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Chapter 7 - Neoplasia

In the year 2000, there were 10 million new cases of cancer and 6 million cancer deaths worldwide.[1] [2] In the United States each year, almost 1.5 million individuals learn for the first time that they have some type of cancer. Not included in these figures are more than 1 million new cases of the most common types of nonpigmented skin cancers and incipient, noninvasive cancers. Not only these noninvasive lesions but many invasive tumors as well can be cured. Nonetheless, according to American Cancer Society estimates, cancer caused approximately 556,000 deaths in 2003, corresponding to 1500 cancer deaths per day, accounting for about 23% of all deaths in the United States.[3] Some good news, however, has emerged: cancer mortality for both men and women in the United States declined during the last decade of the 20th century.[4] Thus, there has been progress, but the problem is still overwhelming. The discussion that follows deals with both benign tumors and cancers; the latter receive more attention. The focus is on the basic morphologic and biologic properties of tumors and on the present understanding of the molecular basis of carcinogenesis. We also discuss the interactions of the tumor with the host and the host response to tumors. Although the discussion of therapy is beyond the scope of this chapter, there are now dramatic improvements in therapeutic responses and 5-year survival rates with many forms of malignancy, notably the leukemias and lymphomas. A greater proportion of cancers is being cured or arrested today than ever before.

Definitions

Neoplasia literally means the process of "new growth," and a new growth is called a *neoplasm*. The term *tumor* was originally applied to the swelling caused by inflammation. Neoplasms also may induce swellings, but by long precedent, the non-neoplastic usage of *tumor* has passed into limbo; thus, the term is now equated with neoplasm. *Oncology* (Greek *oncos* = tumor) is the study of tumors or neoplasms. *Cancer is the common term for all malignant tumors.* Although the ancient origins of this term are somewhat uncertain, it probably derives from the Latin for crab, *cancer*—presumably because a cancer "adheres to any part that it seizes upon in an obstinate manner like the crab."

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[5] 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 *heritable 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 cells within a tumor arises from a single cell that has incurred genetic change, and hence tumors are said to be *clonal*.

Nomenclature

All tumors, benign and malignant, have two basic components: (1) proliferating neoplastic cells that constitute their *parenchyma* and (2) supportive *stroma* made up of connective tissue and blood vessels. Although parenchymal cells represent the proliferating "cutting edge" of neoplasms and so determine their behavior and pathologic consequences, the growth and evolution of neoplasms are critically dependent on their stroma. An adequate stromal blood supply is requisite, and the stromal connective tissue provides the framework for the parenchyma. In addition, there is cross-talk between tumor cells and stromal cells that appears to directly influence the growth of tumors. In some tumors, the stromal support is scant and so the neoplasm is soft and fleshy. Sometimes the parenchymal cells stimulate the formation of an abundant collagenous stroma, referred to as *desmoplasia*. Some tumors—for example, some cancers of the female breast—are stony hard or *scirrhous*. The nomenclature of tumors is, however, based 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 from fibroblastic cells is called a *fibroma*, a cartilaginous tumor is a *chondroma*, and a tumor of osteoblasts is an *osteoma*. In contrast, nomenclature of benign epithelial tumors is more complex. They are variously classified, some based on their cells of origin, others on microscopic architecture, and still others on their macroscopic patterns.

Adenoma is the term applied to a benign epithelial neoplasm that forms glandular patterns as well as to tumors derived from glands but not necessarily reproducing glandular patterns. 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 in no distinctive pattern. Benign epithelial neoplasms producing microscopically or macroscopically visible finger-like or warty projections from epithelial surfaces are referred to as *papillomas* (Fig. 7-1). Those that form large cystic masses, as in the ovary, are referred to as *cystadenomas*.

Figure 7-1 Papilloma of the colon with finger-like projections into the lumen. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

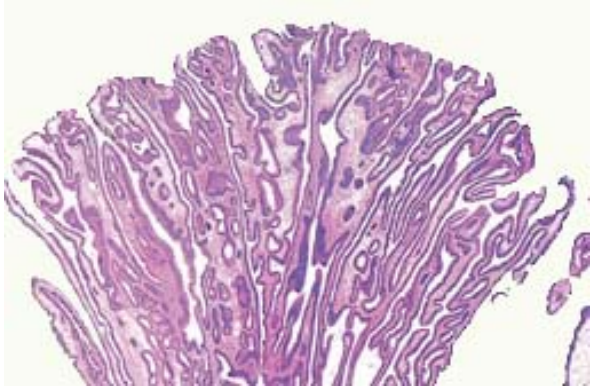

Figure 7-2 Colonic polyp. *A*, This benign glandular tumor (adenoma) is projecting into the colonic lumen and is attached to the mucosa by a distinct stalk. *B*, Gross appearance of several colonic polyps.

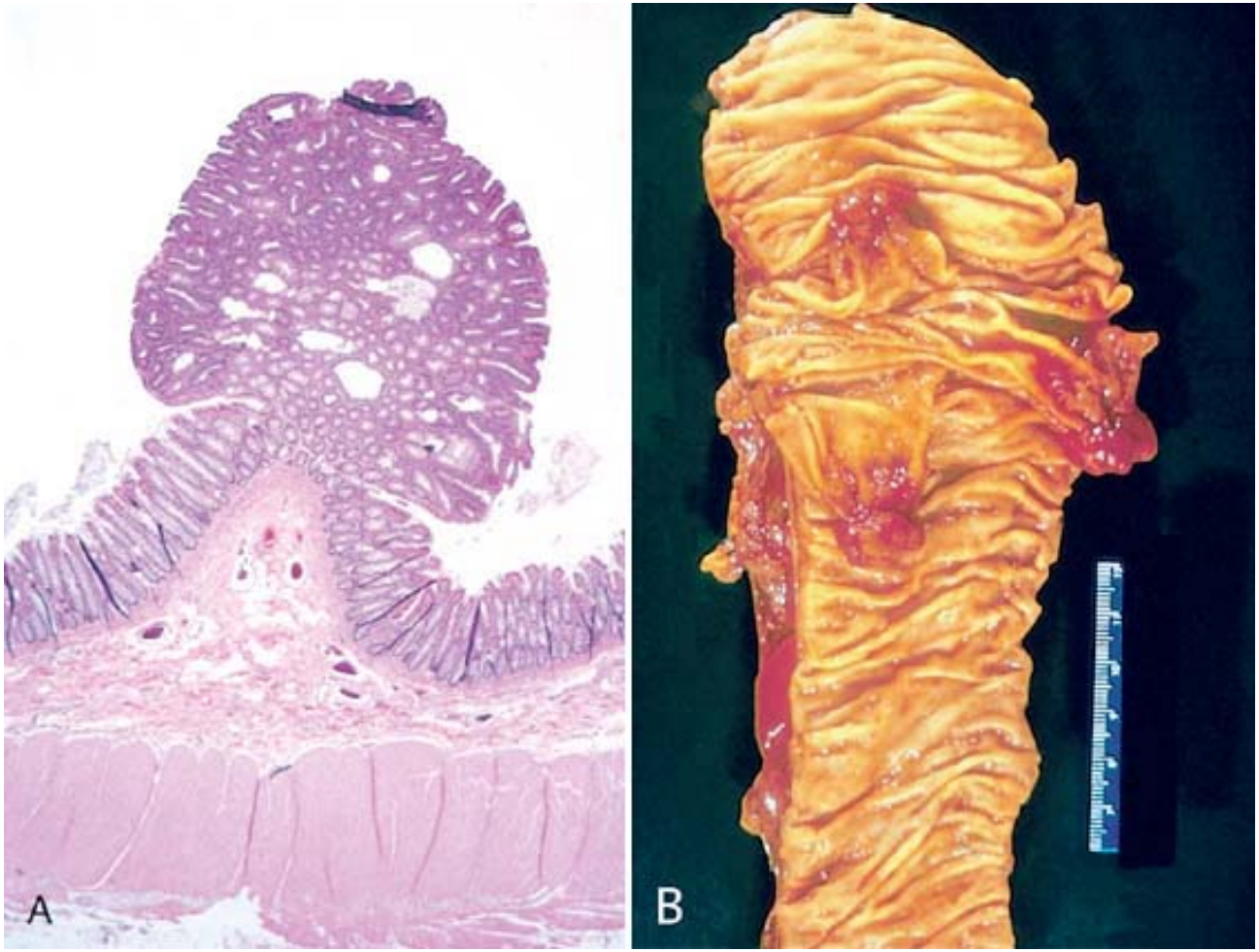

Figure 7-3 This mixed tumor of the parotid gland contains epithelial cells forming ducts and myxoid stroma that resembles cartilage. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

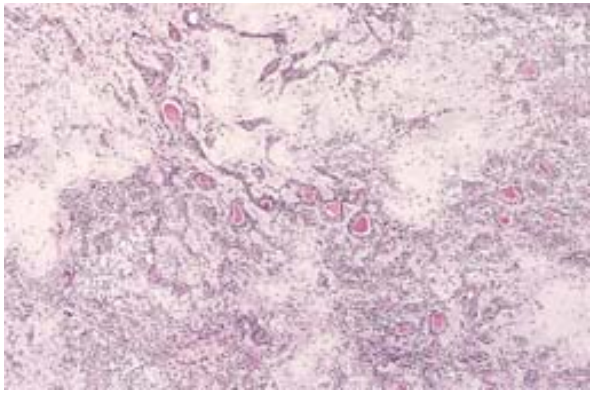

Figure 7-4 *A,* Gross appearance of an opened cystic teratoma of the ovary. Note the presence of hair, sebaceous material, and tooth. *B,* A microscopic view of a similar tumor shows skin, sebaceous glands, fat cells, and a tract of neural tissue *(arrow).*

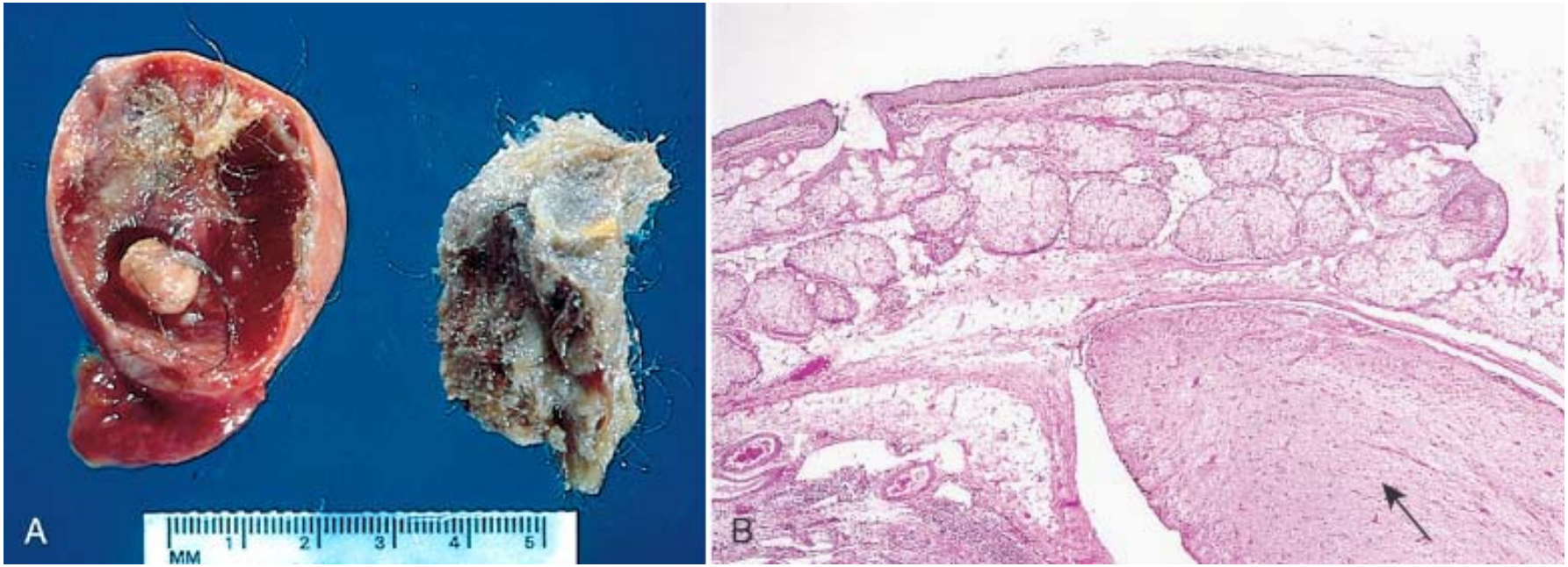

TABLE 7-1 -- Nomenclature of Tumors

Tissue of Origin	Benign	Malignant
Composed of One Parenchymal Cell Type		
Tumors of mesenchymal origin		
••Connective tissue and derivatives	Fibroma	Fibrosarcoma
	Lipoma	Liposarcoma
	Chondroma	Chondrosarcoma
	Osteoma	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		Leukemias
••Lymphoid tissue		Lymphomas
Muscle		
••Smooth	Leiomyoma	Leiomyosarcoma
••Striated	Rhabdomyoma	Rhabdomyosarcoma
Tumors of epithelial origin		
••Stratified squamous	Squamous cell papilloma	Squamous cell or epidermoid carcinoma
••Basal cells of skin or adnexa		Basal cell carcinoma
••Epithelial lining of glands or ducts	Adenoma	Adenocarcinoma
	Papilloma	Papillary carcinomas
	Cystadenoma	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

have primitive-appearing, unspecialized cells. In general, benign tumors are well differentiated (Fig. 7-6). The neoplastic cell in a benign smooth muscle tumor—a leiomyoma—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 massing of these cells into a nodule discloses the neoplastic nature of the lesion. One may get so close to the tree that one loses sight of the forest.

Malignant neoplasms, in contrast, range from well differentiated to undifferentiated. Malignant neoplasms composed of undifferentiated cells are said to be *anaplastic*. Lack of

differentiation, or *anaplasia,* is considered a hallmark of malignant transformation. Anaplasia literally means "to form backward," implying a reversion from a high level of differentiation to a lower level. There is substantial evidence, however, that most cancers do not represent "reverse differentiation" of mature normal cells but, in fact, arise from stem cells that are present in all specialized tissues. The well-differentiated cancer (Fig. 7-7) evolves from maturation or specialization of undifferentiated cells as they proliferate, whereas the undifferentiated malignant tumor derives from proliferation without complete maturation of the transformed cells.

Lack of differentiation, or anaplasia, is marked by a number of morphologic changes.

• *Pleomorphism.* Both the cells and the nuclei characteristically display *pleomorphism*—variation in size and shape (Fig. 7-8). Cells may be found that are many times larger than their neighbors, and other cells may be extremely small and primitive appearing.

• *Abnormal nuclear morphology.* Characteristically the nuclei contain an abundance of DNA and are extremely dark staining *(hyperchromatic).* The nuclei are disproportionately large for the cell, and the nucleus-to-cytoplasm ratio may approach 1:1 instead of the normal 1:4 or 1:6. The nuclear shape is very variable, 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 malignant neoplasia are atypical, bizarre mitotic figures, sometimes producing tripolar, quadripolar, or multipolar spindles (Fig. 7-9).
• *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 nuclei. 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. In the cancer giant cell, the nuclei are hyperchromatic and large in relation to the cell. Although growing tumor cells obviously require a blood

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supply, often the vascular stroma is scant, and in many anaplastic tumors, large central areas undergo ischemic *necrosis.*

Figure 7-5 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.

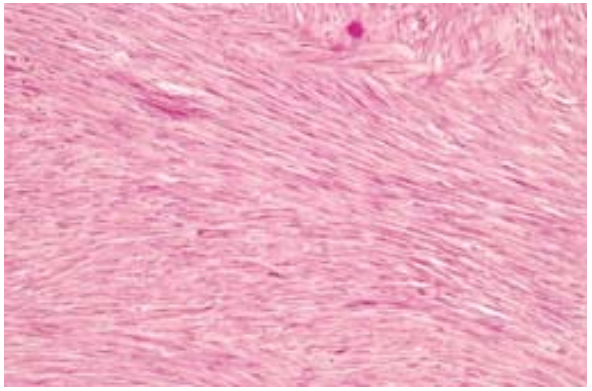

Figure 7-6 Benign tumor (adenoma) of the thyroid. Note the normal-looking (well-differentiated), colloid-filled thyroid follicles. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

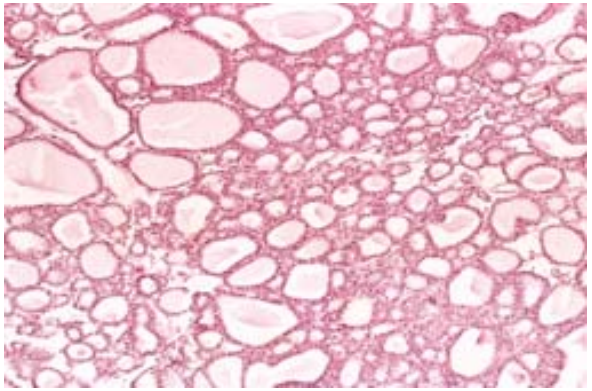

Figure 7-7 Malignant tumor (adenocarcinoma) of the colon. Note that compared with the well-formed and normal-looking glands characteristic of a benign tumor (see Fig. 7-6), the cancerous glands are irregular in shape and size and do not resemble the normal colonic glands. This tumor is considered differentiated 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.)*

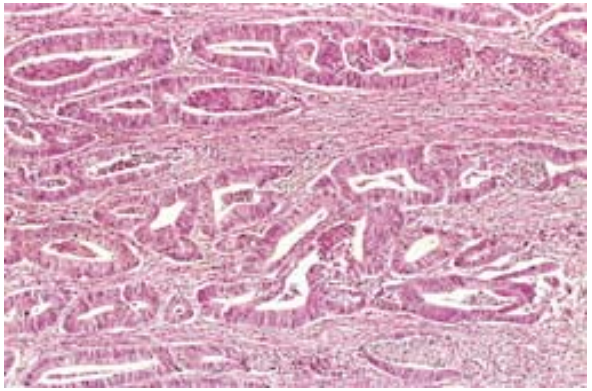

Figure 7-8 Anaplastic tumor of the skeletal muscle (rhabdomyosarcoma). Note the marked cellular and nuclear pleomorphism, hyperchromatic nuclei, and tumor giant cells. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

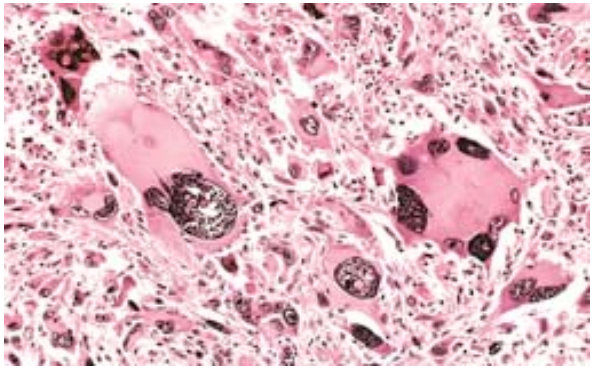

Figure 7-9 Anaplastic tumor showing cellular and nuclear variation in size and shape. The prominent cell in the center field has an abnormal tripolar spindle.

