DNA virus, HCV is also associated with cancer and is discussed briefly here.[157]

Human Papillomavirus. At least 70 genetically distinct types of HPV have been identified. Some types (e.g., 1, 2, 4, and 7) cause benign squamous papillomas (warts) in humans (Chapters 19 and 22). By contrast, high-risk HPVs (e.g., types 16 and 18) have been implicated in the genesis of several cancers, particularly squamous cell carcinoma of the cervix and anogenital region.[158,159] Thus, cervical cancer is a sexually transmitted disease, caused by transmission of HPV. In addition, at least 20% of oropharyngeal cancers are associated with HPV. In contrast to cervical cancers, genital warts have low malignant potential and are associated with low-risk HPVs, predominantly HPV-6 and HPV-11. Interestingly, in benign warts the HPV genome is maintained in a nonintegrated episomal form, while in cancers the HPV genome is integrated into the host genome, suggesting that integration of viral DNA is important for malignant transformation. As with HTLV-1, the site of viral integration in host chromosomes is random, but the pattern of integration is clonal. Cells in which the viral genome has integrated show significantly more genomic instability. Furthermore, since the integration site is random there is no consistent association with a host proto-oncogene. Rather, integration interrupts the viral DNA within the E1/E2 open reading frame, leading to loss of the E2 viral repressor and overexpression of the oncoproteins E6 and E7.

Indeed, the oncogenic potential of HPV can be related to the products of two viral genes, E6 and E7. Together, they interact with a variety of growth-regulating proteins encoded by proto-oncogenes and tumor suppressor genes (Fig. 7–43). The E7 protein binds to the RB protein and displaces the E2F transcription factors that are normally sequestered by RB, promoting progression through the cell cycle. Of note, E7 protein from high-risk HPV types has a higher affinity for RB than does E7 from low-risk HPV types. E7 also inactivates the CDKIs p21 and p27. E7 proteins from high-risk HPV types (types 16, 18, and 31) also bind and presumably activate cyclins E and A. The E6 protein has complementary effects. It binds to and mediates the degradation of p53 and BAX, a pro-apoptotic member of the BCL2 family, and it activates telomerase. Like E7, E6 from high-risk HPV types has a higher affinity for p53 than E6 from low-risk HPV types. Interestingly the E6-p53 interaction may offer some clues regarding polymorphisms and risk factors for development of cervical cancer. Human *p53* is polymorphic at amino acid 72, encoding either a proline or arginine residue at that position. The p53 Arg72 variant is much more susceptible to degradation by E6. Not surprisingly, infected individuals with the Arg72 polymorphism are more likely to develop cervical carcinomas.[160]

To summarize, *high-risk HPV types express oncogenic proteins that inactivate tumor suppressors, activate cyclins, inhibit apoptosis, and combat cellular senescence.* Thus, it is evident that many of the hallmarks of cancer discussed earlier are driven by HPV proteins. The primacy of HPV infection in the causation of cervical cancer is confirmed by the effectiveness of anti-HPV vaccines in preventing cervical cancer. However, infection with HPV itself is not sufficient for carcinogenesis. For example, when human keratinocytes are transfected with DNA from HPV types 16, 18, or 31 in vitro, they are immortalized but do not form tumors in experimental animals. Co-transfection with a mutated *RAS* gene results in full malignant transformation. In addition to such genetic co-factors, HPV in all likelihood also acts in concert with environmental factors (Chapter 22). These include cigarette smoking, coexisting microbial infections, dietary deficiencies, and hormonal changes, all of which have been implicated in the pathogenesis of cervical cancers. A high proportion of women infected with

HPV clear the infection by immunological mechanisms, but some do not for unknown reasons.

Epstein-Barr Virus. EBV, a member of the herpes family, has been implicated in the pathogenesis of several human tumors: the African form of Burkitt lymphoma; B-cell lymphomas in immunosuppressed individuals (particularly in those with HIV infection or undergoing immunosuppressive therapy after organ transplantation); a subset of Hodgkin lymphoma; nasopharyngeal and some gastric carcinomas and rare forms of T cell lymphomas and natural killer (NK) cell lymphomas.[161] Except for nasopharyngeal carcinoma, all others are B-cell tumors. These neoplasms are reviewed elsewhere in this book; therefore, only their association with EBV is discussed here.

EBV infects B lymphocytes and possibly epithelial cells of the oropharynx. EBV uses the complement receptor CD21 to attach to and infect B cells. The infection of B cells is latent; that is, there is no viral replication and the cells are not killed, but the B cells latently infected with EBV are immortalized and acquire the ability to propagate indefinitely in vitro. The molecular basis of B-cell proliferations induced by EBV is complex, but as with other viruses it involves the "hijacking" of several normal signaling pathways.[162] One EBV gene, latent membrane protein-1 (*LMP-1*), acts as an oncogene, in that its expression in transgenic mice induces B-cell lymphomas. LMP-1 behaves like a constitutively active CD40 receptor, a key recipient of helper T-cell signals that stimulate B-cell growth (Chapter 6). LMP-1 activates the NF-κB and JAK/STAT signaling pathways and promotes B-cell survival and proliferation, all of which occur autonomously (i.e., without T cells or other outside signals) in EBV-infected B cells. Concurrently, LMP-1 prevents apoptosis by activating BCL2. Thus, the virus "borrows" a normal B-cell activation pathway to expand the pool of latently infected cells. Another EBV gene, *EBNA-2*, encodes a nuclear protein that mimics a constitutively active Notch receptor. EBNA-2 transactivates several host genes, including cyclin D and the *src* family of proto-oncogenes. In addition, the EBV genome contains a viral cytokine, vIL-10, that was hijacked from the host genome. This viral cytokine can prevent macrophages and monocytes from activating T cells and is required for EBV-dependent transformation of B cells. In immunologically normal individuals EBV-driven polyclonal B-cell proliferation in vivo is readily controlled, and the individual either remains asymptomatic or develops a self-limited episode of infectious mononucleosis (Chapter 8). Evasion of the immune system seems to be a key step in EBV-related oncogenesis.

Burkitt lymphoma is a neoplasm of B lymphocytes that is the most common childhood tumor in central Africa and New Guinea. A morphologically identical lymphoma occurs sporadically throughout the world. The association between endemic Burkitt lymphoma and EBV is quite strong (Fig. 7–44):

- More than 90% of African tumors carry the EBV genome.
- One hundred percent of the patients have elevated antibody titers against viral capsid antigens.
- Serum antibody titers against viral capsid antigens are correlated with the risk of developing the tumor.

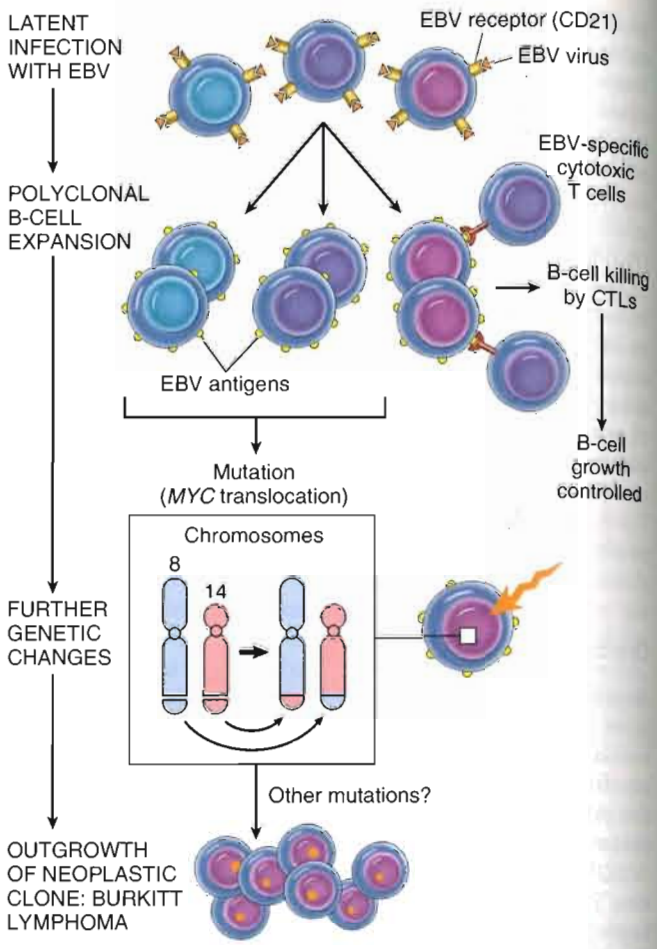


FIGURE 7–44 Possible evolution of EBV-induced Burkitt lymphoma.

Although EBV is intimately involved in the causation of Burkitt lymphoma, several observations suggest that additional factors must also be involved.[163,164] (1) EBV infection is not limited to the geographic locales where Burkitt lymphoma is found, but it is a ubiquitous virus that asymptomatically infects almost all humans worldwide. (2) The EBV genome is found in only 15% to 20% of sufferers of Burkitt lymphoma outside Africa. (3) There are significant differences in the patterns of viral gene expression in EBV-transformed (but not tumorigenic) B-cell lines and Burkitt lymphoma cells. Most notably, Burkitt lymphoma cells do not express LMP-1, EBNA2, and other EBV proteins that drive B-cell growth and immortalization.

Given these observations, how then does EBV contribute to the genesis of endemic Burkitt lymphoma? A plausible scenario is shown in Figure 7–44. In regions of the world where Burkitt lymphoma is endemic, concomitant infections such as malaria impair immune competence, allowing sustained B-cell proliferation. Eventually, however, T-cell immunity directed against EBV antigens such as EBNA2 and LMP-1 eliminates most of the EBV-infected B cells, but a small number of cells downregulate expression of these immunogenic antigens. These cells persist indefinitely, even in the face of normal immunity. Lymphoma cells may emerge from this

population only with the acquisition of specific mutations, most notably translocations that activate the c-*MYC* oncogene. It should be noted that in nonendemic areas 80% of tumors do not harbor the EBV genome, but all tumors possess the t(8;14) or other translocations that dysregulate c-*MYC*. This observation suggests that, although non-African Burkitt lymphomas are triggered by mechanisms other than EBV, they develop through very similar oncogenic pathways.

In summary, in the case of Burkitt lymphoma, it seems that EBV is not directly oncogenic, but by acting as a polyclonal B-cell mitogen, it sets the stage for the acquisition of the t(8;14) translocation and other mutations, which ultimately release the cells from normal growth regulation. In normal individuals, EBV infection is readily controlled by effective immune responses directed against viral antigens expressed on the cell membranes. Hence, the vast majority of infected individuals remain asymptomatic or develop self-limited infectious mononucleosis. In regions of Africa where Burkitt lymphoma is endemic, poorly understood cofactors (e.g., chronic malaria) may favor the acquisition of genetic events (e.g., the t(8;14) translocation) that lead to transformation.

The role played by EBV is more direct in B-cell lymphomas in immunosuppressed patients. Some persons with AIDS and those who receive long-term immunosuppressive therapy for preventing allograft rejection present with multifocal B-cell tumors within lymphoid tissue or in the central nervous system. These tumors are polyclonal at the outset but can develop into monoclonal neoplasms. In contrast to Burkitt lymphoma, the tumors in immunosuppressed patients uniformly express LMP-1 and EBNA2, that are recognized by cytotoxic T cells. These potentially lethal proliferations can be subdued if the immunological status of the host improves, as may occur with withdrawal of immunosuppressive drugs in transplant recipients.

Nasopharyngeal carcinoma is also associated with EBV infection. This tumor is endemic in southern China, in some parts of Africa, and in the Inuit population of the Arctic. In contrast to Burkitt lymphoma, 100% of nasopharyngeal carcinomas obtained from all parts of the world contain EBV DNA.[165] The viral integration in the host cells is clonal, thus ruling out the possibility that EBV infection occurred after tumor development. Antibody titers to viral capsid antigens are greatly elevated, and in endemic areas patients develop IgA antibodies before the appearance of the tumor. The 100% correlation between EBV and nasopharyngeal carcinoma suggests that EBV[110] plays a role in the genesis of this tumor, but (as with Burkitt tumor) the restricted geographic distribution indicates that genetic or environmental cofactors, or both, also contribute to tumor development. LMP-1 is expressed in epithelial cells as well. In these cells, as in B cells, *LMP-1* activates the NF-κB pathway. Furthermore, *LMP-1* induces the expression of pro-angiogenic factors such as VEGF, FGF-2, MMP9, and COX2, which may contribute to oncogenesis. The relationship of EBV to the pathogenesis of Hodgkin lymphoma is discussed in Chapter 13.

Hepatitis B and C Viruses. Epidemiologic studies strongly suggest a close association between HBV infection and the occurrence of liver cancer (Chapter 18). It is estimated that 70% to 85% of hepatocellular carcinomas worldwide are due to infection with HBV or HCV.[111,166–168] HBV is endemic in countries of the Far East and Africa; correspondingly, these areas have the highest incidence of hepatocellular carcinoma. Despite compelling epidemiologic and experimental evidence, the mode of action of these viruses in liver tumorigenesis is not fully elucidated. The HBV and HCV genomes do not encode any viral oncoproteins, and although the HBV DNA is integrated within the human genome, there is no consistent pattern of integration in liver cells. Indeed, the oncogenic effects of HBV and HCV are multifactorial, but the dominant effect seems to be immunologically mediated chronic inflammation with hepatocyte death leading to regeneration and genomic damage. Although the immune system is generally thought to be protective, recent work has demonstrated that in the setting of unresolved chronic inflammation, as occurs in viral hepatitis or chronic gastritis caused by *H. pylori* (see below), the immune response may become maladaptive, promoting tumorigenesis.