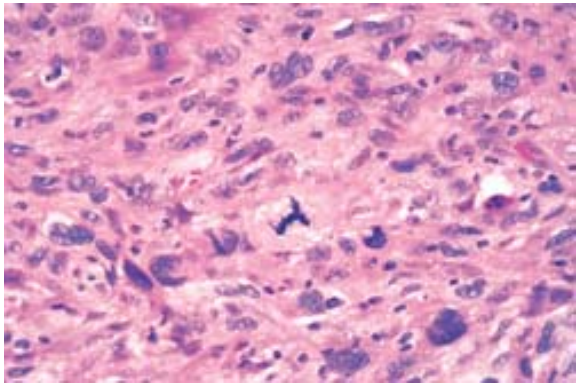

Figure 7-10 Well-differentiated squamous cell carcinoma of the skin. The tumor cells are strikingly similar to normal squamous epithelial cells, with intercellular bridges and nests of keratin pearls *(arrow). (Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

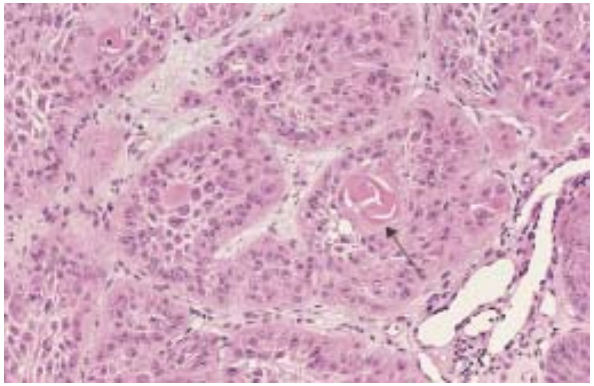

Figure 7-11 *A,* Carcinoma in situ. This low-power view shows that the entire thickness of the epithelium is replaced by atypical dysplastic cells. There is no orderly differentiation of squamous cells. The basement membrane is intact and there is no tumor in the subepithelial stroma. *B,* A high-power 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 *(below)* is not seen in this section.

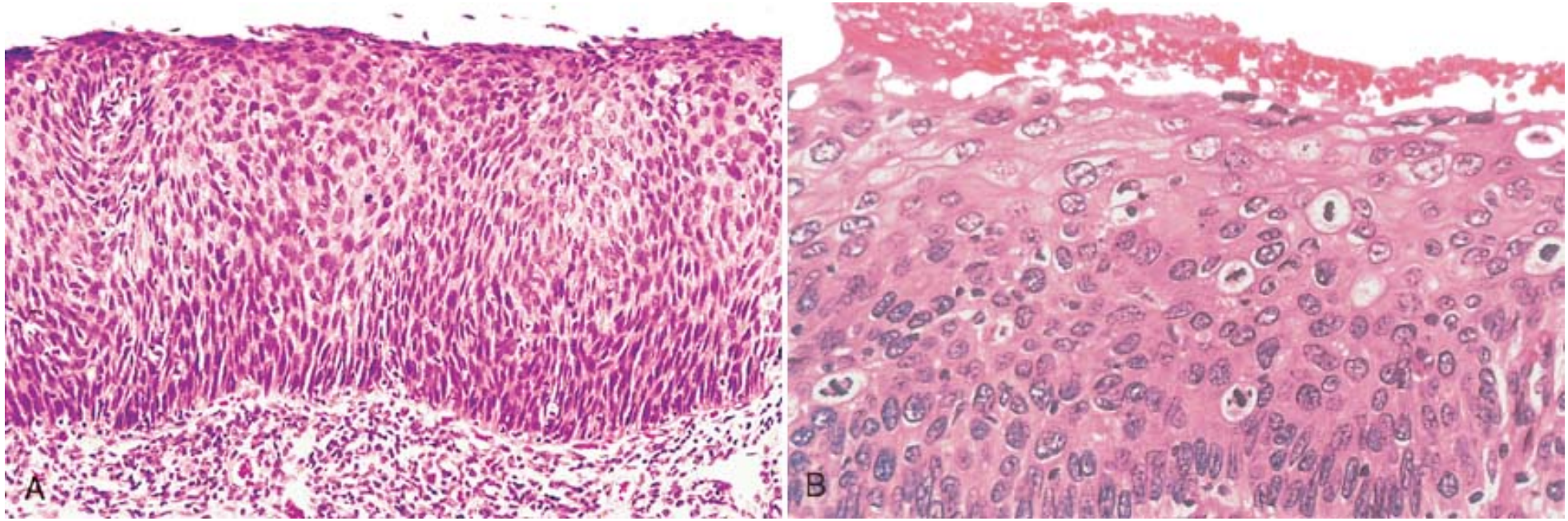

Figure 7-12 Biology of tumor growth. The left panel depicts minimal estimates of tumor cell doublings that precede the formation of a clinically detectable tumor mass. It is evident that by the time a solid tumor is detected, it has already completed a major portion of its life cycle as measured by cell doublings. The right panel illustrates clonal evolution of tumors and generation of tumor cell heterogeneity. New subclones arise from the descendants of the original transformed cell, and with progressive growth the tumor mass becomes enriched for those variants that are more adept at evading host defenses and are likely to be more aggressive. *(Adapted from Tannock IF: Biology of tumor growth. Hosp Pract 18:81, 1983.)*

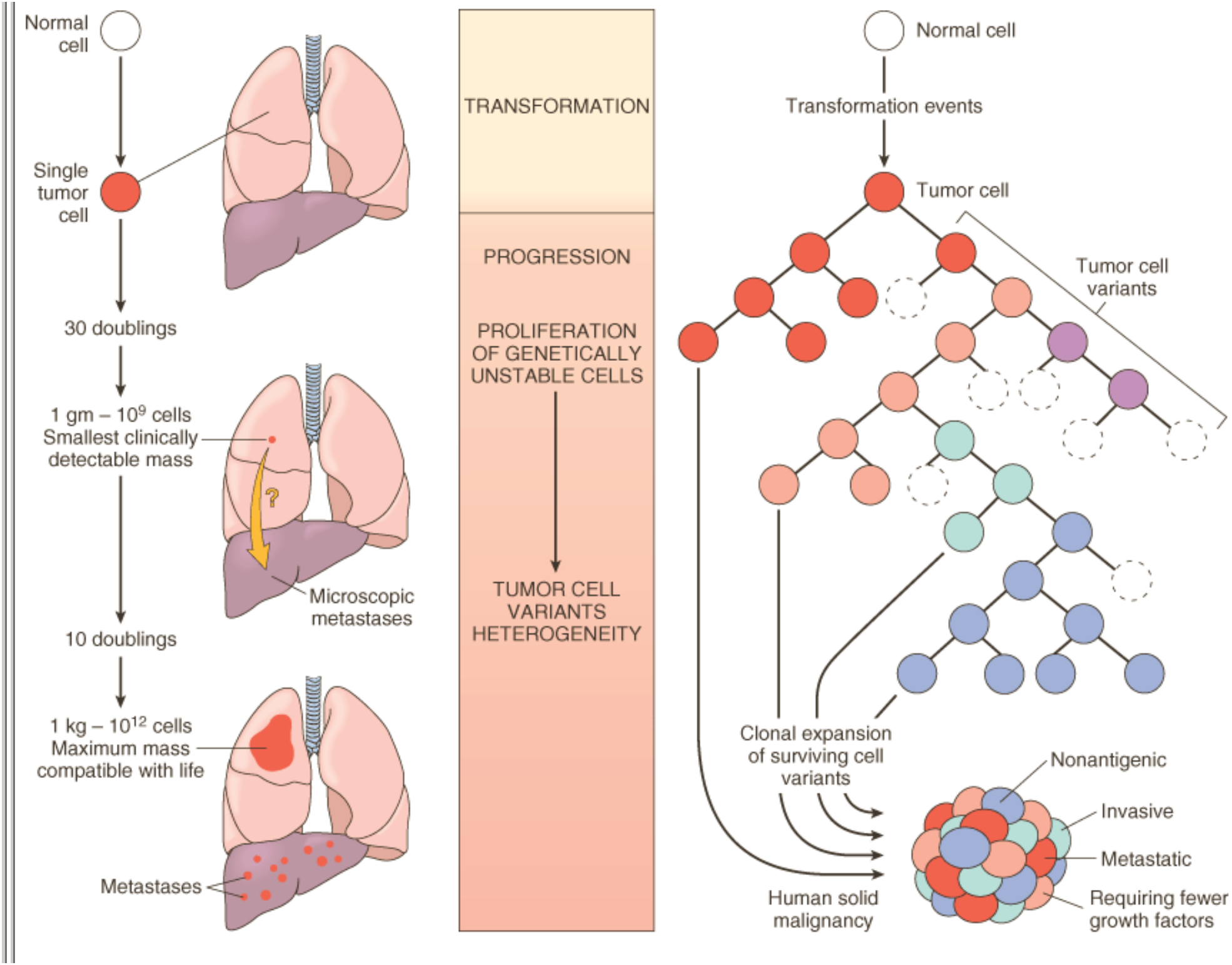
Normal cell
Single tumor cell
30 doublings
1 gm – 10^9 cells
Smallest clinically detectable mass
?
Microscopic metastases
10 doublings
1 kg – 10^{12} cells
Maximum mass compatible with life
Metastases
TRANSFORMATION
PROGRESSION
PROLIFERATION OF GENETICALLY UNSTABLE CELLS
TUMOR CELL VARIANTS HETEROGENEITY
Normal cell
Transformation events
Tumor cell
Tumor cell variants
Clonal expansion of surviving cell variants
Human solid malignancy
Nonantigenic
Invasive
Metastatic
Requiring fewer growth factors

Figure 7-13 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.

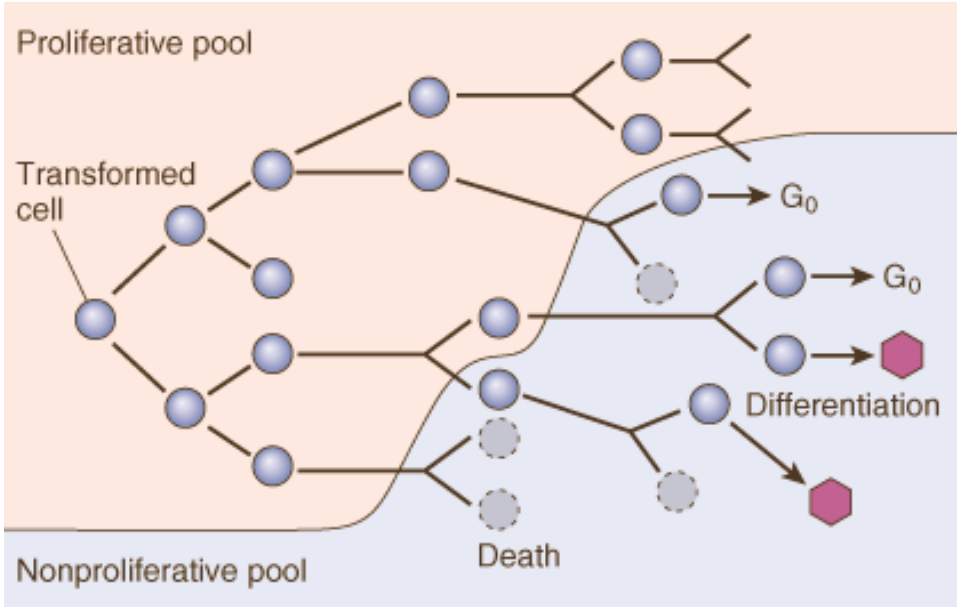

Figure 7-14 Fibroadenoma of the breast. The tan-colored, encapsulated small tumor is sharply demarcated from the whiter breast tissue.

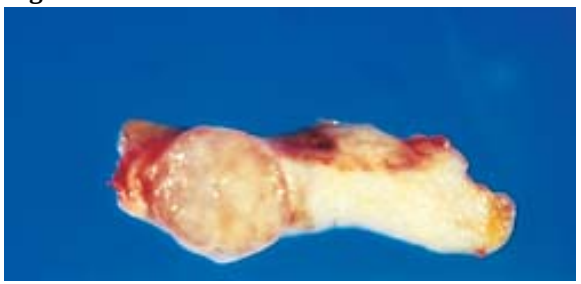

Figure 7-15 Microscopic view of fibroadenoma of the breast seen in Figure 7-14 . The fibrous capsule (*right*) delimits the tumor from the surrounding tissue. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

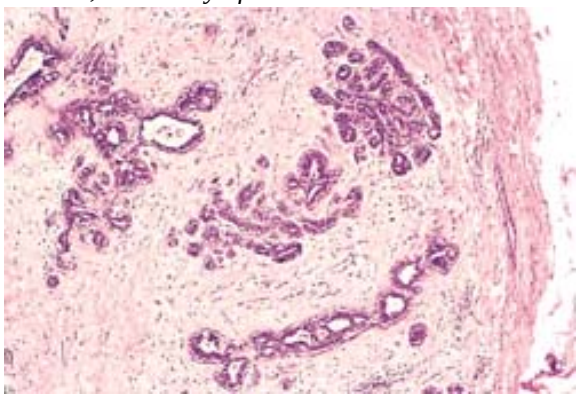

Figure 7-16 Cut section of an invasive ductal carcinoma of the breast. The lesion is retracted, infiltrating the surrounding breast substance, and would be stony hard on palpation.

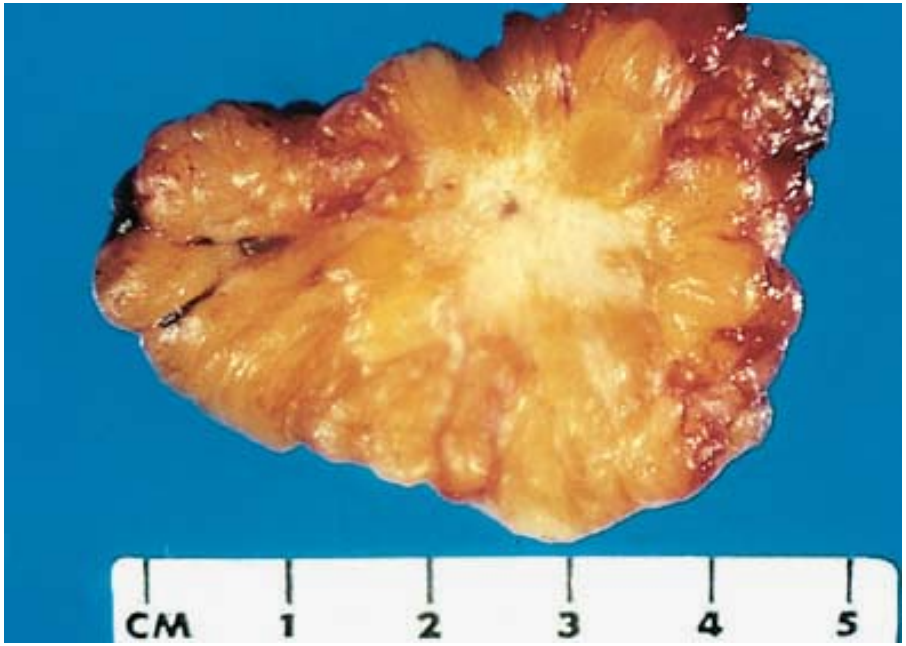

Figure 7-17 The microscopic view of the breast carcinoma seen in Figure 7-16 illustrates the invasion of breast stroma and fat by nests and cords of tumor cells (compare with fibroadenoma shown in Fig. 7-15). The absence of a well-defined capsule should be noted. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

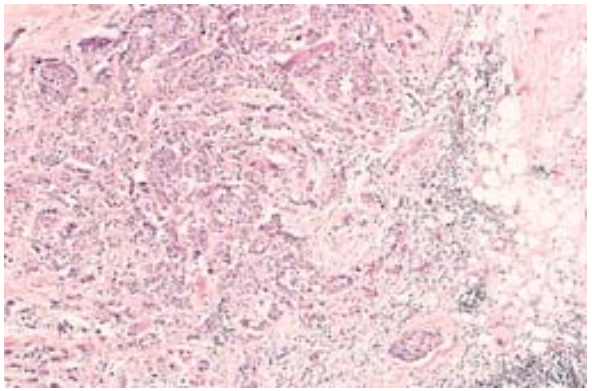

Figure 7-18 Colon carcinoma invading pericolonic adipose tissue. *(Courtesy of Dr. Melissa Upton, University of Washington, Seattle, WA.)*

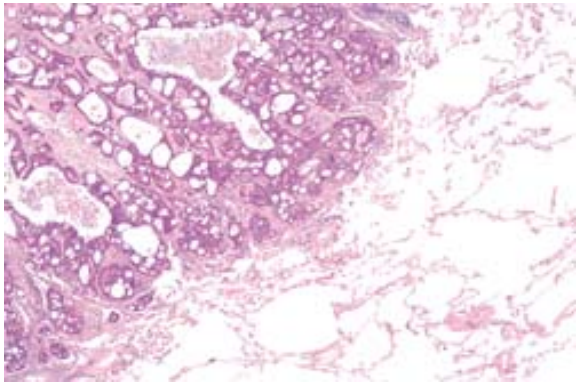

Figure 7-19 Axillary lymph node with metastatic breast carcinoma. The subcapsular sinus *(top)* is distended with tumor cells. Nests of tumor cells have also invaded the subcapsular cortex. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallas, TX.)*

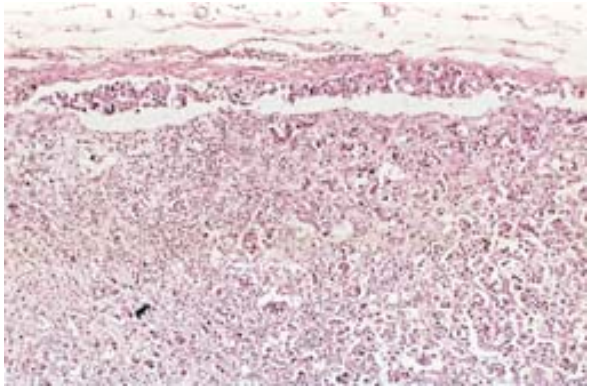

Figure 7-20 A liver studded with metastatic cancer.

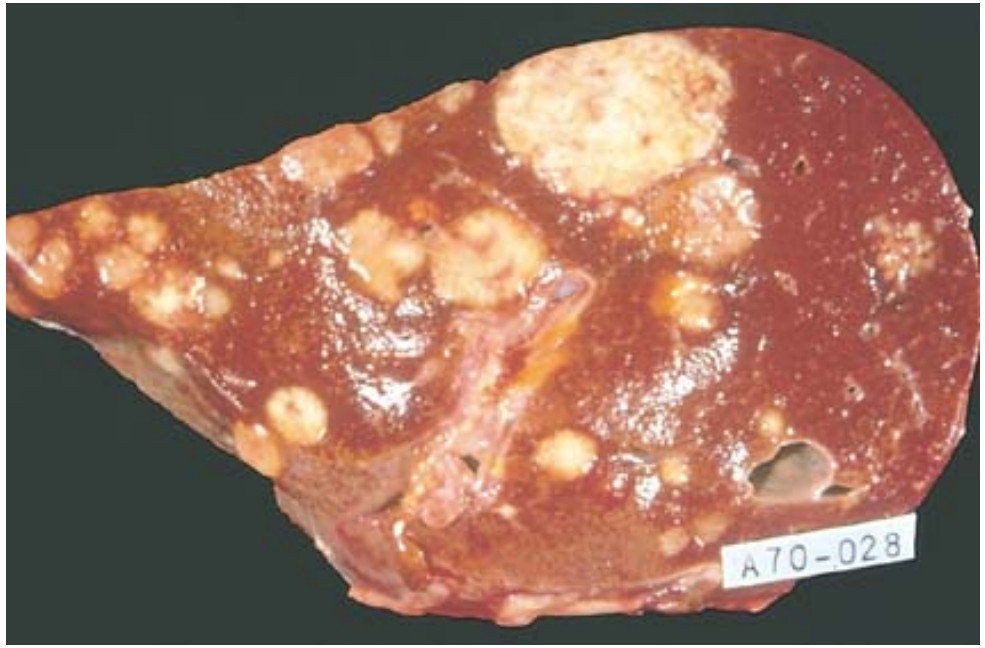

Figure 7-21 Microscopic view of liver metastasis. A pancreatic adenocarcinoma has formed a metastatic nodule in the liver. *(Courtesy of Dr. Trace Worrell, University of Texas Southwestern Medical School, Dallax, TX.)*

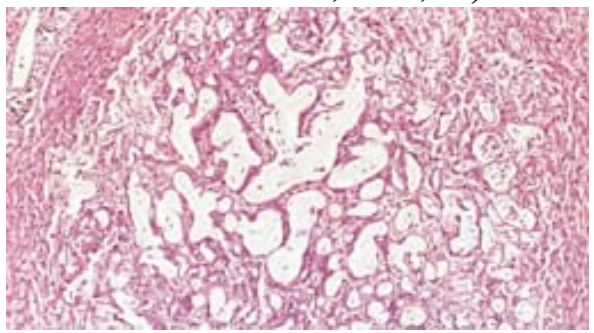

Figure 7-22 Comparison between a benign tumor of the myometrium (leiomyoma) and a malignant tumor of similar origin (leiomyosarcoma).

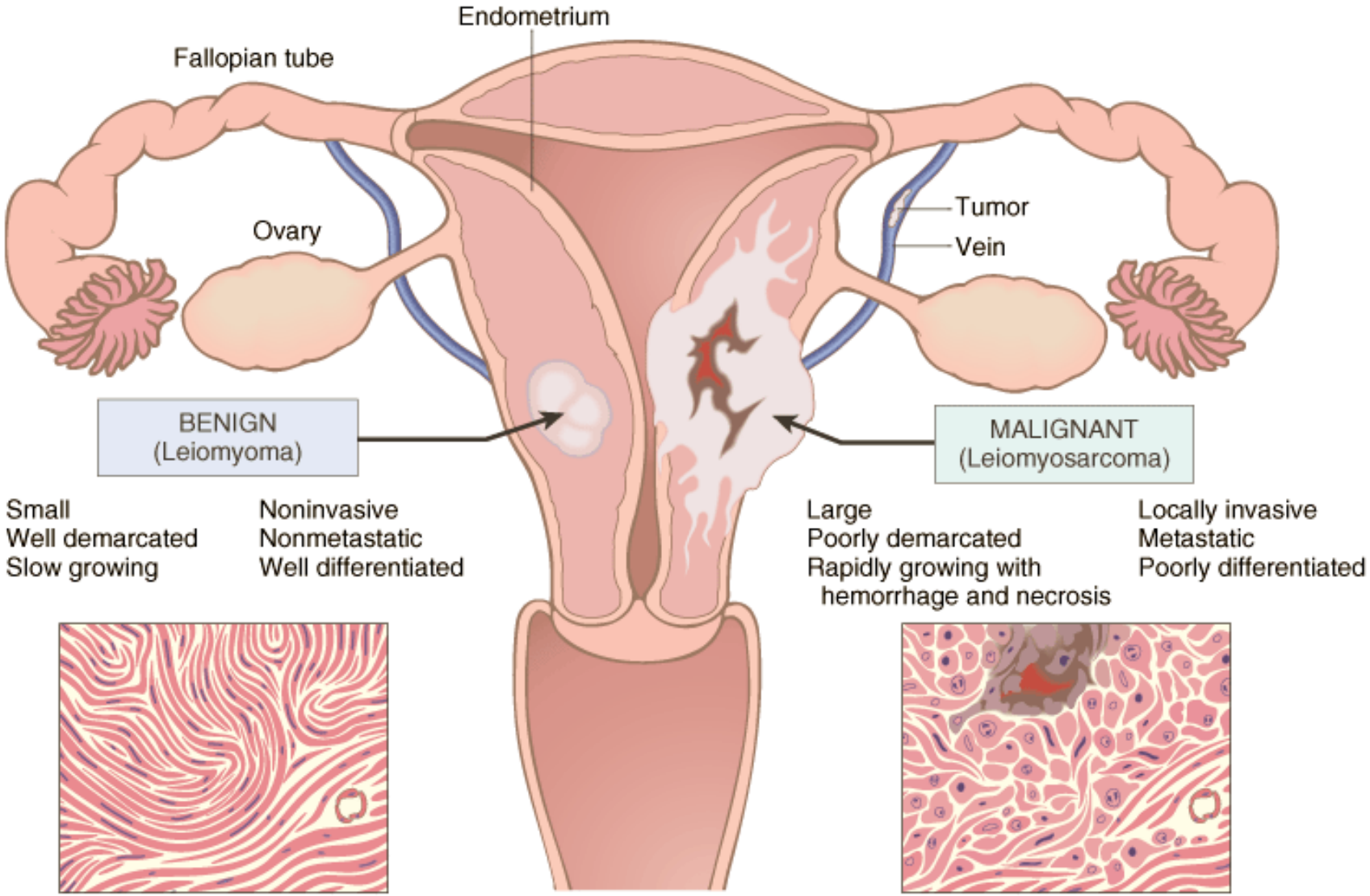

TABLE 7-2 -- Comparisons Between Benign and Malignant Tumors

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

Metastasis	Absent	Frequently present; the larger and more undifferentiated the primary, the more likely are metastases

reasons discussed later, they do not indicate the inevitable development of metastases.

The distinguishing features of benign and malignant tumors discussed in this overview are summarized in Table 7-2 and Figure 7-22 . 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,

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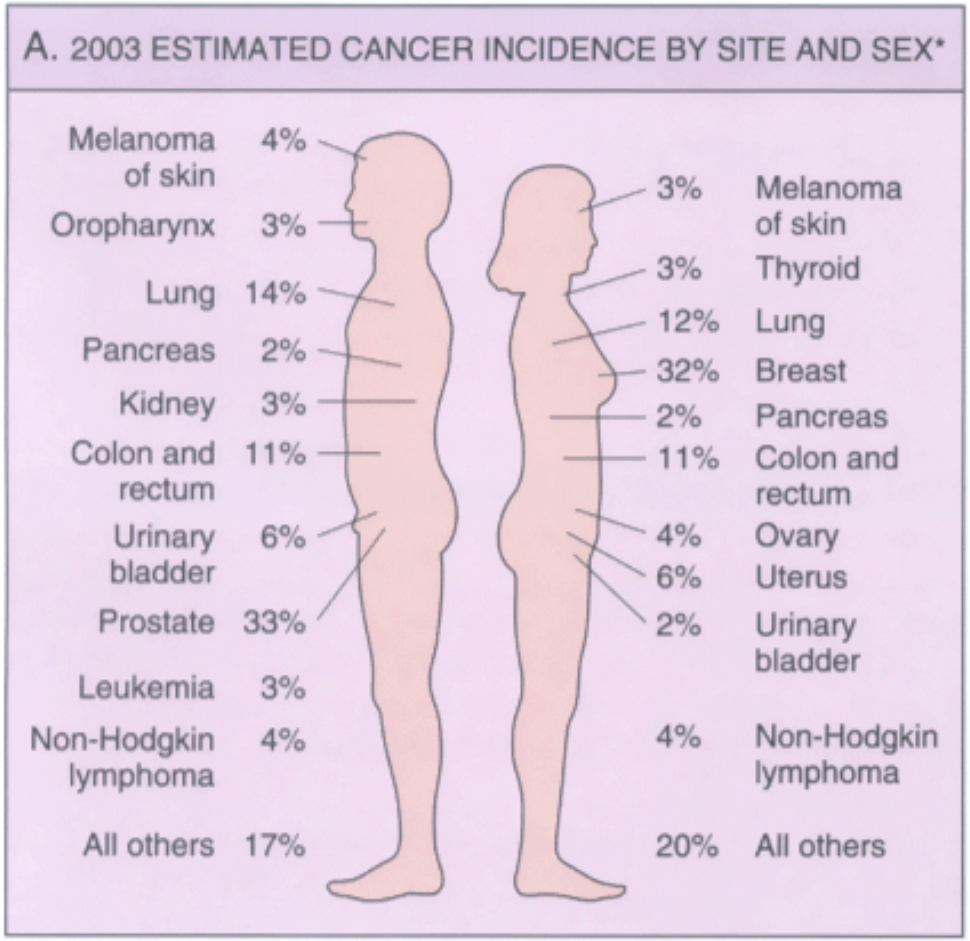
can contribute substantially to knowledge about the origins of cancer. For example, the concept that chemicals can cause cancer arose from the astute observations of Sir Percival Pott, who related the increased incidence of scrotal cancer in chimney sweeps to chronic exposure to soot. Thus, major insights into the cause of cancer can be obtained by epidemiologic studies that relate particular environmental, hereditary, and cultural influences to the occurrence of malignant neoplasms. In addition, certain diseases associated with an increased risk of developing cancer can provide insights into the pathogenesis of malignancy. Therefore, in the following discussion, we first summarize the overall incidence of cancer to provide an insight into the magnitude of the cancer problem, and then review a number of factors relating to both the patient and the environment that influence 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 556,000 deaths from cancer in 2003, representing 23% of all mortality,[3] 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 100,000 cases of carcinoma in situ, largely of the uterine cervix but also of the breast. The major organ sites affected and the estimated frequency of cancer deaths are shown in Figure 7-23 . The most common tumors in men are prostate, lung, and colorectal cancers. 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.[15]

Figure 7-23 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, 2003. CA Cancer J Clin 53:5, 2003.)*



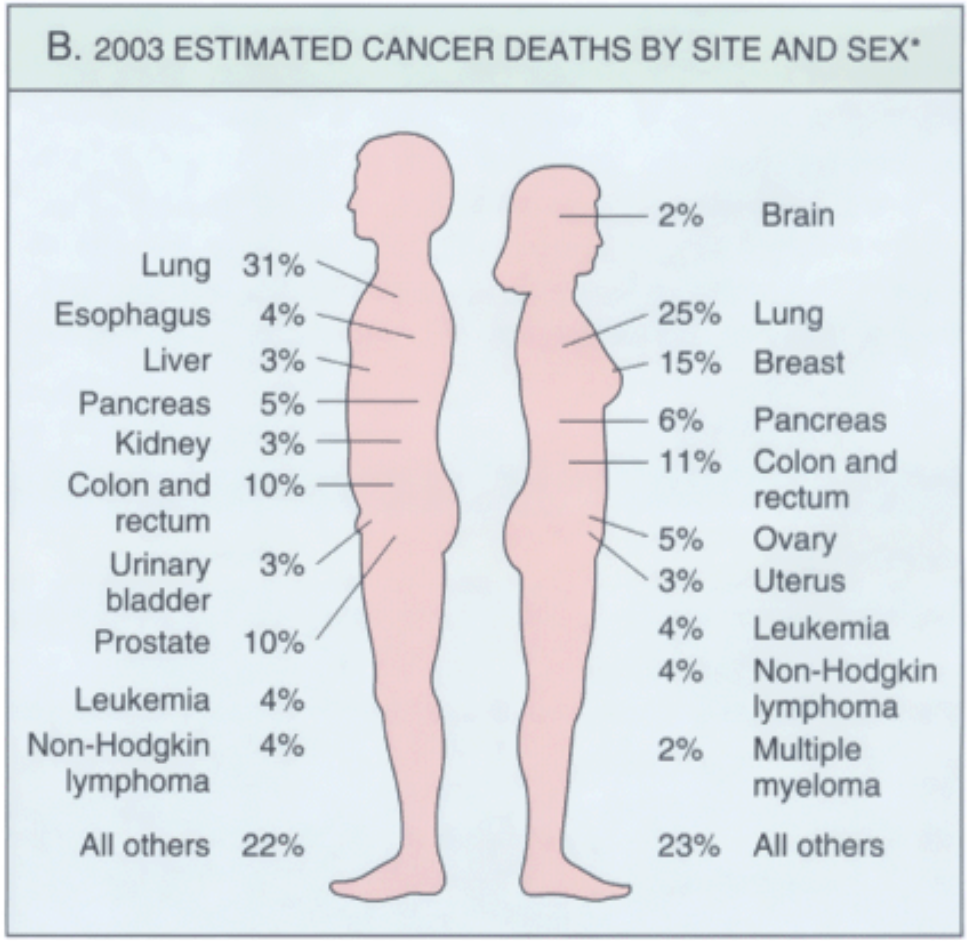
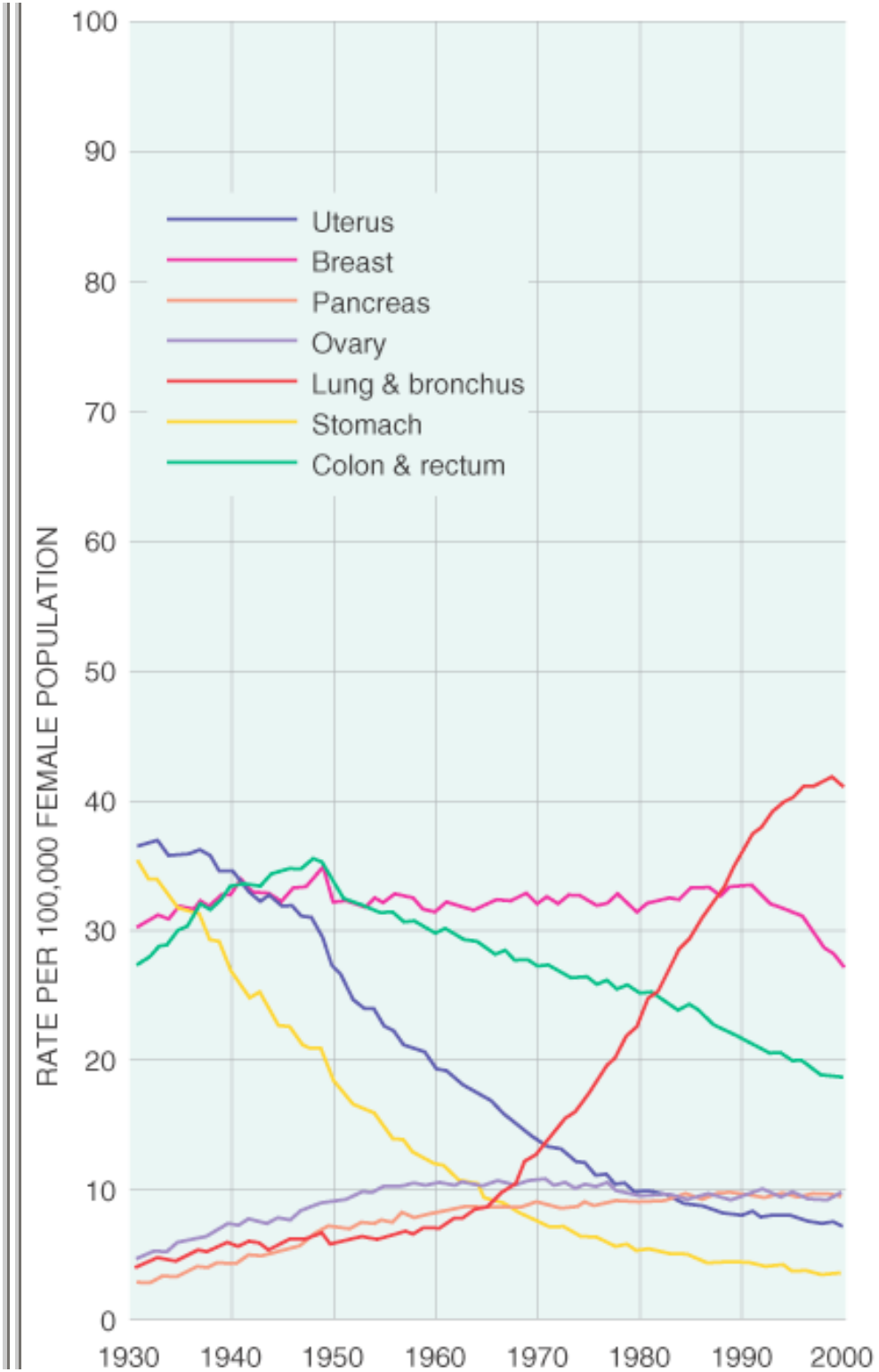
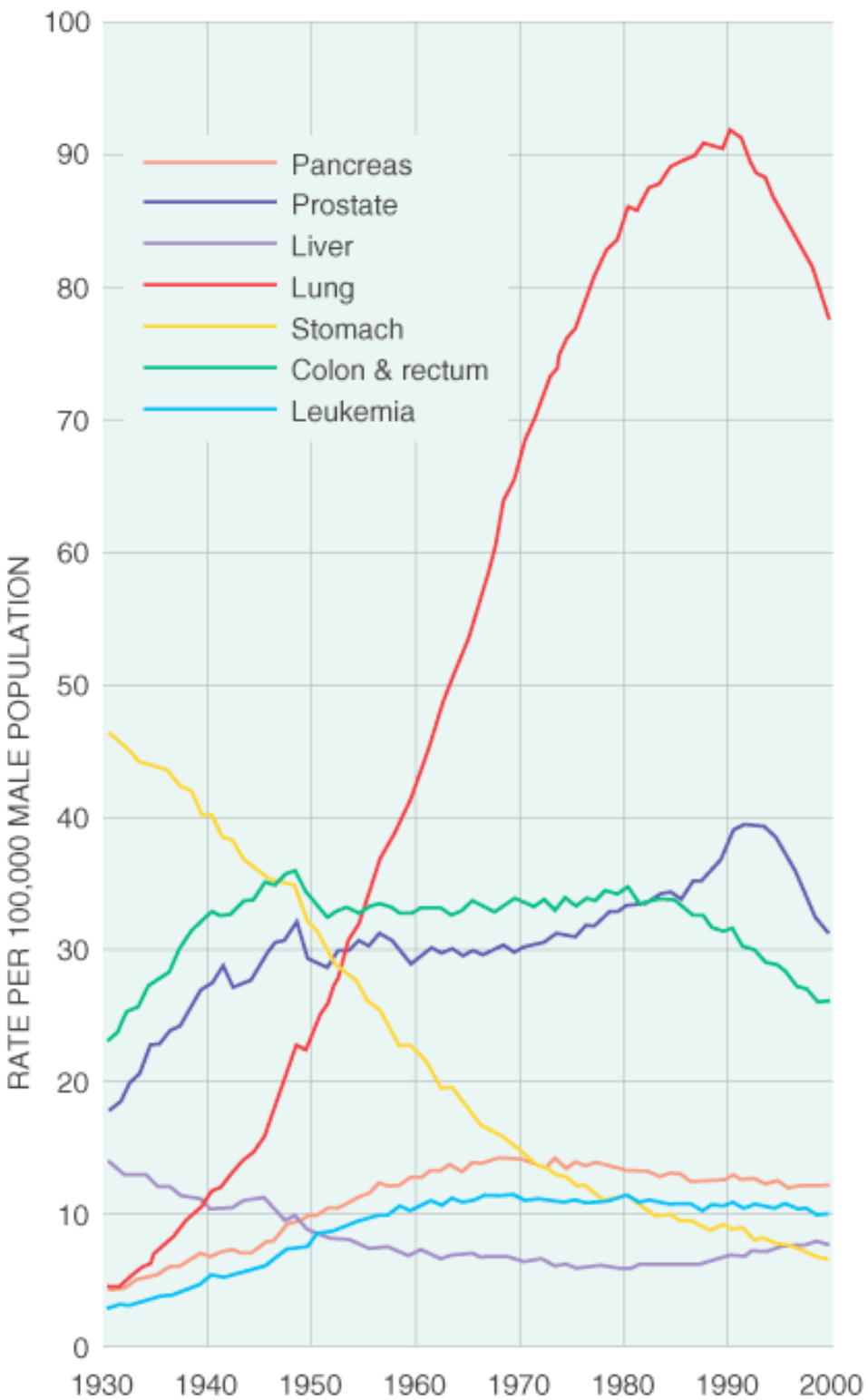