As with any cause of hepatocellular injury, chronic viral infection leads to the compensatory proliferation of hepatocytes. This regenerative process is aided and abetted by a plethora of growth factors, cytokines, chemokines, and other bioactive substances that are produced by activated immune cells and promote cell survival, tissue remodeling, and angiogenesis (Chapter 3). The activated immune cells also produce other mediators, such as reactive oxygen species, that are genotoxic and mutagenic. One key molecular step seems to be activation of the NF-κB pathway in hepatocytes in response to mediators derived from the activated immune cells. Activation of the NF-κB pathway within hepatocytes blocks apoptosis, allowing the dividing hepatocytes to incur genotoxic stress and to accumulate mutations. Although this seems to be the dominant mechanism in the pathogenesis of viral-induced hepatocellular carcinoma, both HBV and HCV also contain proteins within their genomes that may more directly promote the development of cancer. The HBV genome contains a gene known as *HBx* that can directly or indirectly activate a variety of transcription factors and several signal transduction pathways. In addition, viral integration can cause secondary rearrangements of chromosomes, including multiple deletions that may harbor unknown tumor suppressor genes.

Though not a DNA virus, HCV is also strongly linked to the pathogenesis of liver cancer. The molecular mechanisms used by HCV are less well defined than are those of HBV. In addition to chronic liver cell injury and compensatory regeneration, components of the HCV genome, such as the HCV core protein, may have a direct effect on tumorigenesis, possibly by activating a variety of growth-promoting signal transduction pathways.

Helicobacter pylori

First incriminated as a cause of peptic ulcers, *H. pylori* now has acquired the dubious distinction of being the first bacterium classified as a carcinogen. Indeed, *H. pylori* infection is implicated in the genesis of both gastric adenocarcinomas and gastric lymphomas.[169]

The scenario for the development of gastric adenocarcinoma is similar to that of HBV- and HCV-induced liver cancer. It involves increased epithelial cell proliferation in a background of chronic inflammation. As in viral hepatitis, the

inflammatory milieu contains numerous genotoxic agents, such as reactive oxygen species. There is an initial development of chronic gastritis, followed by gastric atrophy, intestinal metaplasia of the lining cells, dysplasia, and cancer. This sequence takes decades to complete and occurs in only 3% of infected patients. Like HBV and HCV, the *H. pylori* genome also contains genes directly implicated in oncogenesis. Strains associated with gastric adenocarcinoma have been shown to contain a "pathogenicity island" that contains cytotoxin-associated A (*CagA*) gene. Although *H. pylori* is noninvasive, *CagA* penetrates into gastric epithelial cells, where it has a variety of effects, including the initiation of a signaling cascade that mimics unregulated growth factor stimulation.

As mentioned above, *H. pylori* is associated with an increased risk for the development of gastric lymphomas as well. The gastric lymphomas are of B-cell origin, and because the tumors recapitulate some of the features of normal Peyer's patches, they are often called lymphomas of mucosa-associated lymphoid tissue, or MALTomas (also discussed in Chapters 13 and 17). Their molecular pathogenesis is incompletely understood but seems to involve strain-specific *H. pylori* factors, as well as host genetic factors, such as polymorphisms in the promoters of inflammatory cytokines such as IL-1β and tumor necrosis factor (TNF). It is thought that *H. pylori* infection leads to the appearance of *H. pylori*–reactive T cells, which in turn stimulate a polyclonal B-cell proliferation. In chronic infections, currently unknown mutations may be acquired that give individual cells a growth advantage. These cells grow out into a monoclonal "MALToma" that nevertheless remains dependent on T-cell stimulation of B-cell pathways that activate the transcription factor NF-κB. At this stage, eradication of *H. pylori* by antibiotic therapy "cures" the lymphoma by removing the antigenic stimulus for T cells. At later stages, however, additional mutations may be acquired, such as an (11;18) translocation, that cause NF-κB to be activated constitutively. At this point, the MALToma no longer requires the antigenic stimulus of the bacterium for growth and survival and develops the capacity to spread beyond the stomach to other tissues.

Host Defense against Tumors—Tumor Immunity

The idea that tumors are not entirely self and may be recognized by the immune system was conceived by Paul Ehrlich, who proposed that immune recognition of autologous tumor cells may be capable of eliminating tumors. Subsequently, Lewis Thomas and Macfarlane Burnet formalized this concept by coining the term *immune surveillance,* which implies that a normal function of the immune system is to survey the body for emerging malignant cells and destroy them.[170,171] This idea has been supported by many observations—the occurrence of lymphocytic infiltrates around tumors and in lymph nodes draining sites of cancer; experimental results, mostly with transplanted tumors; the increased incidence of some cancers in immunodeficient individuals; and the direct demonstration of tumor-specific T cells and antibodies in patients. The fact that cancers occur in immunocompetent individuals suggests that immune surveillance is imperfect; however, that some tumors escape such policing does not preclude the possibility that others may have been aborted.[172] The concept of tumor immune surveillance has recently been expanded to encompass not only the protective role of the immune system in tumor development but also the effect of the immune system in selecting for tumor variants.[173,174] These variants have reduced immunogenicity and can more easily escape immunological detection and rejection. The term *cancer immunoediting* is now being used to describe the effects of the immune system in preventing tumor formation and also in "sculpting" the immunogenic properties of tumors to select tumor cells that escape immune elimination.[175]

In the following section we explore some of the important questions about tumor immunity: What is the nature of tumor antigens? What host effector systems may recognize tumor cells? Is antitumor immunity effective against spontaneous neoplasms? Can immune reactions against tumors be exploited for immunotherapy?

TUMOR ANTIGENS

Antigens that elicit an immune response have been demonstrated in many experimentally induced tumors and in some human cancers.[176] Initially, they were broadly classified into two categories based on their patterns of expression: *tumor-specific antigens*, which are present only on tumor cells and not on any normal cells, and *tumor-associated antigens*, which are present on tumor cells and also on some normal cells. This classification, however, is imperfect because many antigens thought to be tumor-specific turned out to be expressed by some normal cells as well. The modern classification of tumor antigens is based on their molecular structure and source.

The early attempts to purify and characterize tumor antigens relied on producing monoclonal antibodies specific for tumor cells and defining the antigens that these antibodies recognized. An important advance in the field was the development of techniques for identifying tumor antigens that were recognized by cytotoxic T lymphocytes (CTLs), because CTLs are the major immune defense mechanism against tumors. Recall that CTLs recognize peptides derived from cytoplasmic proteins that are displayed bound to class I major histocompatibility complex (MHC) molecules (Chapter 6). Below we describe the main classes of tumor antigens (Fig. 7–45).

Products of Mutated Genes. Neoplastic transformation, as we have discussed, results from genetic alterations in proto-oncogenes and tumor suppressor genes; these mutated proteins represent antigens that have never been seen by the immune system and thus can be recognized as non-self.[177,178] Additionally, because of the genetic instability of tumor cells, many different genes may be mutated in these cells, including genes whose products are not related to the transformed phenotype and have no known function. Products of these mutated genes can also be potential tumor antigens. The products of altered proto-oncogenes, tumor suppressor genes, or other mutated genes not associated with transformation are synthesized in the cytoplasm of tumor cells, and like any cytoplasmic protein, they may enter the class I MHC antigen-processing pathway and be recognized by CD8+ T cells. In addition, these proteins may enter the class II antigen-processing pathway in antigen-presenting cells that have phagocy-

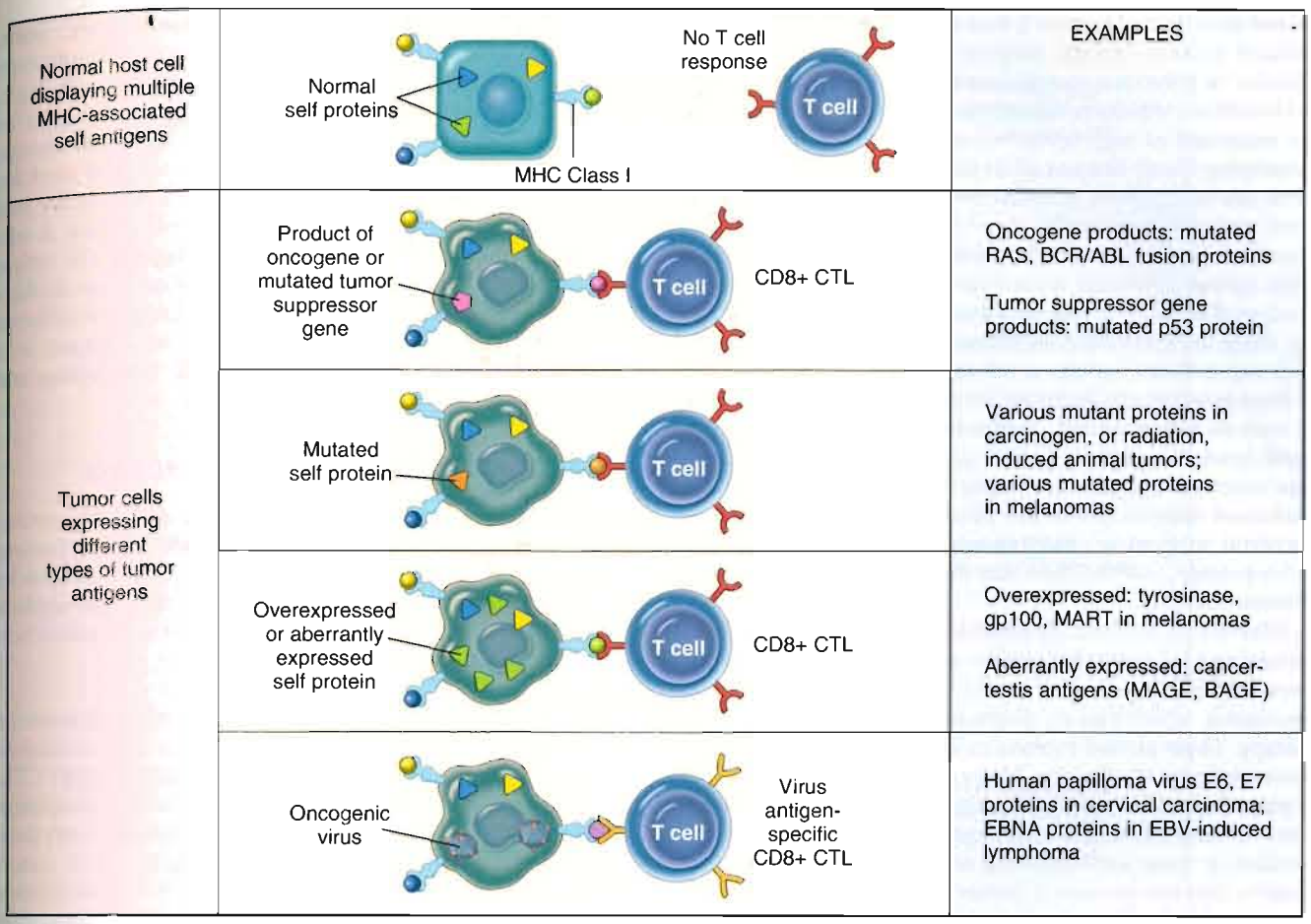


FIGURE 7–45 Tumor antigens recognized by CD8+ T cells. (Modified from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003.)

tosed dead tumor cells, and thus be recognized by CD4+ T cells also. Because these altered proteins are not present in normal cells, they do not induce self-tolerance. Some cancer patients have circulating CD4+ and CD8+ T cells that can respond to the products of mutated oncogenes such as RAS, p53, and BCR-ABL proteins. In animals, immunization with mutated RAS or p53 proteins induces CTLs and rejection responses against tumors expressing these mutants. However, these oncoproteins do not seem to be major targets of tumor-specific CTLs in most patients.

Overexpressed or Aberrantly Expressed Cellular Proteins. Tumor antigens may be normal cellular proteins that are abnormally expressed in tumor cells and elicit immune responses. In a subset of human melanomas some tumor antigens are structurally normal proteins that are produced at low levels in normal cells and overexpressed in tumor cells. One such antigen is tyrosinase, an enzyme involved in melanin biosynthesis that is expressed only in normal melanocytes and melanomas.[179] T cells from melanoma patients recognize peptides derived from tyrosinase, raising the possibility that tyrosinase vaccines may stimulate such responses to melanomas; clinical trials with these vaccines are ongoing. It may be surprising that these patients are able to respond to a normal self-antigen. The probable explanation is that tyrosinase is normally produced in such small amounts and in so few cells that it is not recognized by the immune system and fails to induce tolerance.

Another group, the "cancer-testis" antigens, are encoded by genes that are silent in all adult tissues except the testis—hence their name. Although the protein is present in the testis it is not expressed on the cell surface in an antigenic form, because sperm do not express MHC class I antigens. Thus, for all practical purposes these antigens are tumor specific. Prototypic of this group is the melanoma antigen gene (MAGE) family. Although originally described in melanomas, MAGE antigens are expressed by a variety of tumor types. For example, MAGE-1 is expressed on 37% of melanomas and a variable number of lung, liver, stomach, and esophageal carcinomas.[180] Similar antigens called GAGE, BAGE, and RAGE have been detected in other tumors.

Tumor Antigens Produced by Oncogenic Viruses. As we have discussed, several viruses are associated with cancers. Not surprisingly, these viruses produce proteins that are recognized as foreign by the immune system. The most potent of these antigens are proteins produced by latent DNA viruses; examples in humans include HPV and EBV. There is abundant evidence that CTLs recognize antigens of these viruses and that a competent immune system plays a role in surveillance against virus-induced tumors because of its ability to recognize and kill virus-infected cells. In fact, the concept of immune

surveillance against tumors is best established for DNA virus–induced tumors. Indeed, vaccines against HPV antigens are effective in preventing cervical cancers in young females.

Oncofetal Antigens. Oncofetal antigens are proteins that are expressed at high levels on cancer cells and in normal developing (fetal) but not adult tissues. It is believed that the genes encoding these proteins are silenced during development and are derepressed upon malignant transformation. Oncofetal antigens were identified with antibodies raised in other species, and their main importance is that they provide markers that aid in tumor diagnosis. As techniques for detecting these antigens have improved, it has become clear that their expression in adults is not limited to tumors. Amounts of these proteins are increased in tissues and in the circulation in various inflammatory conditions, and they are found in small quantities even in normal tissues. There is no evidence that oncofetal antigens are important inducers or targets of antitumor immunity. The two most thoroughly characterized oncofetal antigens are carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). These are discussed in the section on "Tumor Markers".

Altered Cell Surface Glycolipids and Glycoproteins. Most human and experimental tumors express higher than normal levels and/or abnormal forms of surface glycoproteins and glycolipids, which may be diagnostic markers and targets for therapy. These altered molecules include gangliosides, blood group antigens, and mucins. Many antibodies have been raised in animals that recognize the carbohydrate groups or peptide cores of these molecules. Although most of the epitopes recognized by these antibodies are not specifically expressed on tumors, they are present at higher levels on cancer cells than on normal cells. This class of antigens is a target for cancer therapy with specific antibodies.

Among the glycolipids expressed at high levels in melanomas are the gangliosides GM_2, GD_2, and GD_3. Clinical trials of anti-GM_2 and anti-GD_3 antibodies and immunization with vaccines containing GM_2 are underway in melanoma patients. Mucins are high-molecular-weight glycoproteins containing numerous O-linked carbohydrate side chains on a core polypeptide. Tumors often have dysregulated expression of the enzymes that synthesize these carbohydrate side chains, which leads to the appearance of tumor-specific epitopes on the carbohydrate side chains or on the abnormally exposed polypeptide core. Several mucins have been the focus of diagnostic and therapeutic studies, including CA-125 and CA-19-9, expressed on ovarian carcinomas, and MUC-1, expressed on breast carcinomas. Unlike many mucins, MUC-1 is an integral membrane protein that is normally expressed only on the apical surface of breast ductal epithelium, a site that is relatively sequestered from the immune system. In ductal carcinomas of the breast, however, the molecule is expressed in an unpolarized fashion and contains new, tumor-specific carbohydrate and peptide epitopes detectable by mouse monoclonal antibodies. The peptide epitopes induce both antibody and T-cell responses in cancer patients and are therefore being considered as candidates for tumor vaccines.

Cell Type–Specific Differentiation Antigens. Tumors express molecules that are normally present on the cells of origin. These antigens are called *differentiation antigens* because they are specific for particular lineages or differentiation stages of various cell types. Such differentiation antigens are typically normal self-antigens, and therefore they do not induce immune response in tumor-bearing hosts. Their importance is as potential targets for immunotherapy and for identifying the tissue of origin of tumors. For example, lymphomas may be diagnosed as B cell–derived tumors by the detection of surface markers characteristic of this lineage, such as CD20. Antibodies against CD20 are also used for tumor immunotherapy. These kill normal B cells as well but because hemopoeitic stem cells are spared, new B cells emerge eventually. The idiotypic determinants of the surface immunoglobulin of a clonal B-cell population are markers for that B-cell clone, because all other B cells express different idiotypes. Therefore, the immunoglobulin idiotype is a highly specific tumor antigen for B-cell lymphomas and leukemias.

ANTITUMOR EFFECTOR MECHANISMS

Cell-mediated immunity is the dominant antitumor mechanism in vivo. Although antibodies can be made against tumors, there is no evidence that they play a protective role under physiologic conditions. The cellular effectors that mediate immunity were described in Chapter 6, so it is necessary here only to characterize them briefly.

- *Cytotoxic T lymphocytes:* The antitumor effect of cytotoxic T cells reacting against tumor antigens is well established in experimentally induced tumors. In humans, CD8+ CTLs play a protective role against virus-associated neoplasms (e.g., EBV- and HPV-induced tumors) and have been demonstrated in the blood and tumor infiltrates of cancer patients. In some cases, such CD8+ T cells do not develop spontaneously in vivo but can be generated by immunization with tumor antigen–pulsed dendritic cells.
- *Natural killer cells:* NK cells are lymphocytes that are capable of destroying tumor cells without prior sensitization and thus may provide the first line of defense against tumor cells.[181] After activation with IL-2 and IL-15, NK cells can lyse a wide range of human tumors, including many that seem to be nonimmunogenic for T cells. T cells and NK cells seem to provide complementary antitumor mechanisms. Tumors that fail to express MHC class I antigens cannot be recognized by T cells, but these tumors may trigger NK cells because the latter are inhibited by recognition of normal autologous class I molecules (Chapter 6). The triggering receptors on NK cells are extremely diverse and belong to several gene families. NKG2D proteins expressed on NK cells and some T cells are important activating receptors. They recognize stress-induced antigens that are expressed on tumor cells and cells that have incurred DNA damage and are at risk for neoplastic transformation.
- *Macrophages:* Activated macrophages exhibit cytotoxicity against tumor cells in vitro. T cells, NK cells, and macrophages may collaborate in antitumor reactivity, because interferon-γ, a cytokine secreted by T cells and NK cells, is a potent activator of macrophages. Activated macrophages may kill tumors by mechanisms similar to those used to kill microbes (e.g., production of reactive oxygen metabolites; Chapter 2) or by secretion of TNF.
- *Antibodies:* Although there is no evidence for the protective effects of antitumor antibodies against spontaneous tumors, administration of monoclonal antibodies against tumor cells can be therapeutically effective. A monoclonal anti-

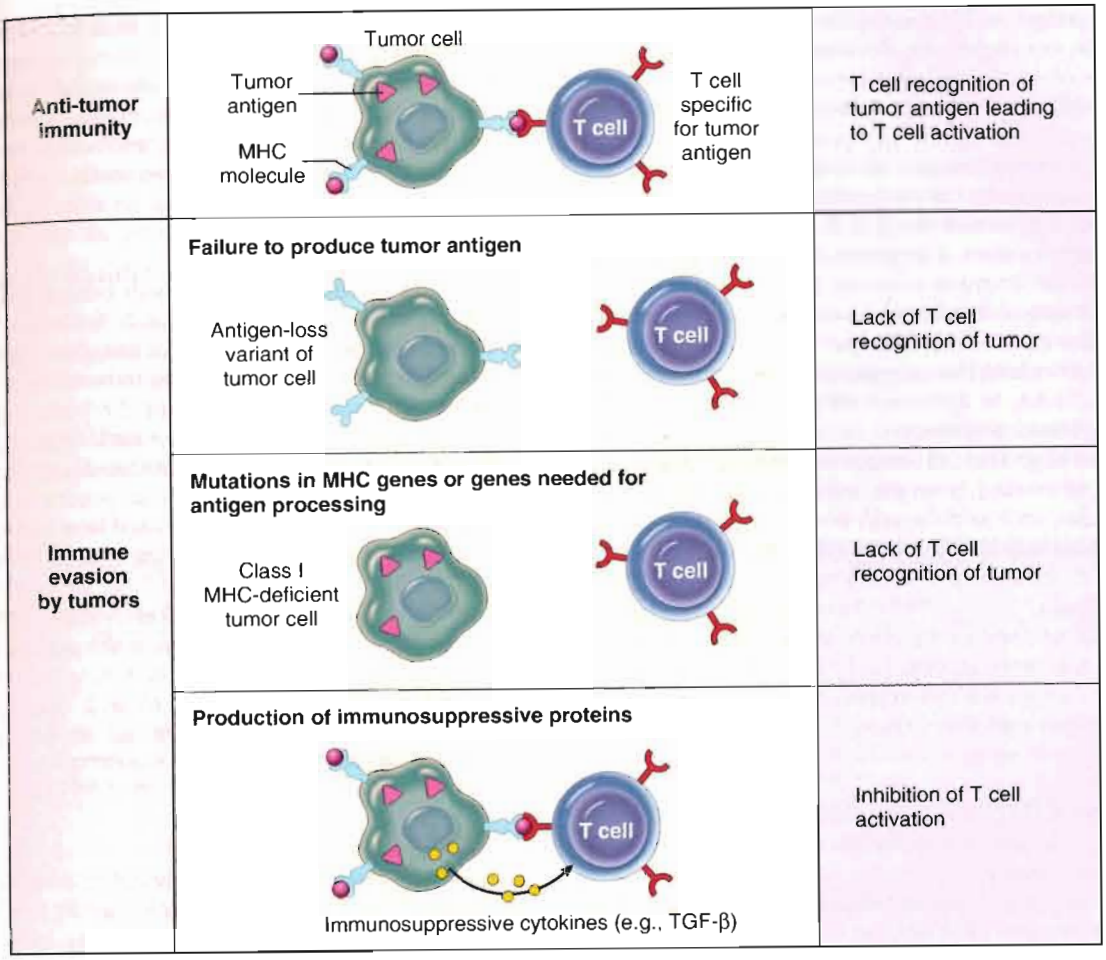


FIGURE 7–46 Mechanisms by which tumors evade the immune system. (Reprinted from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003)

body against CD20, a B-cell surface antigen, is widely used for treatment of lymphomas.

IMMUNE SURVEILLANCE AND ESCAPE

Given the many potential antitumor mechanisms, is there any evidence that they operate in vivo to prevent emergence of neoplasms? The strongest argument for the existence of immune surveillance is the increased frequency of cancers in immunodeficient hosts. About 5% of persons with congenital immunodeficiencies develop cancers, about 200 times the rate in immunocompetent individuals. Immunosuppressed transplant recipients and persons with AIDS also have an increased incidence of malignancies. Most (but not all) of these neoplasms are lymphomas, often diffuse large B-cell lymphomas. Particularly illustrative is the rare X-linked recessive immunodeficiency disorder termed *XLP (X-linked lymphoproliferative syndrome)*, caused by mutations in the gene encoding an adapter protein *(SAP)*, which participates in lymphocyte signaling pathways.[182] When affected boys develop an EBV infection, such infection does not take the usual self-limited form of infectious mononucleosis but instead evolves into a chronic or sometimes fatal form of infectious mononucleosis or, even worse, B-cell lymphoma.

Most cancers occur in persons who do not suffer from any overt immunodeficiency. It is evident, then, that *tumor cells must develop mechanisms to escape or evade the immune system* in immunocompetent hosts. Several such mechanisms may be operative (Fig. 7–46).

- *Selective outgrowth of antigen-negative variants:* During tumor progression, strongly immunogenic subclones may be eliminated.
- *Loss or reduced expression of MHC molecules:* Tumor cells may fail to express normal levels of HLA class I molecules, thereby escaping attack by cytotoxic T cells. Such cells, however, may trigger NK cells.
- *Lack of costimulation:* It may be recalled that sensitization of T cells requires two signals, one by a foreign peptide presented by MHC molecules and the other by costimulatory molecules (Chapter 6); although tumor cells may express peptide antigens with class I molecules, they often do not express costimulatory molecules. This not only prevents sensitization but also may render T cells anergic or, worse, cause them to undergo apoptosis. To bypass this problem, attempts are being made to immunize patients with autologous tumor cells that have been transfected with the gene for the costimulatory molecule B7-1 (CD 80). In another approach, autologous dendritic cells expanded in

vitro and pulsed with tumor antigens (e.g., MAGE1) are infused into cancer patients. Because dendritic cells express high levels of costimulatory molecules, it is expected that such immunization will stimulate antitumor T cells.