Figure 7-24 Age-adjusted cancer death rates for selected sites in the United States, adjusted for the 2000 U.S. population. *(Adapted from Jemal A, et al: Cancer statistics, 2003. CA Cancer J Clin 53:5, 2003.)*


RATE PER 100,000 FEMALE POPULATION
100
90
80
70
60
50
40
30
20
10
0
1930
1940
1950
1960
1970
1980
1990
2000
Uterus
Breast
Pancreas
Ovary
Lung & bronchus
Stomach
Colon & rectum


RATE PER 100,000 MALE POPULATION
100
90
80
70
60
50
40
30
20
10
0
1930
1940
1950
1960
1970
1980
1990
2000
Pancreas
Prostate
Liver
Lung
Stomach
Colon & rectum
Leukemia

1930 1940 1950 1960 1970 1980 1990 2000 1930 1940 1950 1960 1970 1980 1990 2000

Figure 7-25 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.)*

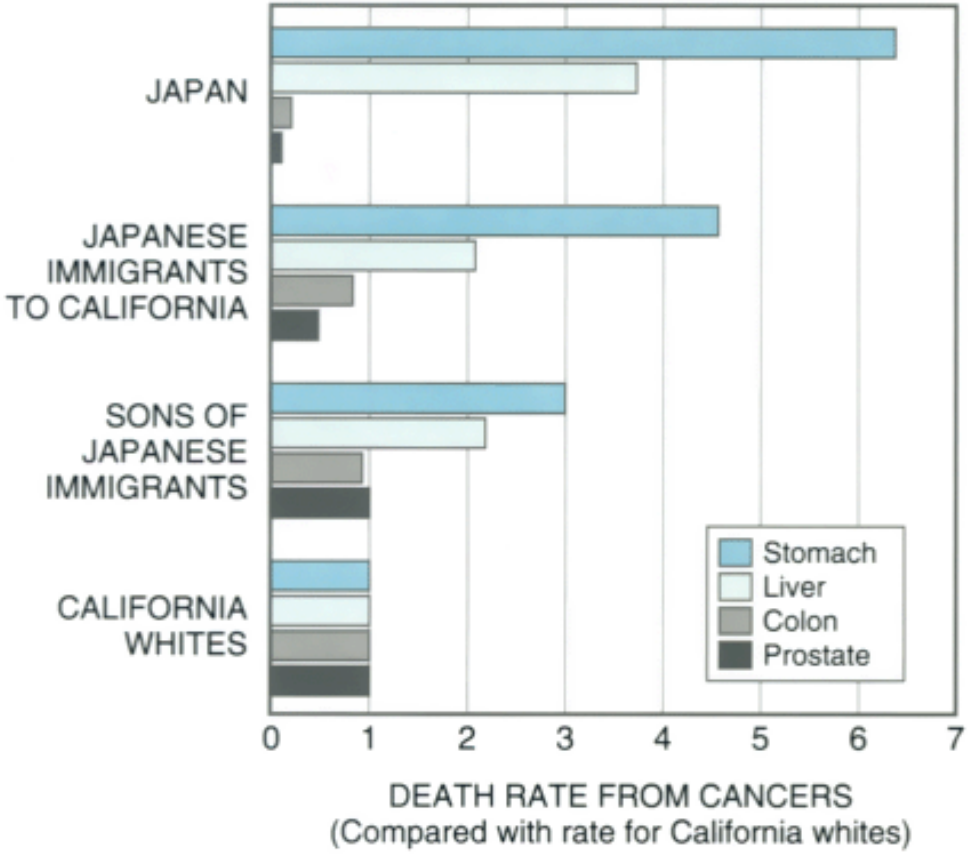


TABLE 7-3 -- Occupational Cancers

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. Although use as solvent is discouraged, 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 mental platings and coatings
Chromium compounds	Lung	Component of metal alloys, paints, pigments, and preservatives
Ethylene oxide	Leukemia	Ripening agent for fruits and nuts. Used in rocket propellant and chemical synthesis, in fumigants for foodstuffs and textiles, and in sterilants for hospital equipment
Nickel compounds	Nose, lung	Nickel plating. Component of ferrous alloys, ceramics, and batteries. Byproduct of stainless steel arc welding
Radon and its decay products	Lung	From decay of minerals containing uranium. Can be 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.		

of the colon and in virtually 100% of cases are fated to develop a carcinoma of the colon by age 50. Other autosomal dominant cancer syndromes are the Li-Fraumeni syndrome resulting from germ line mutations of the *p53* gene, multiple endocrine neoplasia types 1 and 2 (MEN-1 and MEN-2), and hereditary nonpolyposis colon cancer (*HNPCC)*, a condition caused by inactivation of a mismatch repair gene (also listed below among repair defects).

There are several features that characterize inherited cancer syndromes:

- In each syndrome, tumors involve specific sites and tissues, although they may involve more than one site. For example, in MEN-2, caused by a mutation of the *RET* protooncogene, thyroid, parathyroid, and adrenals are involved. There is no increase in predisposition to cancers in general.
- Tumors within this group are often associated with a specific marker phenotype. For example, there may be multiple 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 5).
- 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 cancerpredisposing 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, ataxiatelangectasia, and Bloom syndrome, all rare diseases characterized by genetic instability resulting from defects in DNA repair genes. Also included here is hereditary nonpolypoid colon cancer (HNPCC), an autosomal dominant condition caused by inactivation of a DNA mismatch repair gene.[24] HNPCC is the most common cancer predisposition syndrome, increasing the susceptibility to cancer in the colon and also in some other organs such as 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

TABLE 7-4 -- Reported Deaths for the Five Leading Cancer Types for Males by Age, US, 2000

All Ages	Under Age 20	Age 20–39	Age 40–59	Age 60–70	Age 80+
All cancers	***All cancers***	***All cancers***	***All cancers***	***All cancers***	***All cancers***
286,082	1298	4832	50,069	158,990	70,883
Lung and bronchus	Leukemia	Brain and ONS *	Lung and bronchus	Lung and bronchus	Lung and bronchus
90,415	430	626	15,827	57,470	16,626
Prostate	Brain and ONS *	Leukemia	Colon and rectum	Colon and rectum	Prostate
31,078	307	611	4801	15,420	15,630
Colon and rectum	Bones and joints	Lung and bronchus	Pancreas	Prostate	Colon and rectum
28,484	105	481	2929	14,428	7821
Pancreas	Endocrine system	Non-Hodgkin lymphoma	Esophagus	Pancreas	Urinary Bladder
14,238	104	444	2345	8179	3222
Non-Hodgkin lymphoma	Non-Hodgkin lymphoma	Colon and rectum	Liver	Non-Hodgkin lymphoma	Leukemia
11,812	79	431	2308	6107	3187

"All Cancers" excludes in situ carcinomas except urinary bladder.

Source: US Mortality Public Use Data Tape, 2000, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD 2002.

*ONS = other nervous system.

brain, as well as melanomas. *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 pre-existing benign polyps. The transmission pattern of familial cancers is not clear. In general, siblings have a relative risk between two and three (two to 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. 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. Thus, mutations in *BRCA1* and *BRCA2* cannot account for the large proportion of familial breast cancers.[25] Changes in other genes, probably in low-penetrance susceptibility alleles, appear to be necessary for the development of these tumors. A similar situation occurs in familial melanomas, in which a mutation of the *p16INK4a* 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.[26]

Interactions Between Genetic and Non-Genetic 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 non-genetic 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 non-genetic factors. For instance, breast cancer risk in female carriers of BRCA-1 or BRCA-2 mutations is almost three-fold higher for women born after 1940, compared to the risks for women born before that year.[27] [28] 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 correlations 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. The risk is greater than average, however, under many circumstances, as is evident from the predisposing influences discussed earlier. Certain clinical conditions are also important. Because cell replication is involved in neoplastic transformation, regenerative, hyperplastic, and dysplastic proliferations are fertile soil for the origin of a malignant tumor. There is a well-defined association between certain forms of endometrial hyperplasia and endometrial carcinoma and between cervical dysplasia and cervical carcinoma (Chapter 22). The bronchial mucosal metaplasia and dysplasia of habitual cigarette smokers are ominous

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TABLE 7-5 -- Reported Deaths for the Five Leading Cancer Types for Females by Age, US, 2000

All Ages	Under Age 20	Age 20–39	Age 40–59	Age 60–70	Age 80+
All cancers	***All cancers***	***All cancers***	***All cancers***	***All cancers***	***All cancers***
267,009	973	5617	47,850	131,871	80,697
Lung and bronchus	Leukemia	Breast	Breast	Lung and bronchus	Lung and bronchus
65,016	302	1444	11,937	39,311	14,693
Breast	Brain and ONS *	Uterine cervix	Lung and bronchus	Breast	Colon and rectum
41,872	238	538	10,613	17,842	12,379
Colon and rectum	Endocrine system	Leukemia	Colon and rectum	Colon and rectum	Breast

28,950	82	452	3619	12,612	10,648
Pancreas	Bones and joints	Lung and bronchus	Ovary	Pancreas	Pancreas
15,094	76	397	3033	7825	5319
Ovary	Soft tissue	Brain and ONS *	Pancreas	Ovary	Non-Hodgkin lymphoma
14,060	69	354	1871	7217	4039
"All Cancers" excludes in situ carcinomas except urinary bladder.					
Source: US Mortality Public Use Data Tape, 2000, National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD, 2002.					

*ONS = other nervous system.

antecedents of bronchogenic carcinoma. About 80% of hepatocellular carcinomas arise in cirrhotic livers, which are characterized by active parenchymal regeneration (Chapter 18).

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.[29] This is exemplified by the increased risk of cancer development in patients affected by a

TABLE 7-6 -- Inherited Predisposition to Cancer

Inherited Cancer Syndromes (Autosomal Dominant)	
Gene	***Inherited Predisposition***
••*RB*	Retinoblastoma
••*p53*	Li-Fraumeni syndrome (various tumors)
••*p16INK4A*	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
••*PATCH*	Nevoid basal cell carcinoma syndrome
Familial Cancers	
Familial clustering of cases, but role of inherited predisposition not clear for each individual	

••Breast cancer
••Ovarian cancer
••Pancreatic cancer
Inherited Autosomal Recessive Syndromes of Defective DNA Repair
••Xeroderma pigmentosum
••Ataxia-telangiectasia
••Bloom syndrome
••Fanconi anemia

variety of chronic inflammatory diseases of the gastrointestinal tract. These include ulcerative colitis, Crohn disease, *Helicobacter pylori* gastritis, viral hepatitis, and chronic pancreatitis. The precise mechanisms that link inflammation and cancer development have not been established.[30] Chronic inflammatory reactions may result in the production of cytokines, which stimulate the growth of transformed cells. In some cases, chronic inflammation may increase the pool of tissue stem cells, which become subject to the effect of mutagens. Interestingly, chronic inflammation may also directly promote genomic instability in cells through the production of reactive oxygen species (ROS), thus predisposing to malignant transformation. Whatever the precise mechanism, such a link may have practical implications. For instance, expression of the enzyme *cyclooxygenase-2 (COX-2),* which converts arachidonic acid into prostaglandins (Chapter 2), is induced by inflammatory stimuli and is increased in colon cancers and other tumors.[31] The development of COX-2 inhibitors for cancer treatment is an active and promising area of research.[32]

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, develops cancerous change 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 longstanding 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 non-malignant 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. 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.
• *A tumor is formed by the clonal expansion of a single precursor cell that has incurred the 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 enzymes glucose-6-phosphate dehydrogenase (G6PD), iduronate-2-sulfatase and phosphoglycerate kinase. The principle underlying such an analysis is illustrated in Figure 7-26 . 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 *(HUMARA)*.[33] The frequency of *HUMARA* polymorphism in the general population is more than 90%, so it is easy to establish clonality by showing that all the cells in a tumor express the same allele. For tumors with a specific translocation, such as in myeloid leukemias, the presence of the translocation can be used to assess clonality. 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 protooncogenes, 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 protooncogenes are considered dominant because they transform cells despite the presence of a normal counterpart. In contrast, both normal alleles of the tumor suppressor genes must be damaged for transformation to occur, so this family of genes is sometimes referred to as *recessive oncogenes*. However, there are exceptions to this rule, and some tumor suppressor genes lose their suppressor activity when a single allele is lost or inactivated.[34] This loss of function of a recessive gene caused by damage of a single allele is called *haploinsufficiency*. Genes that regulate apoptosis may be dominant, as are protooncogenes, or they may behave as tumor suppressor genes.
• *DNA repair genes affect cell proliferation or survival indirectly by influencing the ability of the organism to repair nonlethal damage in other genes, including protooncogenes, tumor suppressor genes, and genes that regulate apoptosis.* A disability in the DNA repair genes can predispose to mutations in the genome and *hence to neoplastic transformation*. Such propensity to mutations is called a *mutator phenotype*. [35] With some exceptions, both alleles of DNA repair genes must be inactivated to induce such genomic instability; in this sense, DNA repair genes may also be considered as tumor suppressor genes.
• *Carcinogenesis is a multistep process at both the phenotypic and the genetic levels.* A malignant neoplasm has several phenotypic attributes, such as excessive growth, local invasiveness, and the ability to form distant metastases. These characteristics are acquired in a stepwise fashion, a phenomenon called *tumor progression*. At the molecular level, progression results from accumulation of genetic

lesions that in some instances are favored by defects in DNA repair.

Figure 7-26 Diagram depicting the use of X-linked isoenzyme cell markers as evidence of the monoclonality of neoplasms. Because of random X inactivation, all females are mosaics with two cell populations (with G6PD isoenzyme A or 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.

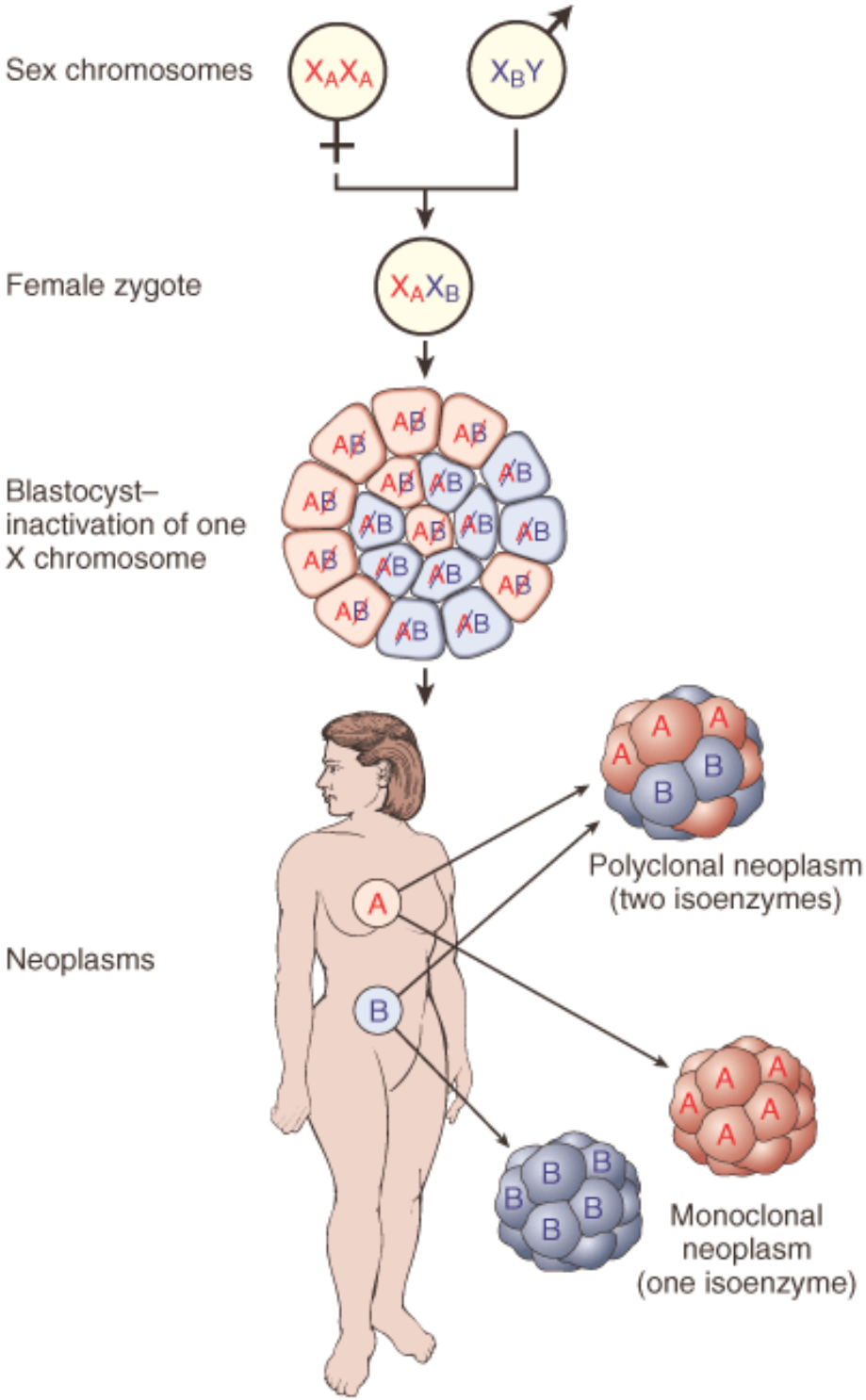
Sex chromosomes
X_AX_A
X_BY
Female zygote
X_AX_B
Blastocyst–
inactivation of one
X chromosome
Neoplasms
A
B
Polyclonal neoplasm
(two isoenzymes)
Monoclonal
neoplasm
(one isoenzyme)

Figure 7-27 Flow chart depicting a simplified scheme of the molecular basis of cancer.