- *Immunosuppression:* Many oncogenic agents (e.g., chemicals and ionizing radiation) suppress host immune responses. Tumors or tumor products also may be immunosuppressive. For example, TGF-β, secreted in large quantities by many tumors, is a potent immunosuppressant. In some cases the immune response induced by the tumor may inhibit tumor immunity. Several mechanisms of such inhibition have been described. For instance, recognition of tumor cells may lead to engagement of the T-cell inhibitory receptor, CTLA4, or activation of regulatory T cells that suppress immune responses.
- *Antigen masking:* The cell surface antigens of tumors may be hidden, or masked, from the immune system by glycocalyx molecules, such as sialic acid–containing mucopolysaccharides. This may be a consequence of the fact that tumor cells often express more of these glycocalyx molecules than normal cells do.
- *Apoptosis of cytotoxic T cells:* Some melanomas and hepatocellular carcinomas express FasL. It has been postulated that these tumors kill Fas-expressing T lymphocytes that come in contact with them, thus eliminating tumor-specific T cells.[183]

Thus, it seems that there is no dearth of mechanisms by which tumor cells can outwit the host and thrive despite an intact immune system.

It is worth mentioning that although much of the focus in the field of tumor immunity has been on the mechanisms by which the host immune system defends against tumors, there is some recent evidence that, paradoxically, the immune system may promote the growth of tumors.[184] It is possible that activated lymphocytes and macrophages produce growth factors for tumor cells, and regulatory T-cells and certain subtypes of macrophages may suppress the host response to tumors. Enzymes, such as MMPs, that enhance tumor invasion, may also be produced. Harnessing the protective actions of the immune system and abolishing its ability to increase tumor growth are obviously important goals of immunologists and oncologists.

Clinical Aspects of Neoplasia

Ultimately the importance of neoplasms lies in their effects on patients. Although malignant tumors are of course more threatening than benign tumors, any tumor, even a benign one, may cause morbidity and mortality. Indeed, both malignant and benign tumors may cause problems because of (1) location and impingement on adjacent structures, (2) functional activity such as hormone synthesis or the development of paraneoplastic syndromes, (3) bleeding and infections when the tumor ulcerates through adjacent surfaces, (4) symptoms that result from rupture or infarction, and (5) cachexia or wasting.

Local and Hormonal Effects

Location is crucial in both benign and malignant tumors. A small (1-cm) pituitary adenoma, though benign and possibly nonfunctional, can compress and destroy the surrounding normal gland and thus lead to serious hypopituatarism. Cancers arising within or metastatic to an endocrine gland may cause an endocrine insufficiency by destroying the gland. Neoplasms in the gut, both benign and malignant, may cause obstruction as they enlarge. Infrequently, peristaltic movement telescopes the neoplasm and its affected segment into the downstream segment, producing an obstructing intussusception (Chapter 17).

Hormone production is seen with benign and malignant neoplasms arising in endocrine glands. Such functional activity is more typical of benign than of malignant tumors, which may be sufficiently undifferentiated to have lost such capability. A benign beta-cell adenoma of the pancreatic islets less than 1 cm in diameter may produce sufficient insulin to cause fatal hypoglycemia. In addition, nonendocrine tumors may elaborate hormones or hormone-like products and give rise to paraneoplastic syndromes (discussed later). The erosive and destructive growth of cancers or the expansile pressure of a benign tumor on any natural surface, such as the skin or mucosa of the gut, may cause ulcerations, secondary infections, and bleeding. Melena (blood in the stool) and hematuria, for example, are characteristic of neoplasms of the gut and urinary tract. Neoplasms, benign as well as malignant, may cause problems in varied ways, but all are far less common than the cachexia of malignancy.

Cancer Cachexia

Individuals with cancer commonly suffer progressive loss of body fat and lean body mass accompanied by profound weakness, anorexia, and anemia, referred to as *cachexia.* Unlike starvation, the weight loss seen in cachexia results equally from loss of fat and lean muscle. There is some correlation between the tumor burden and the severity of the cachexia. However, cachexia is not caused by the nutritional demands of the tumor. In persons with cancer, the basal metabolic rate is increased, despite reduced food intake. This is in contrast to the lower metabolic rate that occurs as an adaptational response in starvation. Although patients with cancer are often anorexic, cachexia probably results from the action of soluble factors such as cytokines produced by the tumor and the host rather than reduced food intake. The basis of these metabolic abnormalities is not fully understood. It is suspected that TNF produced by macrophages in response to tumor cells or by the tumor cells themselves mediates cachexia. TNF at high concentrations may mobilize fats from tissue stores and suppress appetite; both activities would contribute to cachexia. Other cytokines, such as IL-1, interferon-γ, and leukemia inhibitory factor, synergize with TNF. Additionally, other soluble factors produced by tumors, such as proteolysis-inducing factor and a lipid-mobilizing factor, increase the catabolism of muscle and adipose tissue.[185] These factors reduce protein synthesis by decreasing m-RNA translation and by stimulating protein catabolism through the activation of the ATP-dependent ubiquitin-proteasome pathway. It is now thought that there is a balance between factors that regulate muscle hypertrophy, such as IGF, and factors that regulate muscle catabolism. In cachexia these homeostatic mechanisms are disrupted, tilting the scales toward cachectic factors. There is currently no satisfactory treatment for cancer cachexia other than removal of the underlying cause, the

tumor. However, cachexia clearly hampers effective chemotherapy, by reducing the dosages that can be given. Furthermore, it has been estimated that a third of deaths of cancer are attributable to cachexia, rather than directly due to the tumor burden itself. Identification of the molecular mechanisms involved in cancer cachexia may allow treatment of cachexia itself.

Paraneoplastic Syndromes

Symptom complexes in cancer-bearing individuals that cannot readily be explained, either by the local or distant spread of the tumor or by the elaboration of hormones indigenous to the tissue from which the tumor arose, are known as *paraneoplastic syndromes.*[186] These occur in about 10% of persons with malignant disease. Despite their relative infrequency, paraneoplastic syndromes are important to recognize, for several reasons:

- They may represent the earliest manifestation of an occult neoplasm.
- In affected patients they may represent significant clinical problems and may even be lethal.
- They may mimic metastatic disease and therefore confound treatment.

A classification of paraneoplastic syndromes and their presumed origins is presented in Table 7–11. A few comments on some of the more common and interesting syndromes follow.

The *endocrinopathies* are frequently encountered paraneoplastic syndromes.[187] Because the cancer cells are not of

TABLE 7–11 Paraneoplastic Syndromes

Clinical Syndromes	Major Forms of Underlying Cancer	Causal Mechanism
ENDOCRINOPATHIES		
Cushing syndrome	Small-cell carcinoma of lung Pancreatic carcinoma Neural tumors	ACTH or ACTH-like substance
Syndrome of inappropriate antidiuretic hormone secretion	Small-cell carcinoma of lung; intracranial neoplasms	Antidiuretic hormone or atrial natriuretic hormones
Hypercalcemia	Squamous cell carcinoma of lung Breast carcinoma Renal carcinoma Adult T-cell leukemia/lymphoma	Parathyroid hormone–related protein (PTHRP), TGF-α, TNF, IL-1
Hypoglycemia	Ovarian carcinoma Fibrosarcoma Other mesenchymal sarcomas Hepatocellular carcinoma	Insulin or insulin-like substance
Carcinoid syndrome	Bronchial adenoma (carcinoid) Pancreatic carcinoma Gastric carcinoma	Serotonin, bradykinin
Polycythemia	Renal carcinoma Cerebellar hemangioma Hepatocellular carcinoma	Erythropoietin
NERVE AND MUSCLE SYNDROMES		
Myasthenia Disorders of the central and peripheral nervous system	Bronchogenic carcinoma Breast carcinoma	Immunological
DERMATOLOGIC DISORDERS		
Acanthosis nigricans	Gastric carcinoma Lung carcinoma Uterine carcinoma	Immunological; secretion of epidermal growth factor
Dermatomyositis	Bronchogenic, breast carcinoma	Immunological
OSSEOUS, ARTICULAR, AND SOFT-TISSUE CHANGES		
Hypertrophic osteoarthropathy and clubbing of the fingers	Bronchogenic carcinoma	Unknown
VASCULAR AND HEMATOLOGIC CHANGES		
Venous thrombosis (Trousseau phenomenon)	Pancreatic carcinoma Bronchogenic carcinoma Other cancers	Tumor products (mucins that activate clotting)
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Red cell aplasia	Thymic neoplasms	Unknown
OTHERS		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

ACTH, adrenocorticotropic hormone; IL, interleukin; TGF, transforming growth factor; TNF, tumor necrosis factor.

endocrine origin, the functional activity is referred to as *ectopic hormone production.* Cushing syndrome is the most common endocrinopathy. Approximately 50% of individuals with this endocrinopathy have carcinoma of the lung, chiefly the small-cell type. It is caused by excessive production of corticotropin or corticotropin-like peptides. The precursor of corticotropin is a large molecule known as pro-opiomelanocortin. Lung cancer patients with Cushing syndrome have elevated serum levels of pro-opiomelanocortin and of corticotropin. The former is not found in serum of patients with excess corticotropin produced by the pituitary.

Hypercalcemia is probably the most common paraneoplastic syndrome; overtly symptomatic hypercalcemia is most often related to some form of cancer rather than to hyperparathyroidism. Two general processes are involved in cancer-associated hypercalcemia: (1) osteolysis induced by cancer, whether primary in bone, such as multiple myeloma, or metastatic to bone from any primary lesion, and (2) the production of calcemic humoral substances by extraosseous neoplasms. *Hypercalcemia due to skeletal metastases is not a paraneoplastic syndrome.*

Several humoral factors have been associated with paraneoplastic hypercalcemia of malignancy. The most important, parathyroid hormone–related protein (PTHRP), is a molecule related to, but distinct from, parathyroid hormone (PTH). PTHRP resembles the native hormone only in its N terminus.[188] It has some biologic actions similar to those of PTH, and both hormones share a G protein–coupled receptor, known as PTH/PTHRP receptor (often referred to as PTH-R or PTHRP-R). In contrast to PTH, PTHRP is produced in small amounts by many normal tissues, including keratinocytes, muscles, bone, and ovary. It regulates calcium transport in the lactating breast and across the placenta, and seems to regulate development and remodeling in the lung. Tumors most often associated with paraneoplastic hypercalcemia are carcinomas of the breast, lung, kidney, and ovary. In breast cancers, PTHRP production is associated with osteolytic bone disease, bone metastasis, and humoral hypercalcemia. The most common lung neoplasm associated with hypercalcemia is squamous cell bronchogenic carcinoma. In addition to PTHRP, several other factors, such as IL-1, TGF-α, TNF, and dihydroxyvitamin D, have also been implicated in causing the hypercalcemia of malignancy.

The *neuromyopathic paraneoplastic syndromes* take diverse forms, such as peripheral neuropathies, cortical cerebellar degeneration, a polymyopathy resembling polymyositis, and a myasthenic syndrome similar to *myasthenia gravis* (Chapter 27). The cause of these syndromes is poorly understood. In some cases, antibodies, presumably induced against tumor cell antigens (Chapter 28) that cross-react with neuronal cell antigens, have been detected. It is postulated that some neural antigens are ectopically expressed by visceral cancers. For some unknown reason, the immune system recognizes these antigens as foreign and mounts an immune response.

Acanthosis nigricans is characterized by gray-black patches of verrucous hyperkeratosis on the skin. This disorder occurs rarely as a genetically determined disease in juveniles or adults (Chapter 25). In addition, in about 50% of the cases, particularly in those over age 40, the appearance of such lesions is associated with some form of cancer. Sometimes the skin changes appear before discovery of the cancer.

Hypertrophic osteoarthropathy is encountered in 1% to 10% of patients with bronchogenic carcinomas. Rarely, other forms of cancer are involved. This disorder is characterized by (1) periosteal new bone formation, primarily at the distal ends of long bones, metatarsals, metacarpals, and proximal phalanges; (2) arthritis of the adjacent joints; and (3) clubbing of the digits. Although the osteoarthropathy is seldom seen in non-cancer patients, clubbing of the fingertips may be encountered in liver diseases, diffuse lung disease, congenital cyanotic heart disease, ulcerative colitis, and other disorders. The cause of hypertrophic osteoarthropathy is unknown.

Several *vascular and hematologic manifestations* may appear in association with a variety of forms of cancer. As mentioned in the discussion of thrombosis (Chapter 4), *migratory thrombophlebitis* (Trousseau syndrome) may be encountered in association with deep-seated cancers, most often carcinomas of the pancreas or lung. Disseminated intravascular coagulation may complicate a diversity of clinical disorders (Chapter 14). Acute disseminated intravascular coagulation is most commonly associated with acute promyelocytic leukemia and prostatic adenocarcinoma. Bland, small, nonbacterial fibrinous vegetations sometimes form on the cardiac valve leaflets (more often on left-sided valves), particularly in individuals with advanced mucin-secreting adenocarcinomas. These lesions, called *nonbacterial thrombotic endocarditis,* are described further in Chapter 12. The vegetations are potential sources of emboli that can further complicate the course of cancer.

GRADING AND STAGING OF TUMORS

Methods to quantify the probable clinical aggressiveness of a given neoplasm and its apparent extent and spread in the individual patient are necessary for making an accurate prognosis and for comparing end results of various treatment protocols. For instance, the results of treating well-differentiated thyroid adenocarcinoma that is localized to the thyroid gland will be different from those obtained from treating highly anaplastic thyroid cancers that have invaded the neck organs. Systems have been developed to express, at least in semiquantitative terms, the level of differentiation, or *grade*, and extent of spread of a cancer within the patient, or *stage*, as parameters of the clinical gravity of the disease.