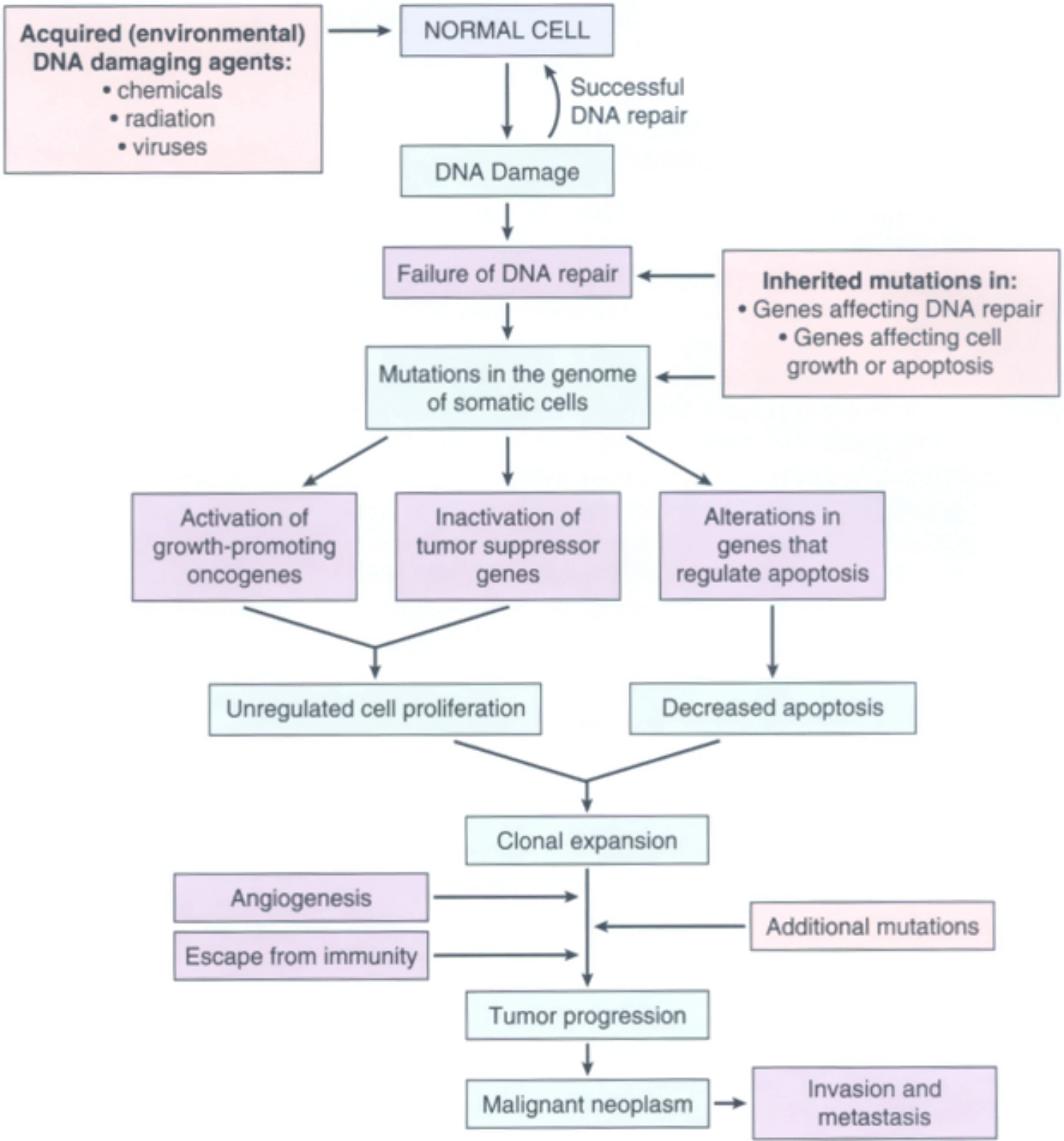

Figure 7-28 Expression of cyclin-cyclin-dependent kinase (CDK) complexes during the cell cycle. The phases of the cycle are indicated inside the arrows. *(Modified from Pollard TD, Earnshaw WC: Cell Biology. Philadelphia, WB Saunders, 2002.)*

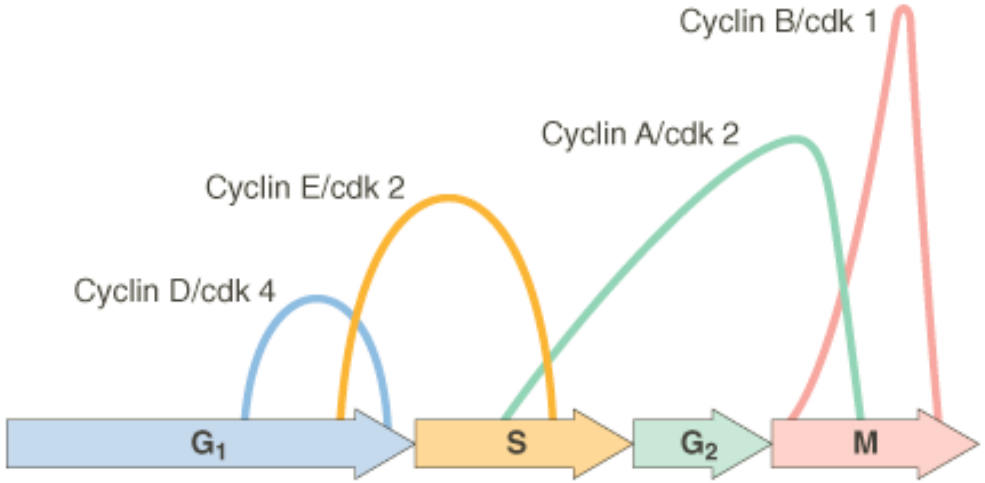

Figure 7-29 Schematic illustration of the role of cyclins, CDKs, and cyclin-dependent kinase inhibitors in regulating the G_1 /S cell-cycle transition. External signals activate multiple signal transduction pathways, including those involving the *MYC* and *RAS* genes, which lead to synthesis and stabilization of cyclin D (there are several D cyclins, but, for simplification, we refer to them as "cyclin D"). Cyclin D binds to CDK4, forming a complex with enzymatic activity (cyclin D can also bind to CDK6, which appears to have a similar role as CDK4). The cyclin D-CDK4 complex phosphorylates RB, located in the E2F/DP1/RB complex in the nucleus, activating the transcriptional activity of E2F (E2F is a family of transcription factors, which we refer to as "E2F"), which leads to transcription of cyclin E, cyclin A and other proteins needed for the cell to go through the late G_1 restriction point. The cell cycle can be blocked by the Cip/Kip inhibitors p21 and p27 *(red boxes)* and the INK4A/ARF inhibitors p16INK4A and p14ARF *(green boxes)*. Cell-cycle arrest in response to DNA damage and other cellular stresses is mediated through p53. The levels of p53 are under negative regulation by MDM2, through a feedback loop that is inhibited by p14ARF.

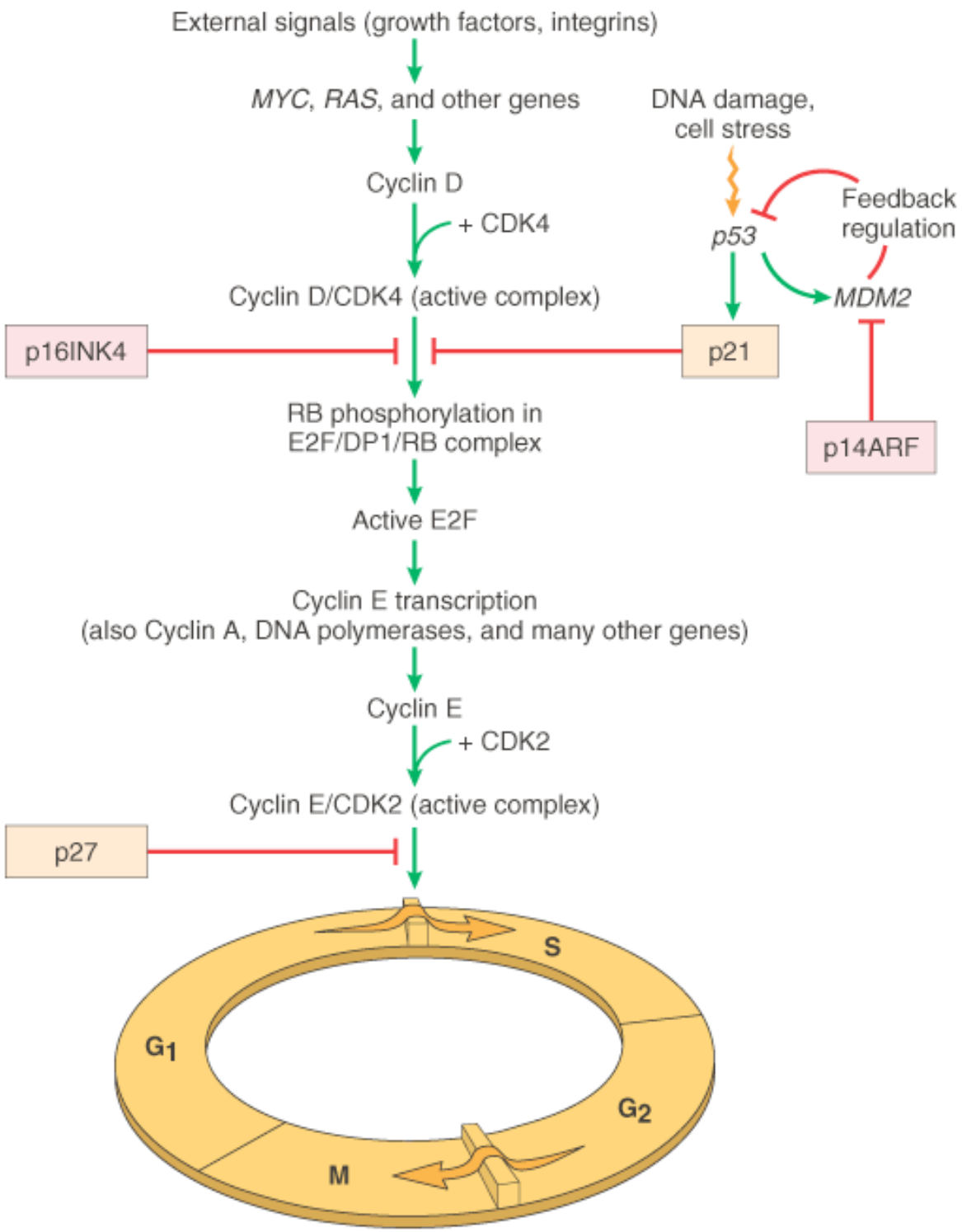
External signals (growth factors, integrins)
MYC, RAS, and other genes
DNA damage, cell stress
Cyclin D
+ CDK4
p53
Feedback regulation
Cyclin D/CDK4 (active complex)
MDM2
p16INK4
p21
RB phosphorylation in E2F/DP1/RB complex
p14ARF
Active E2F
Cyclin E transcription (also Cyclin A, DNA polymerases, and many other genes)
Cyclin E
+ CDK2
Cyclin E/CDK2 (active complex)
p27
S
G1
G2
M

Figure 7-30 Mechanism of cell-cycle regulation by RB. In a resting cell, RB is a component of the E2F/DP1/RB complex, which represses gene transcription through the recruitment of histone deacetylase, an enzyme that alters the conformation of chromatin, making it more compact. Phosphorylation of RB by cyclin D-CDK4 removes histone deacetylase from chromatin, allowing the activation of E2F transcriptional activity (RB can also be phosphorylated by cyclin E-CDK2). E2F-mediated transcription of cyclins E and A, and of genes required for DNA replication, permit the passage through the G_1 restriction point. *(Adapted from Pollard TD, Earnshaw WC: Cell Biology. Philadelphia, WB Saunders, 2002, p. 689.)*

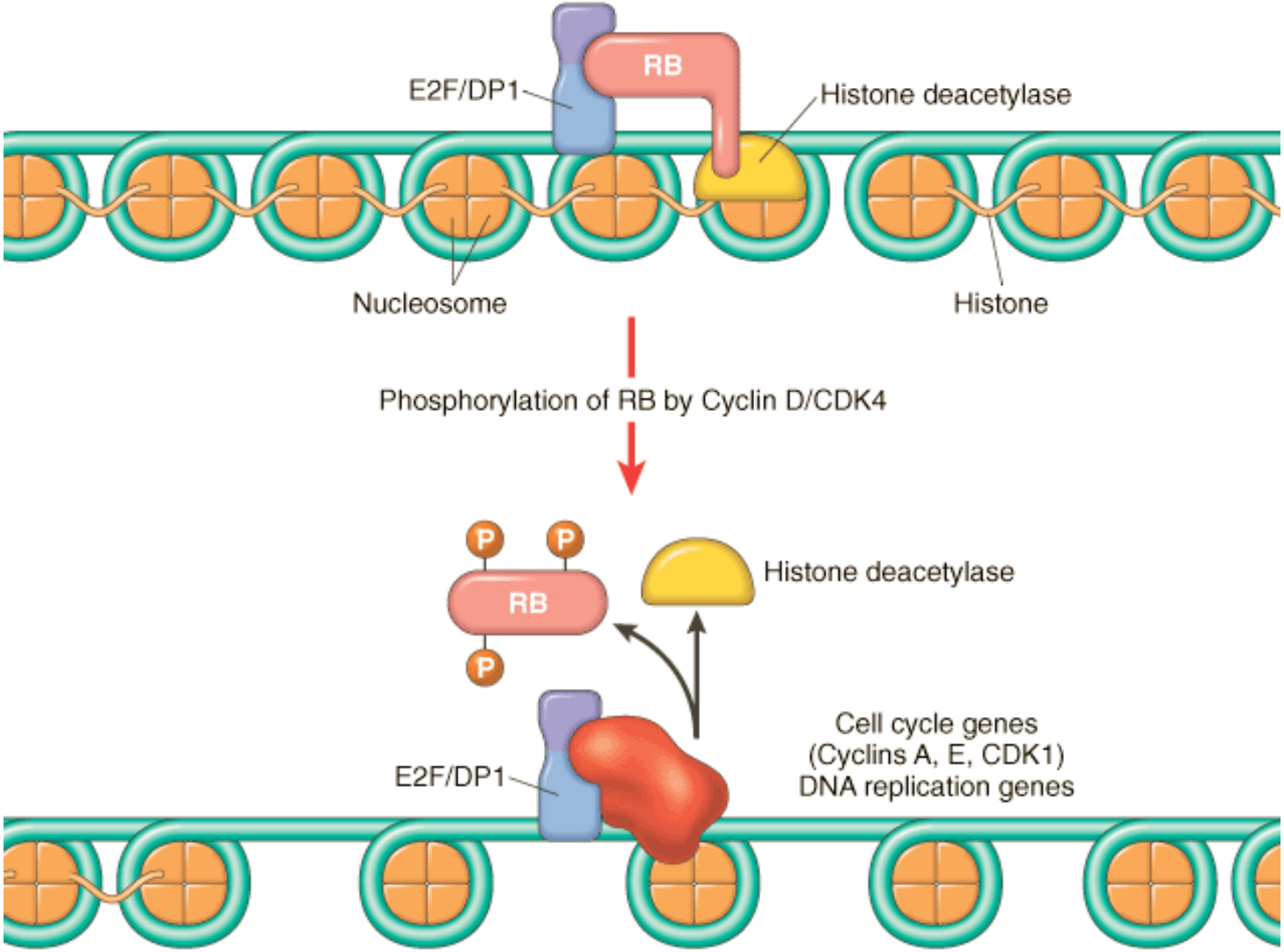

TABLE 7-7 -- Main Cell-Cycle Components and Their Inhibitors

Cell-Cycle Component	Main Function
Cyclin-Dependent Kinases	
• CDK4	Forms a complex with cyclin D. The complex 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 the G_1 /S transition. Forms a complex with cyclin A at the S phase that facilitates the G_2 /M transition.
• CDK1	Forms a complex with cyclin B, which acts on the G_2 /M transition.
Inhibitors	
• Cip/Kip family: p21, p27	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 transforming growth factor-β.
• 1NK4/ARF family: p16INK4A, p14ARF	p16INK4a binds to cyclin D-CDK4 and promotes the inhibitory effects of RB. p14ARF increases p53 levels by inhibiting MDM2 activity.
Checkpoint Components	
• p53	Tumor suppressor 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 (*ATM*)	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.

three components, p21, p27, and p57, which bind to and inactivate the complexes formed between cyclins and CDKs. Transcriptional activation of p21 is under the control of *p53, a tumor suppressor gene that is mutated in a large proportion of human cancers*. The main role of p53 in the cell cycle is one of surveillance, triggering checkpoint controls that slow down or stop cell-cycle progression of damaged cells, or causes apoptosis. The human *INK4a/ARF* locus (a notation for "*in*hibitor of *k*inase *4/a*lternative *r*eading *f*rame") encodes two proteins, p16INK4a and p14ARF, which block the cell cycle and act as tumor suppressors. p16INK4a competes with cyclin D for binding to CDK4 and inhibits the ability of the cyclin D-CDK4 complex to phosphorylate RB, thus causing cell-cycle arrest at late G_1 . It is frequently mutated or inactivated by hypermethylation (discussed later) in human cancers. The *INK4a* locus encodes a second gene product, p14ARF (p19ARF in mice), which acts on p53. p14ARF arises from an alternative reading of the *INK4a* gene, providing for an "economical" way to utilize gene-coding sequences.[42] Although both p16INK4a and p14ARF block the cell cycle, their targets are different; p16INK4a acts on cyclin D-CDK4, whereas p14ARF prevents p53 degradation.

Cell-Cycle Checkpoints.