Grading of a cancer is based on the degree of differentiation of the tumor cells and, in some cancers, the number of mitoses or architectural features. Grading schemes have evolved for each type of malignancy, and generally range from two categories (low grade and high grade) to four categories. Criteria for the individual grades vary with each form of neoplasia and so are not detailed here, but all attempt, in essence, to judge the extent to which the tumor cells resemble or fail to resemble their normal counterparts. Although histologic grading is useful, the correlation between histologic appearance and biologic behavior is less than perfect. In recognition of this problem and to avoid spurious quantification, it is common practice to characterize a particular neoplasm in descriptive terms, for example, well-differentiated, mucin-secreting adenocarcinoma of the stomach, or poorly differentiated pancreatic adenocarcinoma. In general, with a few exceptions, such as soft-tissue sarcomas, grading of cancers has proved of less clinical value than has staging.

The staging of cancers is based on the size of the primary lesion, its extent of spread to regional lymph nodes, and the presence or absence of blood-borne metastases. The major staging system currently in use is the American Joint Committee on Cancer Staging. This system uses a classification called the *TNM system*—*T* for primary tumor, *N* for regional lymph node involvement, and *M* for metastases. The TNM staging varies for each specific form of cancer, but there are general principles. With increasing size the primary lesion is characterized as T1 to T4. T0 is used to indicate an in situ lesion. N0 would mean no nodal involvement, whereas N1 to N3 would denote involvement of an increasing number and range of nodes. M0 signifies no distant metastases, whereas M1 or sometimes M2 indicates the presence of metastases and some judgment as to their number.

LABORATORY DIAGNOSIS OF CANCER

Every year the approach to laboratory diagnosis of cancer becomes more complex, more sophisticated, and more specialized. For virtually every neoplasm mentioned in this text, the experts have characterized several subcategories; we must walk, however, before we can run. Each of the following sections attempts to present the state of the art, avoiding details of method.

Histologic and Cytologic Methods. The laboratory diagnosis of cancer is, in most instances, not difficult. The two ends of the benign-malignant spectrum pose no problems; however, in the middle lies a gray zone that the novices dread and where experts tread cautiously. The focus here is on the roles of the clinician (often a surgeon) and the pathologist in facilitating the correct diagnosis.

Clinical data are invaluable for optimal pathologic diagnosis, but often clinicians underestimate its value. Radiation changes in the skin or mucosa can be similar to those associated with cancer. Sections taken from a healing fracture can mimic an osteosarcoma. Moreover, the laboratory evaluation of a lesion can be only as good as the specimen made available for examination. It must be adequate, representative, and properly preserved. Several sampling approaches are available: (1) excision or biopsy, (2) needle aspiration, and (3) cytologic smears. When excision of a small lesion is not possible, selection of an appropriate site for biopsy of a large mass requires awareness that the periphery may not be representative and the center largely necrotic. Appropriate preservation of the specimen is obvious, yet it involves such actions as prompt immersion in a usual fixative (commonly formalin solution, but other fluids can be used), preservation of a portion in a special fixative (e.g., glutaraldehyde) for electron microscopy, or prompt refrigeration to permit optimal hormone, receptor, or other types of molecular analysis. Requesting "quick-frozen section" diagnosis is sometimes desirable, for example, in determining the nature of a mass lesion or in evaluating the margins of an excised cancer to ascertain that the entire neoplasm has been removed. This method permits histologic evaluation within minutes. In experienced, competent hands, frozen-section diagnosis is highly accurate, but there are particular instances in which the better histologic detail provided by the more time-consuming routine methods is needed—for example, when extremely radical surgery, such as the amputation of an extremity, may be indicated. Better to wait a day or two despite the drawbacks, than to perform inadequate or unnecessary surgery.

Fine-needle aspiration of tumors is another approach that is widely used. The procedure involves aspirating cells and attendant fluid with a small-bore needle, followed by cytologic examination of the stained smear. This method is used most commonly for the assessment of readily palpable lesions in sites such as the breast, thyroid, and lymph nodes. Modern imaging techniques permit extension of the method to lesions in deep-seated structures, such as pelvic lymph nodes and pancreas. Fine-needle aspiration is less invasive and more rapidly performed than are needle biopsies. It obviates surgery and its attendant risks. Although it entails some difficulties, such as small sample size and sampling errors, in experienced hands it is extremely reliable, rapid, and useful.

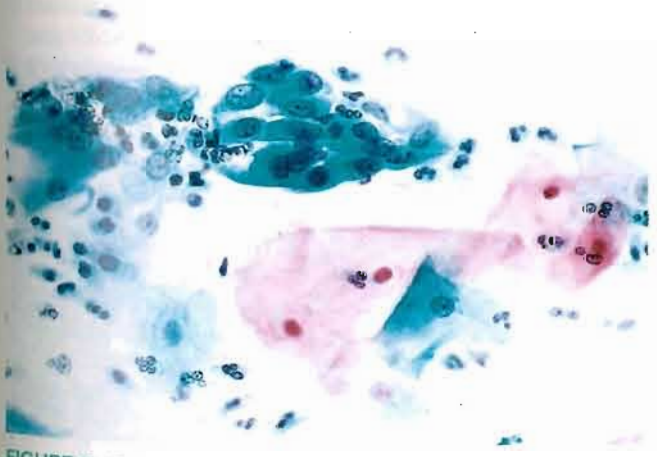


FIGURE 7–47 A normal cervicovaginal smear shows large, flattened squamous cells and groups of metaplastic cells; interspersed are some neutrophils. There are no malignant cells. (Courtesy of Dr. P.K. Gupta, University of Pennsylvania, Philadelphia, PA.)

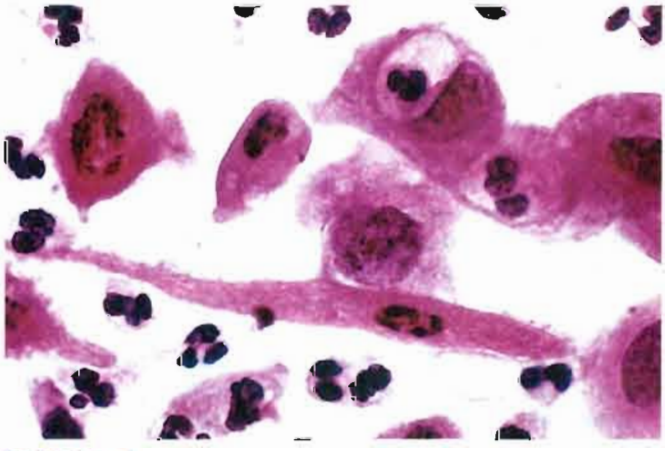


FIGURE 7–48 An abnormal cervicovaginal smear shows numerous malignant cells that have pleomorphic, hyperchromatic nuclei; interspersed are some normal polymorphonuclear leukocytes. (Courtesy of Dr. P.K. Gupta, University of Pennsylvania, Philadelphia, PA.)

Cytologic (Pap) smears provide yet another method for the detection of cancer (Chapter 22). This approach is widely used to screen for carcinoma of the cervix, often at an in situ stage, but it is also used with many other forms of suspected malignancy, such as endometrial carcinoma, bronchogenic carcinoma, bladder and prostatic tumors, and gastric carcinomas; for the identification of tumor cells in abdominal, pleural, joint, and cerebrospinal fluids; and, less commonly, with other forms of neoplasia.

As pointed out earlier, cancer cells have lowered cohesiveness and exhibit a range of morphologic changes encompassed by the term *anaplasia.* Thus, shed cells can be evaluated for the features of anaplasia indicative of their origin from a tumor (Figs. 7–47 and 7–48). In contrast to the histologist's task, judgment here must be rendered based on the features of individual cells or, at most, a clump of cells, without the supporting evidence of loss of orientation of one cell to another, and (most importantly) evidence of invasion. This method permits differentiation among normal, dysplastic, and malignant cells and, in addition, permits the recognition of cellular changes characteristic of carcinoma in situ. The gratifying control of cervical cancer is the best testament to the value of the cytologic method.

Although histology and exfoliative cytology remain the most commonly used methods in the diagnosis of cancer, new techniques are being constantly added to the tools of the surgical pathologist. Some, such as immunohistochemistry, are already well established and widely used; others, including molecular methods, are rapidly finding their way into the "routine" category. Only some highlights of these diagnostic modalities are presented.

Immunohistochemistry. The availability of specific antibodies has greatly facilitated the identification of cell products or surface markers. Some examples of the utility of immunohistochemistry in the diagnosis or management of malignant neoplasms follow.

- *Categorization of undifferentiated malignant tumors:* In many cases malignant tumors of diverse origin resemble each other because of limited differentiation. These tumors are often quite difficult to distinguish on the basis of routine hematoxylin and eosin (H&E)–stained tissue sections. For example, certain anaplastic carcinomas, lymphomas, melanomas, and sarcomas may look quite similar, but they must be accurately identified because their treatment and prognosis are different. Antibodies specific to intermediate filaments have proved to be of particular value in such cases, because solid tumor cells often contain intermediate filaments characteristic of their cell of origin. For example, the presence of cytokeratins, detected by immunohistochemistry, points to an epithelial origin (carcinoma) (Fig. 7–49), whereas desmin is specific for neoplasms of muscle cell origin.
- *Determination of site of origin of metastatic tumors:* Many cancer patients present with metastases. In some the primary site is obvious or readily detected on the basis of clinical or radiologic features. In cases in which the origin of the tumor is obscure, immunohistochemical detection of tissue-specific or organ-specific antigens in a biopsy specimen of the metastatic deposit can lead to the identification of the tumor source. For example, prostate-specific antigen (PSA) and thyroglobulin are markers of carcinomas of the prostate and thyroid, respectively.
- *Detection of molecules that have prognostic or therapeutic significance:* Immunohistochemical detection of hormone (estrogen/progesterone) receptors in breast cancer cells is of prognostic and therapeutic value because these cancers are susceptible to anti-estrogen therapy (Chapter 23). In general, receptor-positive breast cancers have a better prognosis. Protein products of oncogenes such as *ERBB2* in breast cancers can also be detected by immunostaining. Breast cancers with overexpression of ERBB2 protein generally have a poor prognosis. In general practice, the overexpression of ERBB2 is confirmed by fluorescent in situ hybridization (FISH) to confirm amplification of the genomic region containing the *ERBB2* gene.

Flow Cytometry. Flow cytometry can rapidly and quantitatively measure several individual cell characteristics, such as membrane antigens and the DNA content of tumor cells. *Flow cytometry* has also proved useful in the identification and classification of tumors arising from T and B lymphocytes and from mononuclear-phagocytic cells. Monoclonal antibodies directed against various lymphohematopoietic cells are listed in Chapter 13.

Molecular Diagnosis. Several molecular techniques—some established, others emerging—have been used for diagnosis and, in some cases, for predicting behavior of tumors.

- *Diagnosis of malignant neoplasms:* Although molecular methods are not the primary modality of cancer diagnosis, they are of considerable value in selected cases. Molecular techniques are useful in differentiating benign (polyclonal) proliferations of T or B cells from malignant (monoclonal) proliferations. Because each T and B cell has unique rearrangements of its antigen receptor genes (Chapter 6), PCR–based detection of T-cell receptor or immunoglobulin genes allows distinction between monoclonal (neoplastic) and polyclonal (reactive) proliferations. Many hematopoietic neoplasms (leukemias and lymphomas) are associated with specific translocations that activate oncogenes. Detection of such translocations, usually by routine cytogenetic analysis or by FISH technique (Chapter 5), is often extremely

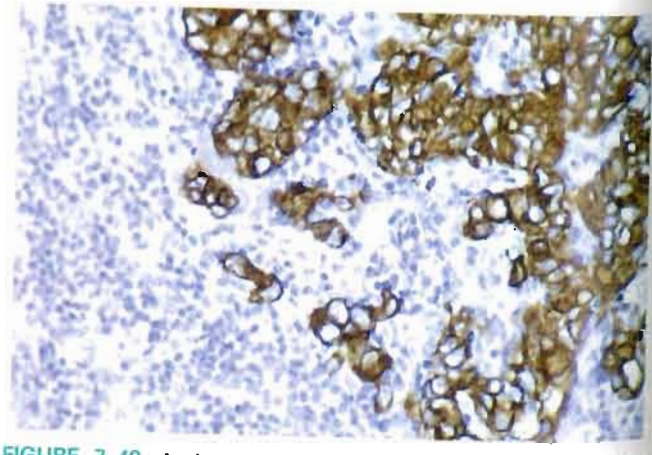


FIGURE 7–49 Anti-cytokeratin immunoperoxidase stain of a tumor of epithelial origin (carcinoma). (Courtesy of Dr. Melissa Upton, University of Washington, Seattle, WA.)

helpful in diagnosis.[189] In some cases, molecular techniques, such as PCR, can detect residual disease in cases that appear negative by conventional analysis. Diagnosis of sarcomas (Chapter 26) with characteristic translocations is also aided by molecular techniques, because chromosome preparations are often difficult to obtain from solid tumors. For example, many sarcomas of childhood, so-called round blue cell tumors (Chapter 10), can be difficult to distinguish from each other on the basis of morphology. However, the presence of the characteristic [t(11;22)(q24;q12)] translocation, established by PCR, in one of these tumors confirms the diagnosis of Ewing sarcoma.[190] A molecular cytogenetic technique called *spectral karyotyping* has great sensitivity and allows the examination of all chromosomes in a single experiment.[191] This technique, which is based on 24-color chromosomal painting with a mixture of fluorochromes, can detect all types of chromosomal rearrangements in tumor cells, even small, cryptic translocations and insertions (Chapter 5; see Fig. 5–35). It can also detect the origin of unidentified chromosomes, called *marker chromosomes*, seen in many hematopoietic malignancies. Another available technique is *comparative genomic hybridization, now more conveniently converted to microarray format*, which allows the analysis of chromosomal gains and losses in tumor cells. The use of DNA microarrays (discussed later), either tiling arrays, which cover the entire human genome, or single-nucleotide polymorphism arrays (SNP chips), also allows analysis of genomic amplifications and deletions at very high resolution.

- *Prognosis of malignant neoplasms:* Certain genetic alterations are associated with poor prognosis, and hence their detection allows stratification of patients for therapy. For example, amplification of the N-*MYC* gene and deletions of 1p bode poorly for patients with neuroblastoma, while amplification of *HER-2/NEU* in breast cancer is an indication that therapy with antibodies against the ERBB2 receptor may be effective. These can be detected by routine cytogenetics and also by FISH or PCR assays. Oligodendrogliomas in which the only genomic abnormality is the loss of chromosomes 1p and 19q respond well to therapy and are associated with long-term survival when compared to tumors with intact 1p and 19q but with EGF receptor amplification.[192]
- *Detection of minimal residual disease:* After treatment of patients with leukemia or lymphoma, the presence of minimal disease or the onset of relapse can be monitored by PCR-based amplification of nucleic acid sequences unique to the malignant clone. For example, detection of *BCR-ABL* transcripts by PCR gives a measure of the residual leukemia cells in treated patients with CML. Similarly, detection of specific *KRAS* mutations in stool samples of persons previously treated for colon cancer can alert the clinician to the possible recurrence of the tumor. The prognostic importance of minimal disease has been established in acute lymphoblastic leukemia, and is being evaluated in other neoplasms.
- *Diagnosis of hereditary predisposition to cancer:* As was discussed earlier, germ-line mutations in several tumor suppressor genes, including *BRCA1*, *BRCA2*, and the *RET* proto-oncogene, are associated with a high risk of developing specific cancers. Thus, detection of these mutated alleles may allow the patient and physician to devise an aggressive screening program, consider the option of prophylactic surgery, and counseling of relatives at risk. Such analysis usually requires detection of a specific mutation (e.g., *RET* gene) or sequencing of the entire gene. The latter is necessitated when several different cancer-associated mutations are known to exist. Although the detection of mutations in such cases is relatively straightforward, the ethical issues surrounding such presymptomatic diagnosis are complex.

Molecular Profiles of Tumors

Until recently, studies of gene expression in tumors involved the analysis of individual genes. These studies have been revolutionized by the introduction of methods that can measure the expression of essentially all the genes in the genome simultaneously.[193,194] The most common method for large-scale analysis of gene expression in use today is based on DNA microarray technology. There are essentially two methods for expression analysis. Either PCR products from cloned genes or oligonucleotides homologous to genes of interest are spotted onto a glass slide. Each method has its advantages and disadvantages. Chips can be purchased from commercial suppliers or produced on the premises, and high-density oligonucleotide arrays can contain more than 2 million elements. The gene chip is then hybridized to "probes" prepared from tumor and control samples (the probes are usually complementary DNA copies of RNAs extracted from tumor and uninvolved tissues) that have been labeled with a fluorochrome. After hybridization the chip is read using a laser scanner (Fig. 7–50); sophisticated software has been developed to measure the intensity of the fluorescence for each spot. A variety of analyses can then be performed with these data; one of the most useful for cancer research has been hierarchical clustering, which can be used in many ways to understand the molecular heterogeneity and biologic behavior of cancer. One can determine the expression profiles of many different individual tumors that have different outcomes, for example, breast cancers that relapsed and those that did not. Using a hierarchical clustering, a (hopefully) short list of genes that are differentially expressed in these two groups can be generated. This "signature" may then be used to predict the behavior of tumors. In this way it is hoped that gene expression profiles will improve our ability to stratify patients' risk and guide treatment beyond the limits of histology and pathologic staging. Indeed, analysis of phenotypically identical large B-cell lymphomas (Chapter 13) from different individuals shows that these tumors are heterogeneous with respect to their gene expression profiles. Importantly, gene expression signatures have been identified that allow segregation of morphologically similar lymphomas into distinct subcategories with markedly different survival rates.[195]

A major problem in the analysis of gene expression in tumors is the heterogeneity of the tissue. In addition to the heterogeneity of the tumor cells, samples may contain variable amounts of stromal connective tissue, inflammatory infiltrates, and normal tissue cells. One way to overcome this problem is to obtain nearly pure tumor cells or small tumors free from associated stroma using *laser capture microdissection.* In this technique, the dissection of tumor cells is made under a microscope through a focused laser. The dissected material

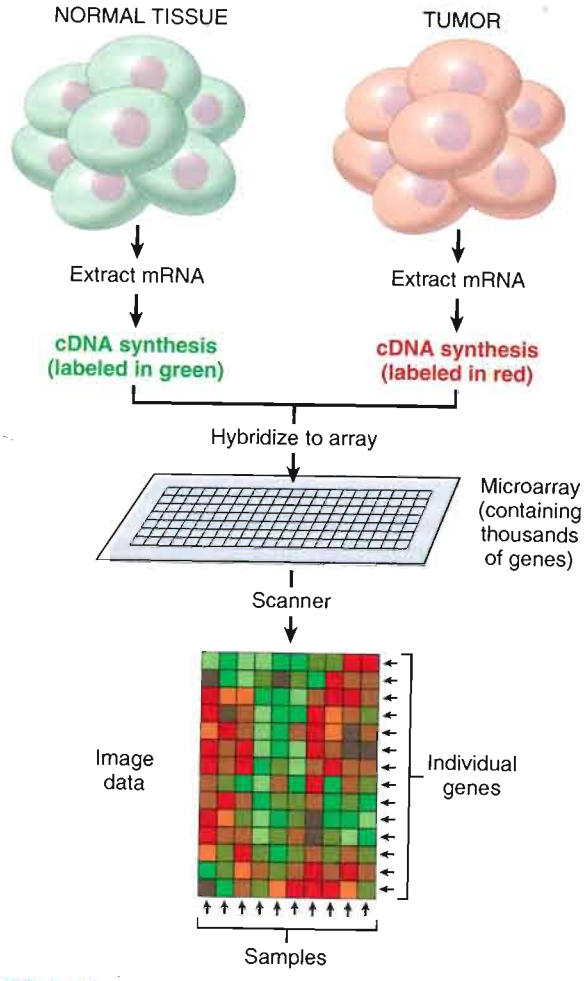


FIGURE 7–50 Steps required for the analysis of global gene expression by DNA microarray. RNA is extracted from tumor and normal tissue. Complementary DNA (cDNA) synthesized from each preparation is labeled with fluorescent dyes (in the example shown, normal tissue cDNA is labeled with a green dye; tumor cDNA is labeled with a red dye). The array consists of a solid support in which DNA fragments from many thousands of genes are spotted. The labeled cDNAs from tumor and normal tissue are combined and hybridized to the genes contained in the array. Hybridization signals are detected using a confocal laser scanner and downloaded to a computer for analysis (*red squares*, expression of the gene is higher in tumor; *green squares*, expression of the gene is higher in normal tissue; *black squares*, no difference in the expression of the gene between tumor and normal tissue). In the display the horizontal rows correspond to each gene contained in the array; each vertical row corresponds to single samples.

is then captured or "catapulted" into a small cap and processed for RNA and DNA isolation.

The applications of molecular profiling technology keep expanding and being refined, but much has already been accomplished. The work that has received the most publicity involves gene expression profiling of breast cancers. In addition to identifying new subtypes of breast cancers, a 70-gene prognosis signature has been established.[196] It has been reported that the signature is a powerful predictor of disease prognosis for young patients and is particularly accurate for predicting metastasis during the first 5 years after diagnosis. Prognosis determined by gene expression profile correlates highly with histologic grade and estrogen receptor status but not with lymphatic spread of the tumor. A smaller panel of 21 genes is currently being used to assess the risk or recurrence and likely benefit of chemotherapy in a subset of breast cancer patients.[197]

The development of new microarray platforms and new technologies, such as high-throughput sequencing, make the methodical categorization of all the genomic changes present in a cancer cell a realistic possibility. Array-based comparative genomic hybridization can be used to look for alterations in genomic structure, such as amplifications and deletions. These changes can then be correlated to changes in gene expression. So-called single nucleotide polymorphism (SNP) chips, which include SNPs that span the entire genome, have been used in genome-wide linkage analysis (Chapter 5) and association studies to identify genes associated with increased risk of cancer.[198–200] Arrays tiled across the entire genome can be used to look for novel transcripts, novel promoters, and novel splice variants. These tiling arrays can also be used to identify epigenetic events, such as DNA methylation, and, when combined with a technique called chromatin immunoprecipitation, can map the genomic site of chromatin marks, as well as genomic binding sites of transcription factors. High-throughput resequencing methods, which can generate hundreds of millions to billions of base pairs in a single run, may allow identification of unknown fusion gene products, as well as efficient resequencing of entire cancer genomes.[201]

Next on the horizon of molecular techniques for the global analysis of cancers is *proteomics*, a technique used to obtain profiles of proteins contained in tissues, serum, or other body fluids. Indeed, with the realization that mRNA levels are regulated post-transcriptionally, it is not clear how closely the levels of proteins, the molecules that execute cellular processes, actually correlate with mRNA levels. Technologies to achieve global protein measurements, such as mass spectroscopy and antibody arrays, are currently being developed.

The excitement created by the development of new techniques for the global molecular analysis of tumors has led some scientists to predict that the end of histopathology is in sight, and to consider existing approaches to tumor diagnosis as the equivalent of magical methods of divination. Indeed, it is hard to escape the excitement generated by the development of entirely new and powerful methods of molecular analysis. However, what lies ahead is not the replacement of one set of techniques by another. On the contrary, the most accurate diagnosis and prognosis of cancer will be arrived at by a combination of morphologic and molecular techniques.

Tumor Markers

Biochemical assays for tumor-associated enzymes, hormones, and other tumor markers in the blood cannot be used for definitive diagnosis of cancer; however, they contribute to the detection of cancer and in some instances are useful in determining the effectiveness of therapy or the appearance of a recurrence.

A host of tumor markers have been described, and new ones are identified every year. Only a few have stood the test of time and proved to have clinical usefulness.

TABLE 7–12 Selected Tumor Markers

HORMONES	
Human chorionic gonadotropin	Trophoblastic tumors, nonseminomatous testicular tumors
Calcitonin	Medullary carcinoma of thyroid
Catecholamine and metabolites	Pheochromocytoma and related tumors
Ectopic hormones	See "Paraneoplastic Syndromes" (Table 7–11)
ONCOFETAL ANTIGENS	
α-Fetoprotein	Liver cell cancer, nonseminomatous germ cell tumors of testis
Carcinoembryonic antigen	Carcinomas of the colon, pancreas, lung, stomach, and heart
ISOENZYMES	
Prostatic acid phosphatase	Prostate cancer
Neuron-specific enolase	Small-cell cancer of lung, neuroblastoma
SPECIFIC PROTEINS	
Immunoglobulins	Multiple myeloma and other gammopathies
Prostate-specific antigen and prostate-specific membrane antigen	Prostate cancer
MUCINS AND OTHER GLYCOPROTEINS	
CA-125	Ovarian cancer
CA-19-9	Colon cancer, pancreatic cancer
CA-15-3	Breast cancer
NEW MOLECULAR MARKERS	
p53, APC, RAS mutants in stool and serum	Colon cancer
p53 and RAS mutants in stool and serum	Pancreatic cancer
p53 and RAS mutants in sputum and serum	Lung cancer
p53 mutants in urine	Bladder cancer

The application of several markers, listed in Table 7–12, is considered in the discussion of specific forms of neoplasia in other chapters, so only a few widely used examples suffice here. PSA, used to screen for prostatic adenocarcinoma, may be one of the most used, and most successful, tumor markers in clinical practice.[202] Prostatic carcinoma can be suspected when elevated levels of PSA are found in the blood. However, PSA screening also highlights problems encountered with virtually every tumor marker. Although PSA levels are often elevated in cancer, PSA levels also may be elevated in benign prostatic hyperplasia (Chapter 18). Furthermore, there is no PSA level that ensures that a person does not have prostate cancer. *Thus, the PSA test suffers from both low sensitivity and low specificity.* Other tumor markers occasionally used in clinical practice include CEA, which is elaborated by carcinomas of the colon, pancreas, stomach, and breast, and AFP, which is produced by hepatocellular carcinomas, yolk sac remnants in the gonads, and occasionally teratocarcinomas and embryonal cell carcinomas. Unfortunately, like PSA, both of these markers can be produced by a variety of non-neoplastic conditions as well. Thus, as with PSA levels, CEA and AFP assays lack both specificity and sensitivity required for the early detection of cancers. They are still useful in the detection of recurrences after excision. With successful resection of the tumor, these markers disappear from the serum; their reappearance almost always signifies the beginning of the end.