The cell cycle has its own internal controls, called *checkpoints*. There are two main checkpoints, one at the G_1 /S transition and another at G_2 /M.[43] [44] The S phase is the point of no return in the cell cycle, and before a cell makes the final commitment to replicate, the G_1 /S checkpoint checks for DNA damage. If DNA 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. [44] The sensors and transducers of DNA damage appear 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. 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 independent mechanisms. *Defect in cell-cycle checkpoint components is a major cause of genetic instability in cancer cells.*

With this background on the cell cycle and its control, we now proceed to discuss the genes that determine the malignant phenotype. This discussion will take place in the context of the seven fundamental changes in cell physiology (listed earlier) that are the hallmarks of malignant cells.

SELF-SUFFICIENCY IN GROWTH SIGNALS: ONCOGENES

Genes that promote autonomous cell growth in cancer cells are called *oncogenes*, and their normal cellular counterparts are called *protooncogenes*. Protooncogenes are physiologic regulators of cell proliferation and differentiation; oncogenes are characterized by the ability to promote cell growth in the

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absence of normal mitogenic signals. Their products, called *oncoproteins*, resemble the normal products of protooncogenes with the exception that oncoproteins are devoid of important regulatory elements. Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other 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 generally located on the cell membrane
- 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 signal transduction molecules that directly activate transcription
- 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. We start with a description of oncogenes and their protein products, and how these were discovered.

Protooncogenes, Oncogenes, and Oncoproteins

As often happens in science, the discovery of protooncogenes was not straightforward. These cellular genes were first discovered in their mutated or "oncogenic" forms as "passengers" within the genome of *acute transforming retroviruses* by the 1989 Nobel laureates Harold Varmus and Michael Bishop. These retroviruses cause rapid induction of tumors in animals and can also transform animal cells in vitro. Molecular dissection of their genomes revealed the presence of unique transforming sequences (viral oncogenes [v-*onc*]) not found in the genomes of nontransforming retroviruses. Most surprisingly, molecular hybridization revealed that the v-*onc* sequences were almost identical to sequences found in normal cellular DNA. From this evolved the concept that during evolution, cellular oncogenes were *transduced* (captured) by the virus through a chance recombination with the DNA of a (normal) host cell that had been infected by the virus. Because they were discovered initially as *viral genes*, these protooncogenes were named after their viral homologues. Each v-*onc* is designated by a three-letter word that relates the oncogene to the virus from which it was isolated. Thus, the v-*onc* contained in *fe*line sarcoma virus is referred to as v-*FES*, whereas the oncogene in *si*mian sarcoma virus is called v-*SIS*. The corresponding protooncogenes are referred to as *FES* and *SIS*, dropping the prefix.

The viral oncogenes are not present in several cancer-causing RNA viruses. One such example is a group of so-called slow transforming viruses that cause leukemias in rodents after a long latent period. The mechanism by which they cause neoplastic transformation implicates protooncogenes. Molecular dissection of the cells transformed by these leukemia viruses revealed

that the proviral DNA is always integrated (inserted) near a protooncogene. One consequence of proviral insertion near a protooncogene is to induce a structural change in the cellular gene, thus converting it into a cellular oncogene (c-*onc*, or *onc*). This mode of protooncogene activation is called *insertional mutagenesis*. Alternatively, strong retroviral promoters inserted in the vicinity of the protooncogenes lead to dysregulated expression of the cellular gene.

Although the study of transforming animal retroviruses provided the first glimpse of protooncogenes, these investigations did not explain the origin of human tumors, which (with rare exceptions) are not caused by infection with retroviruses. Hence the question was raised: Do nonviral tumors contain oncogenic DNA sequences? The answer was provided by experiments involving DNA-mediated gene transfer (DNA transfection). When DNA extracted from several different human tumors was transfected into mouse fibroblast cell lines in vitro, the recipient cells acquired some properties of neoplastic cells. The conclusion from such experiments was inescapable: DNA of spontaneously arising cancers contains oncogenic sequences, or oncogenes. One of the first oncogenic sequences detected in cancers was a mutated form of the *RAS* protooncogene. This protooncogene is the forbear of v-*oncs* contained in Harvey (H) and Kirsten (K) sarcoma viruses.

A large number of protooncogenes have been identified during the past 20 years, most of which do not have a viral counterpart. Protooncogenes have multiple roles, participating in cellular functions related to growth and proliferation. Proteins encoded by protooncogenes may function as growth factor ligands and receptors, signal transducers, transcription factors, and cell-cycle components (Fig. 7-31). Oncoproteins encoded by oncogenes generally serve similar functions as their normal counterparts (Table 7-8). However, because they are constitutively expressed, *oncoproteins endow the cell with self-sufficiency in growth*.[45]

To summarize, protooncogenes may be converted into cellular oncogenes (c-oncs) that are involved in tumor development. Two questions follow: (1) What are the functions of oncogene products, the oncoproteins? (2) How do the normally "civilized" protooncogenes turn into "enemies within"? These issues are discussed below.

Growth Factors.

Many cancer cells develop growth self-sufficiency by acquiring the ability to synthesize the same growth factors to which they are responsive. The protooncogene *SIS*, which encodes the β chain of platelet-derived growth factor (PDGF), is overproduced in many tumors, especially low-grade astrocytomas and osteosarcomas. Furthermore, it appears that the same tumors also express receptors for PDGF and are hence responsive to autocrine stimulation. 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 such as *RAS* (that lie along many signal transduction pathways) cause overexpression of growth factor genes, thus forcing the cells to secrete large amounts of growth factors, such as transforming growth factor-α (TGF-α). This growth factor is related to epidermal growth factor (EGF) and induces proliferation by binding to the EGF receptor. TGF-α is often

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Figure 7-31 Subcellular localization and functions of major classes of cancer-associated genes. The protooncogenes are colored red, cancer suppressor genes blue, DNA repair genes green, and genes that regulate apoptosis purple.

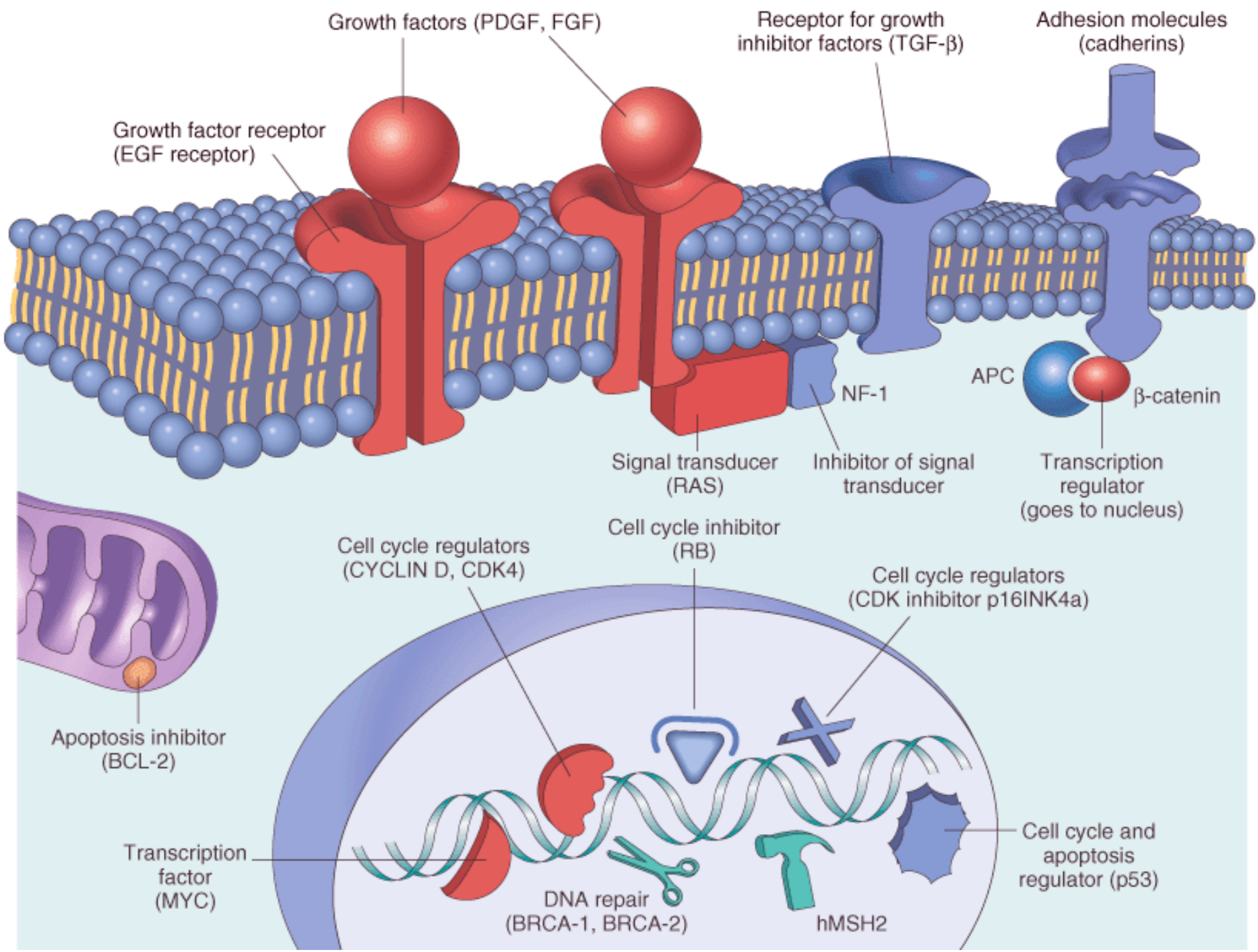

TABLE 7-8 -- Selected Oncogenes, Their Mode of Activation, and Associated Human Tumors

Category	Protooncogene	Mode of Activation	Associated Human Tumor
Growth Factors			
PDGF-β chain	*SIS*	Overexpression	Astrocytome
			Osteosarcoma
Fibroblast growth factors	*HST-1*	Overexpression	Stomach cancer
	INT-2	Amplification	Bladder cancer
			Breast cancer
			Melanoma
TGFα	TGFα	Overexpression	Astrocytomas
			Hepatocellular carcinomas
HGF	HGF	Overexpression	Thyroid cancer
Growth Factor Receptors			
EGF-receptor family	*ERB-B1 (ECFR)*	Overexpression	Squamous cell carcinomas of lung, gliomas
	ERB-B2	Amplification	Breast and ovarian cancers
CSF-1 receptor	*FMS*	Point mutation	Leukemia
Receptor for neurotrophic factors	*RET*	Point mutation	Multiple endocrine neoplasia 2A and B, familial medullary thyroid carcinomas
PDGF receptor	*PDGF-R*	Overexpression	Gliomas
Receptor for stem cell (steel) factor	*KIT*	Point mutation	Gastrointestinal stromal tumors and other soft tissue tumors
Proteins Involved in Signal Transduction			
GTP-binding	*K-RAS*	Point mutation	Colon, lung, and pancreatic tumors
	H-RAS	Point mutation	Bladder and kidney tumors
	N-RAS	Point mutation	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	Hepatoblastomas, hepatocellular carcinoma
		Overexpression	
Nuclear Regulatory Proteins			
Transcriptional activators	*C-MYC*	Translocation	Burkitt lymphoma

	N-MYC	Amplification	Neuroblastoma, small cell carcinoma of lung
	L-MYC	Amplification	Small cell carcinoma of lung
Cell-Cycle Regulators			
Cyclins	*CYCLIN D*	Translocation	Mantle cell lymphoma
		Amplification	Breast and esophageal cancers
	CYCLIN E	Overexpression	Breast cancer
Cyclin-dependent kinase	*CDK4*	Amplification or point mutation	Glioblastoma, melanoma, sarcoma

domain alter the substrate specificity of the tyrosine kinase and lead to thyroid and adrenal tumors but no involvement of the parathyroid. Complete loss of RET function results in Hirschsprung disease (Chapter 17), in which there is lack of development of intestinal nerve plexuses. In all these familial conditions, the affected individuals inherit the *RET* mutation in the germ line. Sporadic medullary carcinomas of the thyroid are associated with somatic rearrangements of the *RET* gene, generally similar to those found in MEN 2B.[46] [47]

Oncogenic conversions by mutations and rearrangements have been found in other growth factor receptor genes. Point mutations that activate c-*FMS*, the gene encoding the colony-stimulating factor 1 (CSF-1) receptor, have been detected in myeloid leukemias. In certain chronic myelomonocytic leukemias with the t(12;9) translocation, the entire cytoplasmic domain of the PDGF receptor is fused with a segment of the ETS family transcription factor, resulting in permanent dimerization of the PDGF receptor.

Far more common than mutations of these protooncogenes is overexpression of normal forms of growth factor receptors. In sporadic papillary thyroid carcinomas, c-MET is overexpressed in almost every case.[48] In these tumors, increased expression of c-MET is not caused by gene mutation but results from enhanced transcription of the gene. In some tumors, increased receptor expression results from gene amplification, but in many cases, the molecular basis of

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increased receptor expression is not fully known. Two members of the EGF receptor family are most commonly involved. The normal form of *ERB B1*, the EGF receptor gene, usually referred to as *EGFR*, is overexpressed in up to 80% of squamous cell carcinomas of the lung, in 50% or more of high-grade astrocytomas called *glioblastomas* (Chapter 28), in 80% to 100% of head and neck tumors, and less commonly, in carcinomas of the urinary bladder and the gastrointestinal tract.[49] [50] In contrast, the *ERB B2* 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.[51] Because the molecular alteration in *ERB B2* is specific for the cancer cells, new therapeutic agents consisting of monoclonal antibodies against ERB B2 have been developed and are currently in use clinically.[49] [51] This type of therapy, directed to a specific alteration in the cancer cell, is called *targeted therapy*. [52] Another example of very successful targeted cancer therapy is the blockage of receptor tyrosine kinase activity of c-KIT in stromal tumors of the gastrointestinal tract.[53] In these tumors, a mutation in c-*KIT*, the gene encoding the receptor for stem cell factor (also known as *steel factor*), constitutively activates the receptor tyrosine kinase, independent of ligand binding.

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 best and 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 proteins were discovered as products of viral oncogenes. *Point mutation of RAS family genes is the single most common abnormality of dominant oncogenes in human tumors.* Approximately 15% to 20% of all human tumors contain mutated versions of RAS proteins.[54] Several distinct mutations of *RAS* have been identified in cancer cells, all of which dramatically reduce the GTPase activity of the RAS proteins. The mutations generally involve codons 12, 59, or 61 of *HRAS, KRAS,* and *NRAS*. The frequency of such mutations varies with different tumors, but in some types 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.[55] [56] [57] 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, particularly those arising in the uterine cervix or breast.

Several studies indicate that RAS plays an important role in mitogenesis induced by growth factors. For example, blockade of RAS function by microinjection of specific antibodies blocks the proliferative response to EGF, PDGF, and CSF-1. Normal RAS proteins are tethered to the cytoplasmic aspect of the plasma membrane, and they flip back and forth between an activated, signal-transmitting form and an inactive, quiescent state. Recently it was found that these proteins may also be found in the endoplasmic reticulum and Golgi membranes, where they can be activated by growth factor binding to the plasma membrane, through a still-uncertain mechanism. [58] In the inactive state, RAS proteins bind guanosine diphosphate (GDP); when cells are stimulated by growth factors or other receptor-ligand interactions, RAS becomes activated by exchanging GDP for GTP (Fig. 7-32). Activated RAS, in turn, acts on the MAP kinase pathway by recruiting the cytosolic protein RAF-1. The MAP kinases so activated target nuclear transcription factors and thus promote mitogenesis. In normal cells, the activated signal-transmitting stage of the RAS protein is transient because its intrinsic GTPase activity hydrolyzes GTP to GDP, thereby returning RAS to its quiescent ground state (described below).

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 enzymatically regulated. The removal of GDP and its replacement by GTP during RAS activation are catalyzed by a family of guanine nucleotide-releasing proteins that are recruited to the cytosolic domain of activated growth factor receptors by adapter proteins. More importantly, 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 rapid hydrolysis of GTP to GDP and termination of signal transduction. Thus, GAPs function as "brakes" that prevent uncontrolled RAS activity. The response to this braking action of GAPs seems to falter when mutations affect the *RAS* gene. *Mutant RAS proteins bind GAP, but their GTPase activity fails to be augmented.* Hence the mutant proteins are "trapped" in their excited GTP-bound form, causing, in turn, a pathologic activation of the mitogenic signaling pathway. The importance of GTPase activation in normal growth control is underscored by the fact that a disabling mutation of neurofibromin *(NF-1),* a GTPase-activating protein, is also associated with neoplasia (see discussion of tumor suppressor genes below).

In addition to RAS, other members of the RAS signaling cascade (RAS/RAF/MAP kinase) may also be altered in cancer cells. 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.[59] [60] 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.

Recent studies have revealed that, in addition to its role in transducing growth factor signals, RAS is also involved in regulation of the cell cycle. As described above, the passage of cells from G_1 to the S phase is modulated by cyclins and CDKs. RAS proteins can indirectly regulate the levels of cyclins by activating the MAP kinase pathway and the AP-1 transcription factor.

Because *RAS* is so frequently mutated in human cancers, much effort has been spent to develop anti-RAS modalities of targeted therapy. Several such strategies for cancer treatment are being evaluated. The specific targets include blockade of

Figure 7-32 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 mutant 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.