Other widely used markers include human chorionic gonadotropin for testicular tumors, CA-125 for ovarian tumors, and immunoglobulins in multiple myeloma and other secretory plasma cell tumors. The development of tests to detect cancer markers in blood and body fluids is an active area of research. Some of the markers being evaluated include the detection of mutated *APC, p53,* and *RAS* in the stool of individuals with colorectal carcinomas; the presence of mutated *p53* and of hypermethylated genes in the sputum of persons with lung cancer and in the saliva of persons with head and neck cancers; and the detection of mutated *p53* in the urine of patients with bladder cancer.

REFERENCES

1. Jemal A et al.: Cancer Statistics, 2008. CA Cancer J Clin 58:71, 2008.
2. Willis R: The Spread of Tumors in the Human Body. London, Butterworth, 1952.
3. Fusco A, Fedele M: Roles of HMGA proteins in cancer. Nat Rev Cancer 7:899, 2007.
4. Morrison SJ, Spradling C: Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132:598, 2008.
5. Jordan C, Guzman M, Noble M: Cancer stem cells. N Engl J Med 355:1253, 2006.
6. Ward R, Dirks P: Cancer stem cells: at the headwaters of tumor development. Annu Rev Pathol 2:175, 2007.
7. Al-Hajj M et al.: Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 100:3983, 2003.
8. O'Brien CA et al.: A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445:106, 2007.
9. Quintana E et al.: Efficient tumor formation by single human melanoma cells. Nature 456:593, 2008.
10. Park IK et al.: Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature 423:302, 2003.
11. Padera T et al.: Lymphatic metastasis in the absence of functional intratumor lymphatics. Science 296:1883, 2002.
12. Choi S-H et al.: Clinicopathologic analysis of sentinel lymph node mapping in early breast cancer. Breast J 9:153, 2003.

13. Covens A: Sentinel lymph nodes. Cancer 97:2945, 2003.
14. Ghafoor A et al.: Cancer statistics for African Americans. CA Cancer J Clin 52:326, 2002.
15. O'Brien K et al.: Cancer statistics for Hispanics, 2003. CA Cancer J Clin 53:208, 2003.
16. Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol 2:533, 2001.
17. Parkin DM et al.: Global cancer statistics, 2002. CA Cancer J Clin 55:74, 2005.
18. Calle E, Kaaks R: Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 4:579, 2004.
19. Knudson AG: Cancer genetics. Am J Med Genet 111:96, 2002.
20. Narod S: Modifiers of risk of hereditary breast cancer. Oncogene 25:5832, 2005.
21. Rustgi A: The genetics of hereditary colon cancer. Genes Dev 21:2525, 2007.
22. Easton D et al.: Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447:1087, 2007.
23. Pho LG et al.: Melanoma genetics: a review of genetic factors and clinical phenotypes in familial melanoma. Curr Opin Oncol 18:173, 2006.
24. Tlsty TD, Coussens LM: Tumor stroma and regulation of cancer development. Annu Rev Pathol 1:119, 2006.
25. Sinicrope FA: Targeting cyclooxygenase-2 for prevention and therapy of colorectal cancer. Mol Carcinog 45:447, 2006.
26. Howe LR, Dannenberg AJ: A role for cyclooxygenase-2 inhibitors in the prevention and treatment of cancer. Semin Oncol 29:111, 2002.
27. Gale RE: Evaluation of clonality in myeloid stem-cell disorders. Semin Hematol 36:361, 1999.
28. Santarosa M, Ashworth A: Haploinsufficiency for tumour suppressor genes: when you don't need to go all the way. Biochim Biophys Acta 1654:105, 2004.
29. Zhang W et al.: MicroRNAs in tumorigenesis: a primer. Am J Pathol 171:728, 2007.
30. Rana TM: Illuminating the silence: understanding the structure and function of small RNAs. Nat Rev Mol Cell Biol 8:23, 2007.
31. Loeb LA et al.: Multiple mutations and cancer. Proc Natl Acad Sci U S A 100:776, 2003.
32. Weinberg RA, Hanahan D: The hallmarks of cancer. Cell 100:57, 2000.
33. Halazonetis TD et al.: An oncogene-induced DNA damage model for cancer development. Science 319:1352, 2008.
34. Kern SE: Progressive genetic abnormalities in human neoplasia. In Mendelsohn J, Howley PM, Israel MA, et al (eds): The Molecular Basis of Cancer, 2nd ed. Philadelphia, WB Saunders, 2001, p 41.
35. Plaza-Menacho I et al.: Current concepts in RET-related genetics, signaling and therapeutics. Trends Genet 22:627, 2006.
36. Lakhani VT et al.: The multiple endocrine neoplasia syndromes. Annu Rev Med 58:253, 2007.
37. Badalamenti G et al.: Gastrointestinal stromal tumors (GISTs): focus on histopathological diagnosis and biomolecular features. Ann Oncol 18 (Suppl 6):vi36, 2007.
38. Rowinsky EK: The erbB family: targets for therapeutic development against cancer and therapeutic strategies using monoclonal antibodies and tyrosine kinase inhibitors. Annu Rev Med 55:433, 2004.
39. Hudis C: Trastuzumab—mechanism of action and use in clinical practice. N Engl J Med 357:39, 2007.
40. Malumbres M, Barbacid M: *RAS* oncogenes: the first 30 years. Nat Rev Cancer 3:459, 2003.
41. Jaffee EM et al.: Focus on pancreas cancer. Cancer Cell 2:25, 2002.
42. Minna JD et al.: Focus on lung cancer. Cancer Cell 1:49, 2002.
43. Hingorani SR, Tuveson DA: Ras redux: rethinking how and where Ras acts. Curr Opin Genet Dev 13:6, 2003.
44. Michaloglou C et al.: BRAFE600 in benign and malignant human tumours. Oncogene 27:877, 2007.
45. Pollock P et al.: High frequency of BRAF mutations in nevi. Nat Genet 33:19, 2003.
46. Krause DS, Van Eetten RA: Tyrosine kinases as targets for cancer therapy. N Engl J Med 353:172, 2005.
47. Goldman JM, Melo JV: Chronic myeloid leukemia—advances in biology and new approaches to treatment. N Engl J Med 349:1451, 2003.
48. Kurzrock R et al.: Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. Ann Intern Med 138:819, 2003.
49. Sattler M, Griffin JD: Molecular mechanisms of transformation by the *BCR-ABL* oncogene. Semin Hematol 40:4, 2003.
50. Sharma SV and Settleman J: Oncogene addiction: setting the stage for molecularly targeted cancer therapy. Genes and Development 21:3214, 2007.
51. Campbell PJ, Green AR: The myeloproliferative disorders. N Engl J Med 355:2452, 2006.
52. Patel JH et al.: Analysis of genomic targets reveals complex functions of MYC. Nat Rev Cancer 4:562, 2004.
53. Adhikary S, Eilers M: Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 6:635, 2005.
54. Dominguez-Sola D et al.: Non-transcriptional control of DNA replication by c-Myc. Nature 448:445, 2007.
55. Meyer N et al.: The Oscar-worthy role of Myc in apoptosis. Semin Cancer Biol 16:275, 2006.
56. Chu I et al.: The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev Cancer 8:253, 2008.
57. Kim WY, Sharpless NE: The regulation of INK4/ARF in cancer and aging. Cell 127:265, 2006.
58. Bartek J, Lukas J: Mammalian G_1- and S-phase checkpoints in response to DNA damage. Curr Opin Cell Biol 13:738, 2001.
59. Kastan MB, Bartek J: Cell cycle checkpoints and cancer. Nature 432:316, 2004.
60. Knudson A: Two genetic hits (more or less) to cancer. Nat Rev Cancer 1:157, 2001.
61. Kaelin WG: von Hippel-Lindau disease. Annu Rev Pathol 2:145, 2007.
62. Massague J: G_1 cell-cycle control and cancer. Nature 432:298, 2004.
63. Ji P et al.: An Rb–Skp2–p27 pathway mediates acute cell cycle inhibition by Rb and is retained in a partial-penetrance Rb mutant. Mol Cell 16:47, 2004.
64. Binne UK et al.: Retinoblastoma protein and anaphase-promoting complex physically interact and functionally cooperate during cell-cycle exit. Nature Cell Biol 9:225, 2007.
65. Skapek SX et al.: Regulation of cell lineage specification by the retinoblastoma tumor suppressor. Oncogene 25:5268, 2006.
66. Macaluso M et al.: Rb family proteins as modulators of gene expression and new aspects regarding the interaction with chromatin remodeling enzymes. Oncogene 25:5263, 2006.
67. Ajioka I et al.: Differentiated horizontal interneurons clonally expand to form metastatic retinoblastoma in mice. Cell 131:378, 2007.
68. Sherr CJ, McCormick F: The RB and p53 pathways in cancer. Cancer Cell 2:103, 2002.
69. Vousden K, Lane D: p53 in health and disease. Nat Rev Mol Cell Biol 8:275, 2007.
70. Frebourg T et al.: Germ-line p53 mutations in 15 families with Li-Fraumeni syndrome. Am J Hum Genet 56:608, 1995.
71. Nichols KE et al.: Germ-line p53 mutations predispose to a wide spectrum of early-onset cancers. Cancer Epidemiol Biomarkers Prev 10:83, 2001.
72. Onel K, Cordon-Cardo C: MDM2 and prognosis. Mol Cancer Res 2:1, 2004.
73. Shmueli A, Oren M: Regulation of p53 by Mdm2: fate is in the numbers. Mol Cell 13:4, 2004.
74. Wei CL et al.: A global map of p53 transcription-factor binding sites in the human genome. Cell 124: 207, 2006.
75. Riley T et al.: Transcriptional control of human p53-regulated genes. Nature Rev Mol Cell Biol 402:402, 2008.
76. He L et al.: microRNAs join the p53 network—another piece in the tumour-suppression puzzle. Nat Rev Cancer 7:819, 2007.
77. Shiloh Y: The ATM-mediated DNA-damage response: taking shape. Trends Biochem Sci 31:402, 2006.
78. Cimprich KA, Cortez D: ATR: an essential regulator of genome integrity. Nature Rev Med 9:616, 2008.
79. Di Micco R et al.: Breaking news: high-speed race ends in arrest—how oncogenes induce senescence. Trends Cell Biol 17:529, 2007.
80. Murray-Zmijewski et al.: A complex barcode underlies the heterogenous response of p53 to stress. Nature Rev Med 9:702, 2008.
81. Deyoung M, Ellisen L: p63 and p73 in human cancer: defining the network. Oncogene 26:5169, 2007.
82. Ratovitski E et al.: p63 and p73: teammates or adversaries? Cancer Cell 9:1, 2006.
83. Leong C et al.: The p63/p73 network mediates chemosensitivity to cisplatin in a biologically defined subset of primary breast cancers. J Clin Invest 117:1370, 2007.
84. Shibata H et al.: Rapid colorectal adenoma formation initiated by conditional targeting of the *Apc* gene. Science 278:120, 1997.

85. Polakis P: The many ways of Wnt in cancer. Curr Opin Genet Dev 17:45, 2007.
86. Wei Y et al.: Activation of β-catenin in epithelial and mesenchymal hepatoblastomas. Oncogene 19:498, 2000.
87. Hirohashi S, Kanai Y: Cell adhesion system and human cancer morphogenesis. Cancer Sci 94:575, 2003.
88. Thiery J, Sleeman J: Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7:131, 2006.
89. Bierie B, Moses H: Tumour microenvironment: TGFβ: the molecular Jekyll and Hyde of cancer. Nat Rev Cancer 6:506, 2006.
90. Jiang B-H, Liu L-Z: PI3K/PTEN signaling in tumorigenesis and angiogenesis. Biochim Biophys Acta 1784:150, 2008.
91. Chaloub N, Baker SJ: PTEN and PI3-kinase pathway in cancer. Ann Rev Path Mech Dis 4:97, 2009.
92. Gutmann D, Collins F (eds): Neurofibromatosis 1, 2nd ed. In Vogelstein B, Kinzler K (eds): The Genetic Basis of Human Cancer. New York, McGraw-Hill, 2002, p 417–437.
93. MacCollin M, Gusella J (eds): Neurofibromatosis 2. In Vogelstein B, Kinzler K (eds): The Genetic Basis of Human Cancer. New York, McGraw-Hill, 2002, p 439.
94. Harvey K, Tapon N: The Salvador-Warts-Hippo pathway—an emerging tumour-suppressor network. Nat Rev Cancer 7:182, 2007.
95. Haber D (ed): Wilms tumor. In Vogelstein B, Kinzler K (eds): The Genetic Basis of Human Cancer. New York, McGraw-Hill, 2002, p 403.
96. Beachy PA et al.: Tissue repair and stem cell renewal in carcinogenesis. Nature 432:324, 2004.
97. Evan GI, Vousden KH: Proliferation, cell cycle and apoptosis in cancer. Nature 411:342, 2001.
98. Korsmeyer SJ: Programmed cell death and the regulation of homeostasis. Harvey Lect 95:21, 1999.
99. Igney FH, Krammer PH: Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2:277, 2002.
100. Green D, Kroemer G: The pathophysiology of mitochondrial cell death. Science 305:626, 2004.
101. Danial NN, Korsmeyer SJ: Cell death: critical control points. Cell 116:205, 2004.
102. Deng Y et al.: Telomere dysfunction and tumor suppression: the senescence connection. Nature Rev. Cancer 8:450, 2008.
103. Sharpless N, DePinho R: Telomeres, stem cells, senescence, and cancer. J Clin Invest 113:160, 2004.
104. Nagy J et al.: VEGF-A and the induction of pathological angiogenesis. Annu Rev Pathol 2:251, 2007.
105. Bergers G, Benjamin L: Tumorigenesis and the angiogenic switch. Nat Rev Cancer 3:401, 2003.
106. Sonpavde G et al.: Bevacizumab in colorectal cancer. N Engl J Med 351:1690, 2004.
107. Noguera-Troise I et al.: Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. Nature 444:1032, 2006.
108. Ridgway J et al.: Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. Nature 444:1083, 2006.
109. Fidler IJ: The pathogenesis of cancer metastasis: the "seed and soil" hypothesis revisited. Nat Rev Cancer 3:453, 2003.
110. Bissell MJ, Radisky D: Putting tumours in context. Nat Rev Cancer 1:46, 2001.
111. Radisky D, Muschler J, Bissell MJ: Order and disorder: the role of extracellular matrix in epithelial cancer. Cancer Invest 20:139, 2002.
112. Overall CM, Kleifeld O: Validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nature Rev Cancer 6:227, 2006.
113. Sahai E: Illuminating the metastatic cascade. Nature Rev Cancer 7:737, 2007.
114. Epstein RJ: The CXCL12-CXCR4 chemotactic pathway as a target of adjuvant breast cancer therapies. Nat Rev Cancer 4:901, 2004.
115. Steeg P: Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 12:895, 2006.
116. Ramaswamy S et al.: A molecular signature of metastasis in primary solid tumors. Nat Genet 33:49, 2003.
117. Nguyen D, Massague J: Genetic determinants of cancer metastasis. Nat Rev Genet 8:341, 2007.
118. Steeg PZ: Metastasis suppressors alter the signal transduction of cancer cells. Nat Rev Cancer 3:55, 2003.
119. Tavazoie SF et al.: Endogenous human microRNAs that suppress breast cancer metastases. Nature 451:157, 2008.
120. Ma L et al.: Tumor invasions and metastases initiated by microRNA-10b in breast cancer. Nature 449:682, 2008.
121. Peindao H et al.: Snail, ZEB and bHLH factors in tumour progression; an alliance against the epithelial phenotype? Nature Rev Cancer 7:415, 2007.
122. Hoeijmakers JH: Genome maintenance mechanisms for preventing cancer. Nature 411:366, 2001.
123. Lynch HT, de la Chapelle A: Hereditary colorectal cancer. N Engl J Med 348:919, 2003.
124. Jiricny J, Marra G: DNA repair defects in colon cancer. Curr Opin Genet Dev 13:61, 2003.
125. Friedberg EC: How nucleotide excision repair protects against cancer. Nat Rev Cancer 1:22, 2001.
126. Wang W: Emergence of a DNA-damage response network consisting of Fanconi anaemia and BRCA proteins. Nat Rev Genet 8:735, 2007.
127. Hickson ID et al.: Role of the Bloom's syndrome helicase in maintenance of genome stability. Biochem Soc Trans 29:201, 2001.
128. Venkatiraman AR: Linking the cellular functions of *BRCA* gene to cancer pathogenesis and treatment. Ann Rev Path Mech Dis 4:435, 2009.
129. Condeelis J, Pollard JW: Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 124:263, 2006.
130. Cunha GR et al.: Role of stromal microenvironment in carcinogenesis of prostate. Int J Cancer 107:1, 2003.
131. Finak G et al.: Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14:518, 2008.
132. Yeung SJ et al.: Roles of p53, Myc, and HIF1 in regulating glycolysis—the seventh hallmark of cancer. Cell Mol Life Sci 2008. Advance Online Publication.
133. DeBerardinis RJ et al.: Brick by brick: metabolism and tumor cell growth. Curr Opin Gen Devel 18:54, 2008.
134. Hsu PP, Sabatini DM: Cancer cell metabolism: warburg and beyond. Cell 134:703, 2008.
135. Dang CV et al.: The interplay between MYC and HIF in cancer. Nature Rev Cancer 8:51, 2008.
136. Denko NC: Hypoxia, HIF1 and glucose metabolism in the solid tumor. Nature Rev Cancer 8:705, 2008.
137. Tomlins SA et al.: Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 310:644, 2005.
138. Kumar-Sinha C et al.: Recurrent gene fusions in prostate cancer. Nature Rev Cancer 8:497, 2008.
139. Hogarty MD, Brodeur GM: Gene amplification in human cancers: biological and clinical significance. In Vogelstein B, Kinzler KW (eds): The Genetic Basis of Human Cancer, 2nd ed. New York, McGraw-Hill, 2002, pp 115–128.
140. Ting A et al.: The cancer epigenome—components and functional correlates. Genes Dev 20:3215, 2006.
141. Esteller M: Epigenetics in cancer. N Engl J Med 358:1148, 2008.
142. Dutta A, Lee YS: MicroRNA in cancer. Ann Rev Path Mech Dis 4:175, 2009.
143. Hahn W, Weinberg R: Rules for making human tumor cells. N Engl J Med 347:1593, 2002.
144. Wood LD et al.: The genomic landscapes of human breast and colorectal cancers. Science. 318(5853):1108, 2007.
145. Cichowski K, Hahn WC: Unexpected pieces of the senescence puzzle. Cell 133:958, 2008.
146. Tennant R: Chemical carcinogenesis. In Franks LM, Teich NM (eds): An Introduction to the Cellular and Molecular Biology of Cancer, 3rd ed. Oxford, Oxford University Press, 1997, pp 106–125.
147. Perera F: Environment and cancer: who are susceptible? Science 278:1068, 1997.
148. Vineis P et al.: CYP1A1 T3801 C polymorphism and lung cancer: a pooled analysis of 2,451 cases and 3,358 controls. Int J Cancer 104:650, 2003.
149. Hecht SS: Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol 3:461, 2002.
150. Palli D et al.: Biomarkers of dietary intake of micronutrients modulate DNA adduct levels in healthy adults. Carcinogenesis 24:739, 2003.
151. Hussain S et al.: *TP53* mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 26:2166, 2007.
152. Preston DL et al.: Radiation effects on breast cancer risk: a pooled analysis of eight cohorts. Radiat Res 158:220, 2002.
153. Cleaver JE, Crowley E: UV damage, DNA repair and skin carcinogenesis. Front Biosci 7:1024, 2002.

154. Neronova E et al.: Chromosome alterations in cleanup workers sampled years after the Chernobyl accident. Radiat Res 160:46, 2003.
155. Matsuoka M, Jeang K.-T: Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. Nat Rev Cancer 7:270, 2007.
156. Grassmann R et al.: Molecular mechanisms of cellular transformation by HTLV-1 Tax. Oncogene 24:5976, 2005.
157. McLaughlin-Drubin ME, Munger K: Viruses associated with human cancer. Biochim Biophys Acta 1782:127, 2008.
158. Woodman C et al.: The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer 7:11, 2007.
159. zur Hausen H: Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2:342, 2002.
160. Zehbe I et al.: Codon 72 polymorphism of and its association with cervical cancer. The Lancet 354:218, 1999.
161. Kutok J, Wang F: Spectrum of Epstein-Barr virus–associated diseases. Annu Rev Pathol 1:375, 2006.
162. Thorley-Lawson D: Epstein-Barr virus: exploiting the immune system. Nat Rev Immunol 1:75, 2001.
163. Thorley-Lawson DA, Gross A: Mechanism of disease: persistence of Epstein-Barr virus and the origins of associated lymphomas. N Engl J Med 350:1328, 2004.
164. Lindstrom MS, Wiman KG: Role of genetic and epigenetic changes in Burkitt lymphoma. Semin Cancer Biol 12:381, 2002.
165. Raab-Traub N: Epstein-Barr virus in the pathogenesis of NPC. Semin Cancer Biol 12:431, 2002.
166. Tang H et al.: Molecular functions and biological roles of hepatitis B virus x protein. Cancer Sci 97:977, 2006.
167. Kremsdorf D et al.: Hepatitis B virus–related hepatocellular carcinoma: paradigms for viral-related human carcinogenesis. Oncogene 25:3823, 2006.
168. Levrero M: Viral hepatitis and liver cancer: the case of hepatitis C. Oncogene 25:3834, 2006.
169. Peek RM Jr, Crabtree JE: *Helicobacter* infection and gastric neoplasia. J Pathol 208:233, 2006.
170. Burnet FM: The concept of immunological surveillance. Prog Exper Tumor Res 13:1, 1970.
171. Dunn GP et al.: Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991, 2002.
172. Aptsiauri N et al.: MHC class I antigens and immune surveillance in transformed cells. Int Rev Cytol 256:139, 2007.
173. Zitvogel L et al.: Cancer despite immunosurveillance: immunoselection and immunosubversion. Nat Rev Immunol 6:715, 2006.
174. Kim R et al.: Cancer immunoediting from immune surveillance to immune escape. Immunology 121:1, 2007.
175. Dunn GP et al.: Interferons, immunity and cancer immunoediting. Nat Rev Immunol 6(11):836, 2006.
176. Coulie PG, Hanagiri T, Takenoyama M: From tumor antigens to immunotherapy. Int J Clin Oncol 6:163, 2001.
177. Pardoll D: Does the immune system see tumors as foreign or self? Annu Rev Immunol 21:807, 2003.
178. Boon T, Van den Eynde B: Tumour immunology. Curr Opin Immunol 15:129, 2003.
179. Castelli C et al.: T-cell recognition of melanoma-associated antigens. J Cell Physiol 182:323, 2000.
180. Barker PA, Salehi A: The MAGE proteins: emerging roles in cell cycle progression, apoptosis, and neurogenetic disease. J Neurosci Res 67:705, 2002.
181. Cerwenka A, Lanier LL: Natural killer cells, viruses and cancer. Nat Rev Immunol 1:41, 2001.
182. Latour S, Veillette A: Molecular and immunological basis of X-linked lymphoproliferative disease. Immunol Rev 192:212, 2003.
183. Strand S, Galle PR: Immune evasion by tumours: involvement of the CD95 (APO-1/Fas) system and its clinical implications. Mol Med Today 4:63, 1998.
184. Hanahan D, Lanzavecchia A, Mihich E: The novel dichotomy of immune interactions with tumors. Cancer Res 63:3005, 2003.
185. Acharyya S, Guttridge D: Cancer cachexia signaling pathways continue to emerge yet much still points to the proteasome. Clin Cancer Res 13:1356, 2007.
186. Darnell R, Posner J: Paraneoplastic syndromes involving the nervous system. N Engl J Med 349:1543, 2003.
187. Mazzone PJ, Arroliga AC: Endocrine paraneoplastic syndromes in lung cancer. Curr Opin Pulm Med 9:313, 2003.
188. Hoey RP et al.: The parathyroid hormone-related protein receptor is expressed in breast cancer bone metastases and promotes autocrine proliferation in breast carcinoma cells. Br J Cancer 88:567, 2003.
189. Swansbury J: Some difficult choices in cytogenetics. Methods Mol Biol 220:245, 2003.
190. Rowland JM: Molecular genetic diagnosis of pediatric cancer: current and emerging methods. Pediatr Clin North Am 49:1415, 2002.
191. Bayani J, Squire JA: Advances in the detection of chromosomal aberrations using spectral karyotyping. Clin Genet 59:65, 2001.
192. Louis DN, Pomeroy SL, Cairncross JG: Focus on central nervous system neoplasia. Cancer Cell 1:125, 2002.
193. Lakhani SR, Ashworth A: Microarray and histopathological analysis of tumours: the future and the past? Nat Rev Cancer 1:151, 2001.
194. Riggins GJ, Morin PJ: Gene expression profiling in cancer. In Vogelstein B, Kinzler KW (eds): The Genetic Basis of Human Cancers, 2nd ed. New York, McGraw-Hill, 2002, pp 131–141.
195. Rosenwald A et al.: The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. New Engl J Med 346:1937, 2002.
196. van de Vijver MJ et al.: A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999, 2002.
197. Paik S et al.: A Multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351(27):2817, 2004.
198. Eeles RA et al.: Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 40(3):316, 2008.
199. Thomas G et al.: Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 40(3):310, 2008.
200. Hunter DJ et al.: A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet 39(7):870, 2007.
201. Campbell PJ et al.: Identification of somatically acquired rearrangements in cancer using genome-wide massively parallel paired-end sequencing. Nat Genet 40(6):722, 2008.
202. Lilja H et al.: Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nature Rev Cancer 8(4):268, 2008.

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