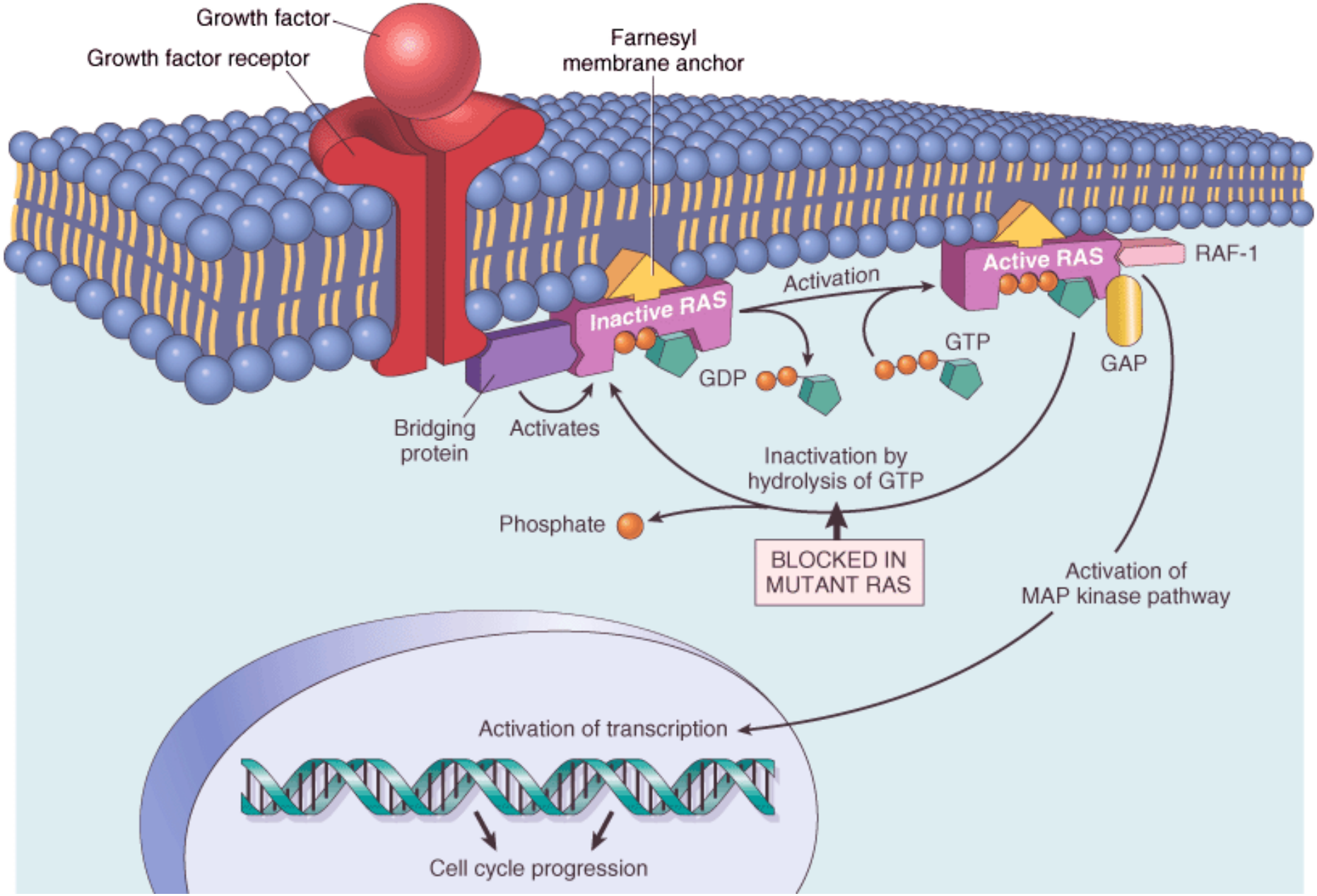
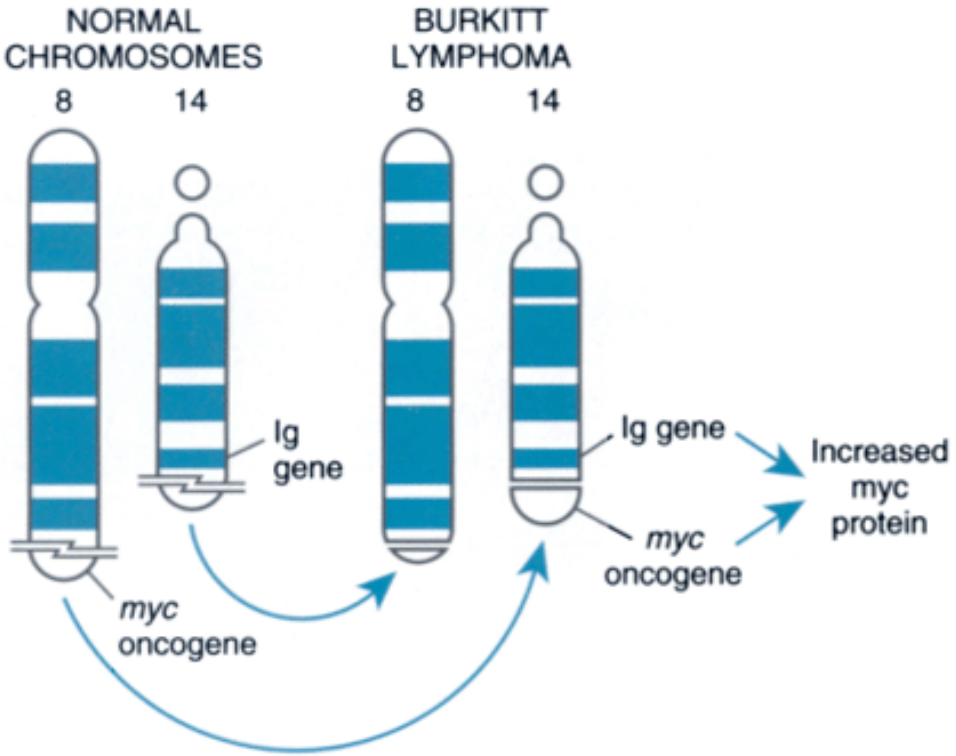


Figure 7-33 The chromosomal translocation and associated oncogenes in Burkitt lymphoma and chronic myelogenous leukemia.



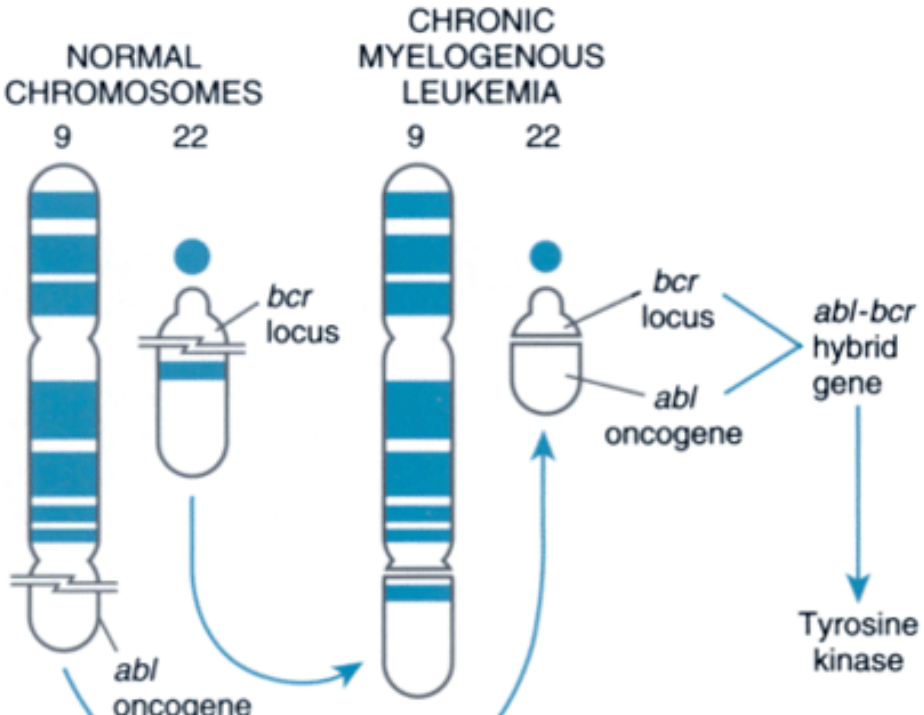


Figure 7-34 Amplification of the N-*MYC* gene in human neuroblastomas. The N-*MYC* gene, normally present on chromosome 2p, becomes amplified and is seen either as extra chromosomal double minutes or as a chromosomally integrated, homogeneous staining region. The integration involves other autosomes, 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, pp. 229–256.)*

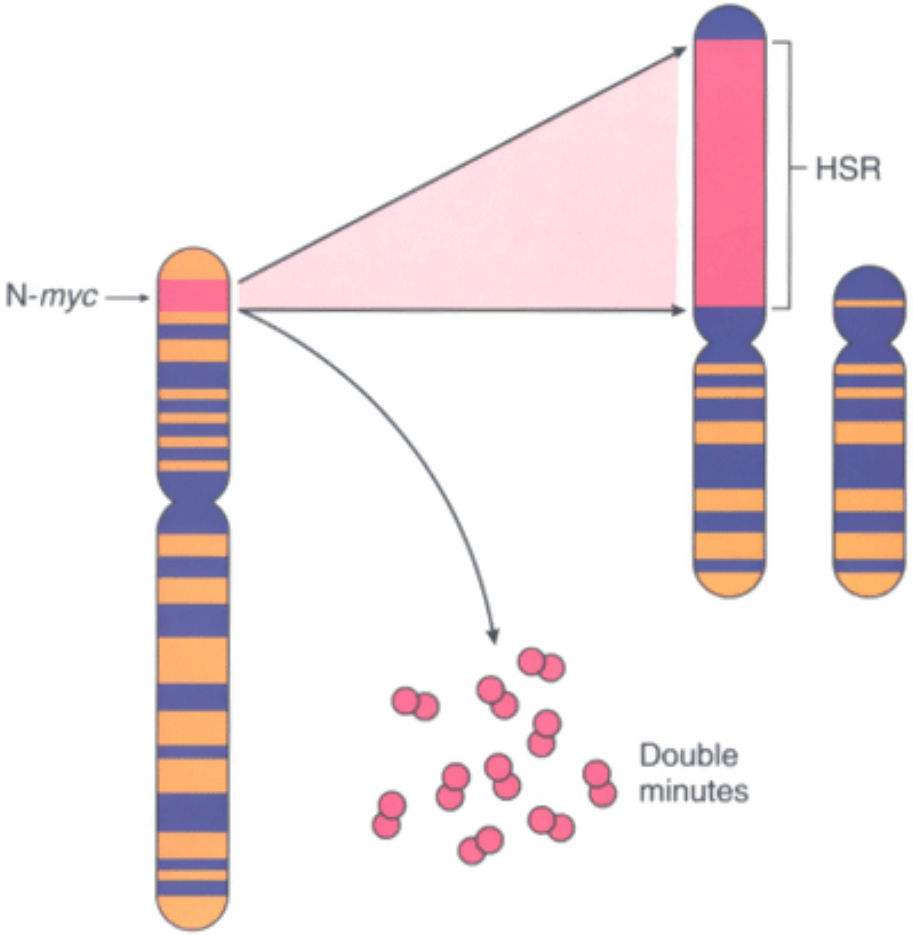


TABLE 7-9 -- Selected Tumor Suppressor Genes Involved in Human Neoplasms

Subcellular Location	Gene	Function	Tumors Associated with Somatic Mutations	Tumors Associated 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	*NF-1*	Inhibition of RAS signal transduction and of p21 cell-cycle inhibitor	Neuroblastomas	Neurofibromatosis type 1 and sarcomas
Cytoskeleton	*NF-2*	Cytoskeletal stability	Schwannomas and meningiomas	Neurofibromatosis 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	PI-3 kinase signal transduction	Endometrial and prostate cancers	Unknown
	SMAD 2 and *SMAD 4*	TGF-β signal transduction	Colon, pancreas tumors	Unknown
Nucleus	*RB*	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
	WT-1	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
	BRCA-1 and *BRCA-2*	DNA repair	Unknown	Carcinomas of female breast and ovary; carcinomas of male breast
	KLF6	Transcription factor	Prostate	Unknown

(Fig. 7-36). When cells enter the S phase, they can continue to cell division independent of growth factors. It should be obvious from this discussion that if RB is absent (owing to gene deletions) or its ability to regulate E2F transcription factors is derailed, the molecular brakes on the cell cycle are released, and the cells move into the S phase followed by cell replication. 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.

It was mentioned previously that germ-line loss or mutations of the *RB* gene predispose to occurrence of retinoblastomas and to a lesser extent osteosarcomas. Furthermore, somatically acquired 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 germ line mutation of the *RB* locus develop mainly retinoblastomas? (2) Why are inactivating mutations of *RB* not much more common in human cancer? The basis for the occurrence of tumors restricted to the retina in patients 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* mutation may be a critical initiating event for retinoblastomas but may be only an accessory factor for malignancies at other sites.

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 CDK inhibitors 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 (p16INK4a, CYCLIN D, CDK4, RB) is dysregulated in the vast majority of human cancers*.[38] 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.[45]

Several other pathways of cell growth regulation, some to be discussed in more detail later, also converge on RB (Fig. 7-36):

• TGF-β induces inhibition of cellular proliferation. This effect of TGF-β is mediated, at least in part, by up-regulation of the CDK inhibitor p27.

• 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 deleted 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 human papillomavirus (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 16, which confer high risk for the development of

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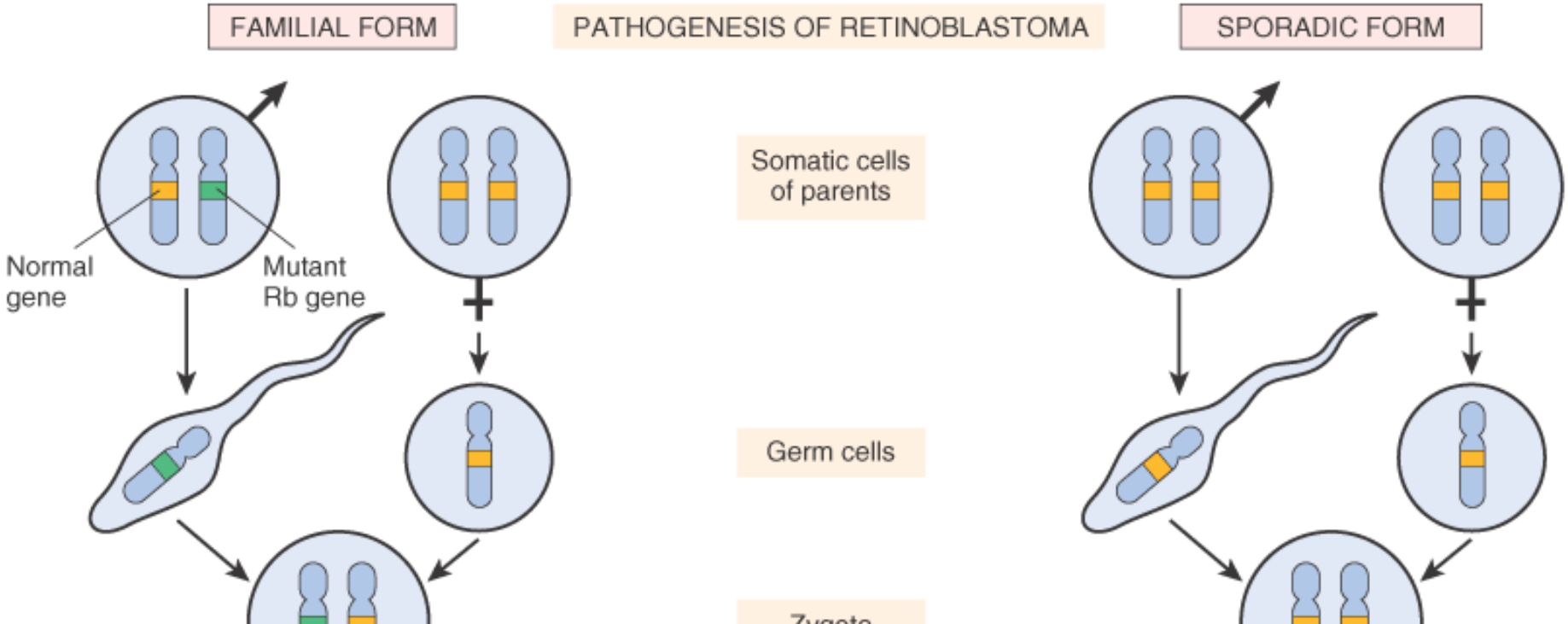
cervical carcinomas. Thus, the RB protein, unable to bind the E2F transcription factors, is functionally deleted, and the transcription factors are free to cause cell-cycle progression.

• The *p53* tumor suppressor gene exerts its growth-inhibiting effects at least in part by up-regulating the synthesis of the CDK inhibitor p21 (see Fig. 7-29 and Fig. 7-36).

Figure 7-35 Pathogenesis of retinoblastoma. Two mutations of the *RB* locus on chromosome 13q14 lead to neoplastic proliferation of the retinal cells. 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. In the sporadic form, on the other hand, both mutations at the *RB* locus are acquired by the retinal cells after birth.



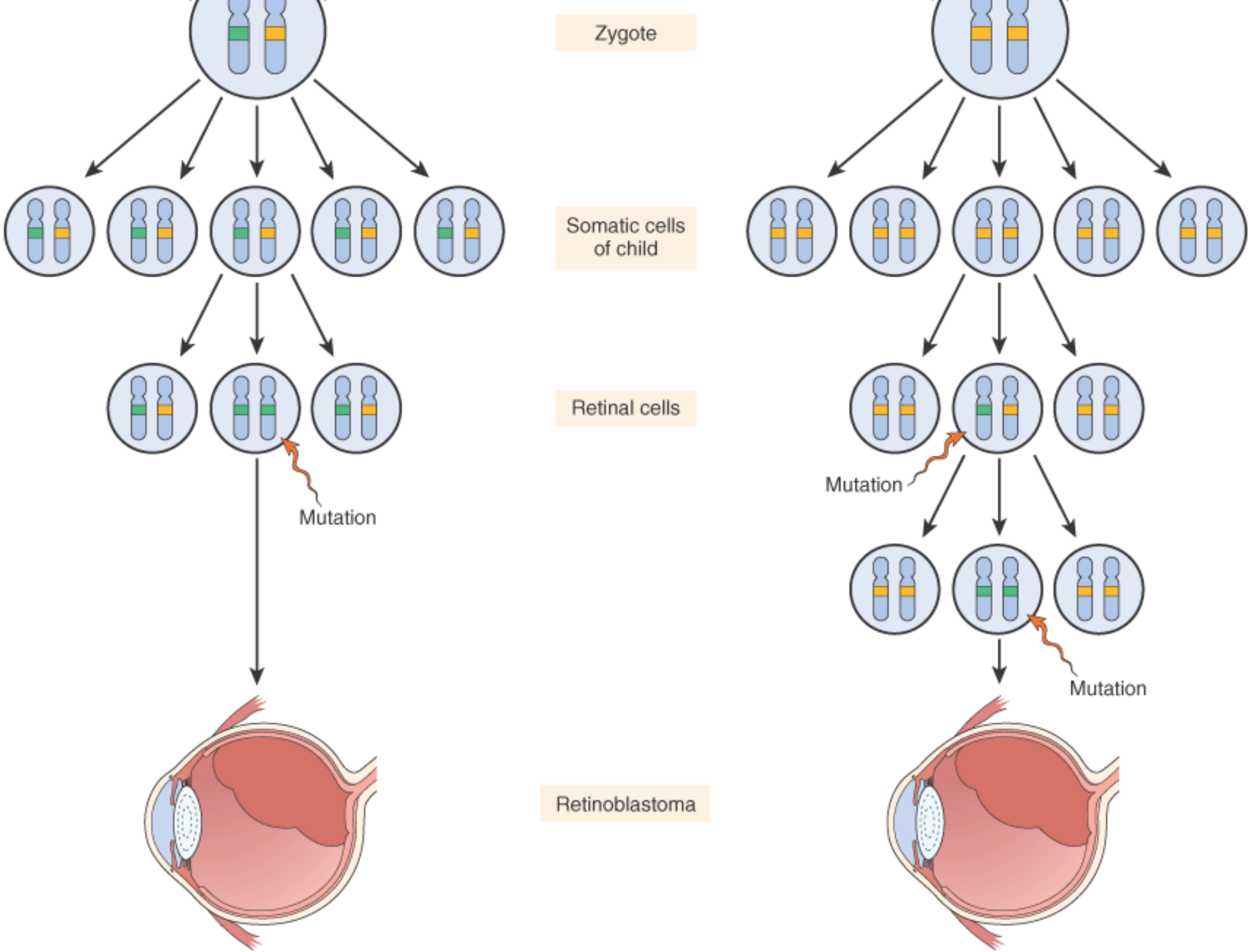

Zygote
Somatic cells of child
Retinal cells
Mutation
Retinoblastoma
Mutation
Mutation

Figure 7-36 Role of RB as a cell-cycle regulator. Various growth factors promote the formation of the cyclin D-CDK4 complex. This complex (and to some extent cyclin E-CDK2) phosphorylates RB, changing it from an active (hypophosphorylated) to an inactive state (hyperphosphorylation). RB inactivation allows the cell to pass the G_1 /S restriction point. Growth inhibitors such as TGF-β and *p53* and the Cip/Kip (e.g., p21, p57) and INK4a (p161NK4a and p19ARF) cell-cycle inhibitors prevent RB activation. Transforming proteins of oncogenic viruses bind hypophosphorylated RB and cause its functional inactivation. Virtually all cancers show dysregulation of the cell cycle by affecting the four genes marked by an asterisk.

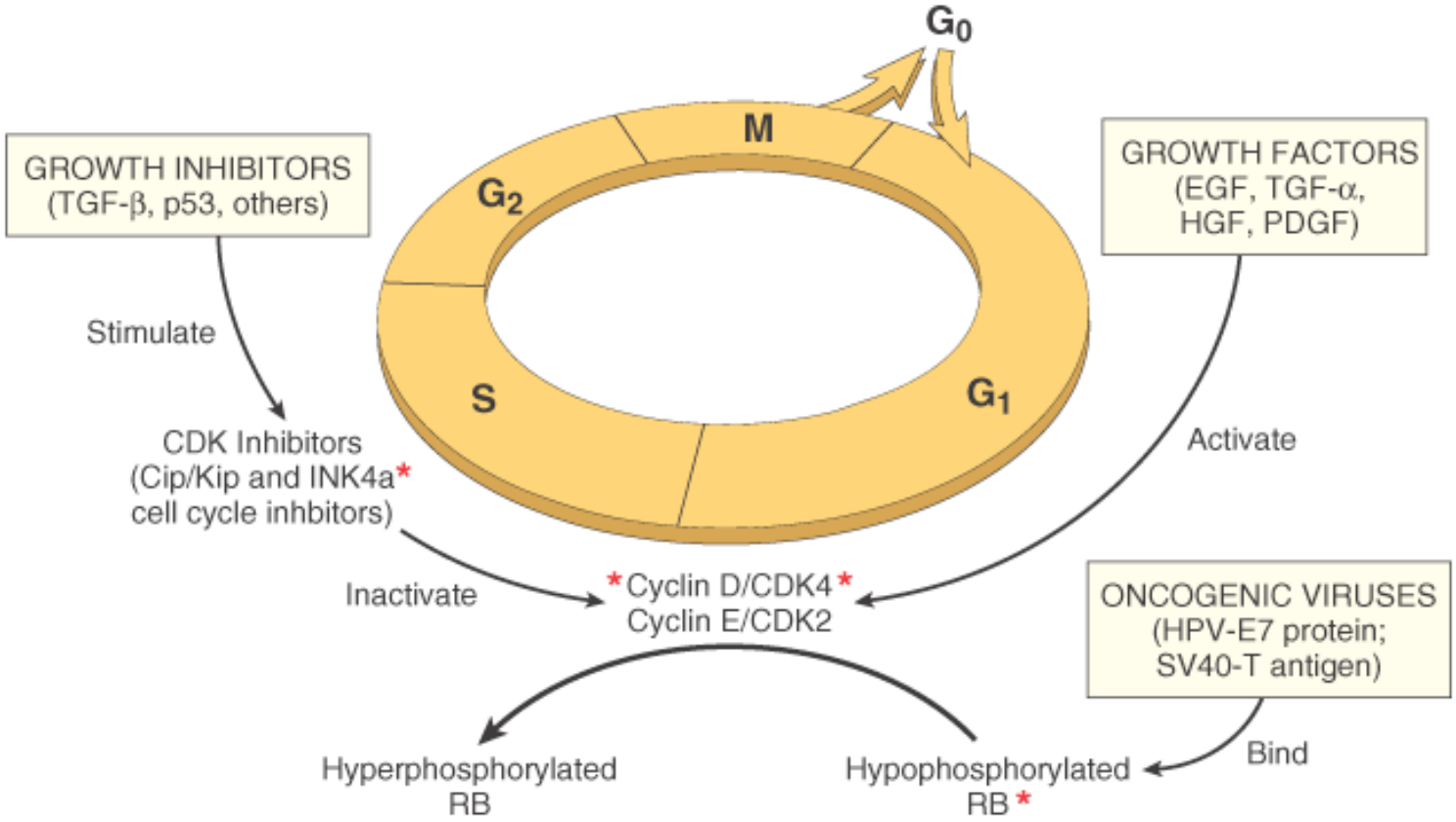
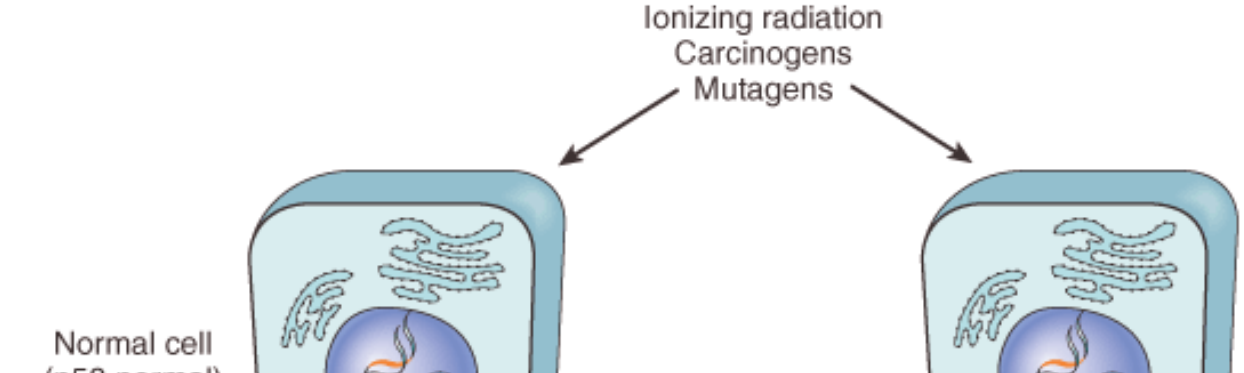


Figure 7-37 The role of *p53* in maintaining the integrity of the genome. Activation of normal *p53* by DNA-damaging agents or by hypoxia leads to cell-cycle arrest in G_1 and induction of DNA repair, by transcriptional up-regulation of the cyclin-dependent kinase inhibitor *p21*, and the *GADD45* genes, respectively. Successful repair of DNA allows cells to proceed with the cell cycle; if DNA repair fails, *p53*-induced activation of the *BAX* gene promotes apoptosis. In cells with loss or mutations of *p53*, DNA damage does not induce cell-cycle arrest or DNA repair, and hence genetically damaged cells proliferate, giving rise eventually to malignant neoplasms.



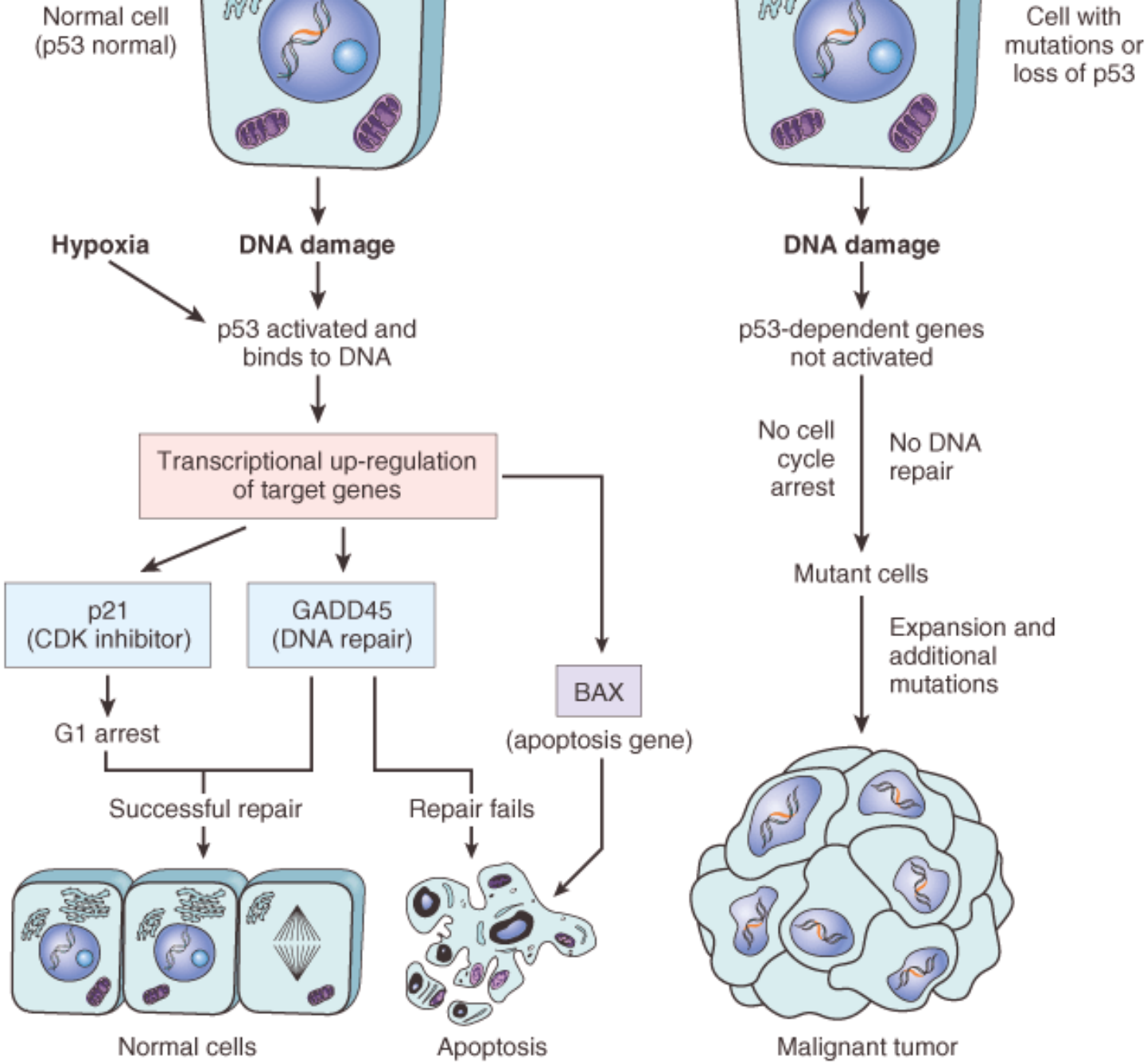


Figure 7-38 *A,* The role of APC in regulating the stability and function of β-catenin. APC and β-catenin are components of the WNT signaling pathway. In resting cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low. *B,* When cells are stimulated by secreted 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 several genes involved in the cell cycle. *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 the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

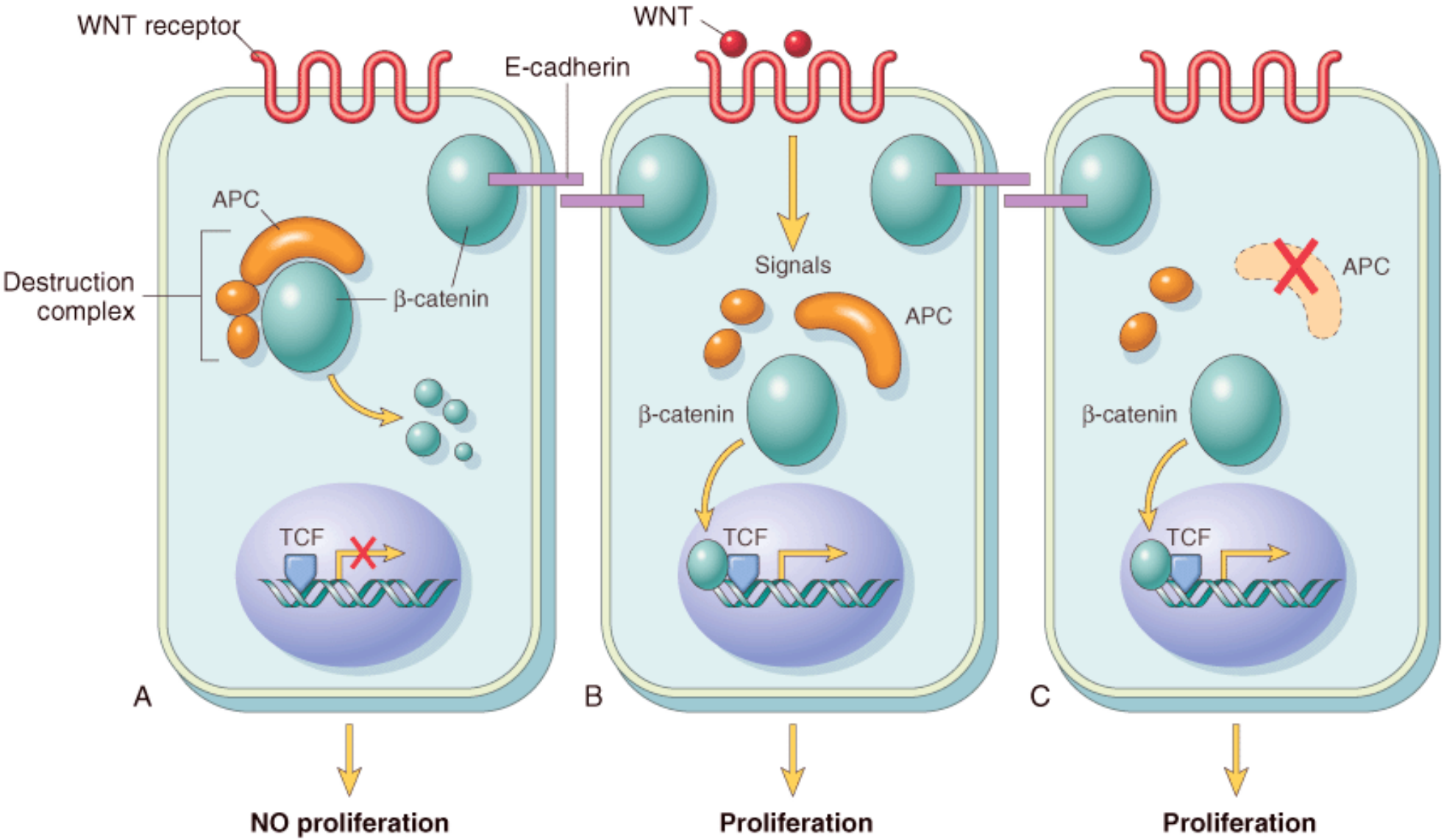


Figure 7-39 Interaction between cancer susceptibility genes and DNA repair. *ATM* (ataxia-telangiectasia mutated) senses a double-strand break in DNA, induced by agents such as ionizing radiation. *ATM* and *CHEK2* phosphorylate *BRCA1*, promoting its migration to the break site. The Fanconi's anemia protein complex (proteins A, C, E, F, G) triggers the ubiquitination and colocalization of the Fanconi protein D2 with *BRCA1* at the break site. *BRCA2* carries RAD51, an enzyme involved in DNA recombination repair, to the same site. BRCA1, BRCA2, and RAD51 repair the DNA break by an error-free recombination mechanism. RAD51 is a component of cell cycle check points. *(Redrawn from Venkitaraman AR: A growing network of cancer-susceptibility genes. N Engl J Med 348:1917, 2003.)*

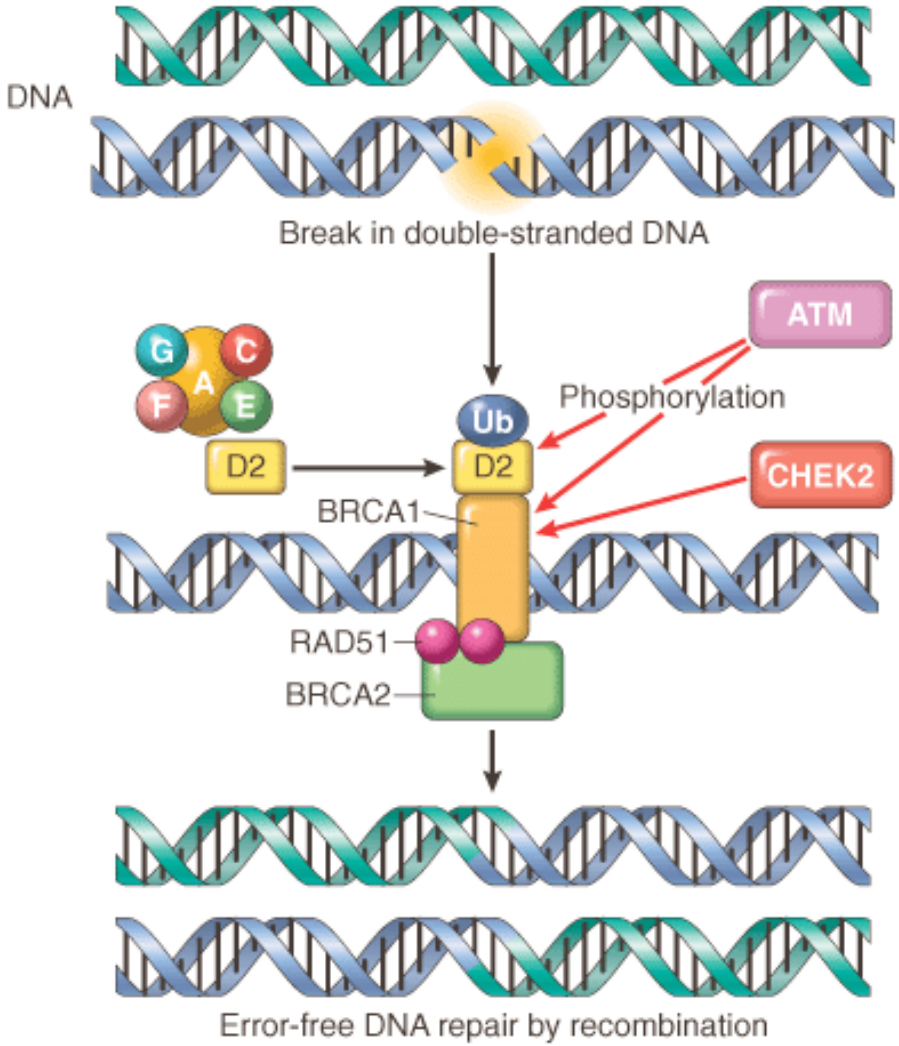


Figure 7-40 Cellular responses to telomere shortening. The figures show the responses of normal cells, which have intact cell-cycle checkpoints and of cells with checkpoint defects. *(From Wong JMY, Collins K: Telomere maintenance and disease. Lancet 362:983, 2003.)*

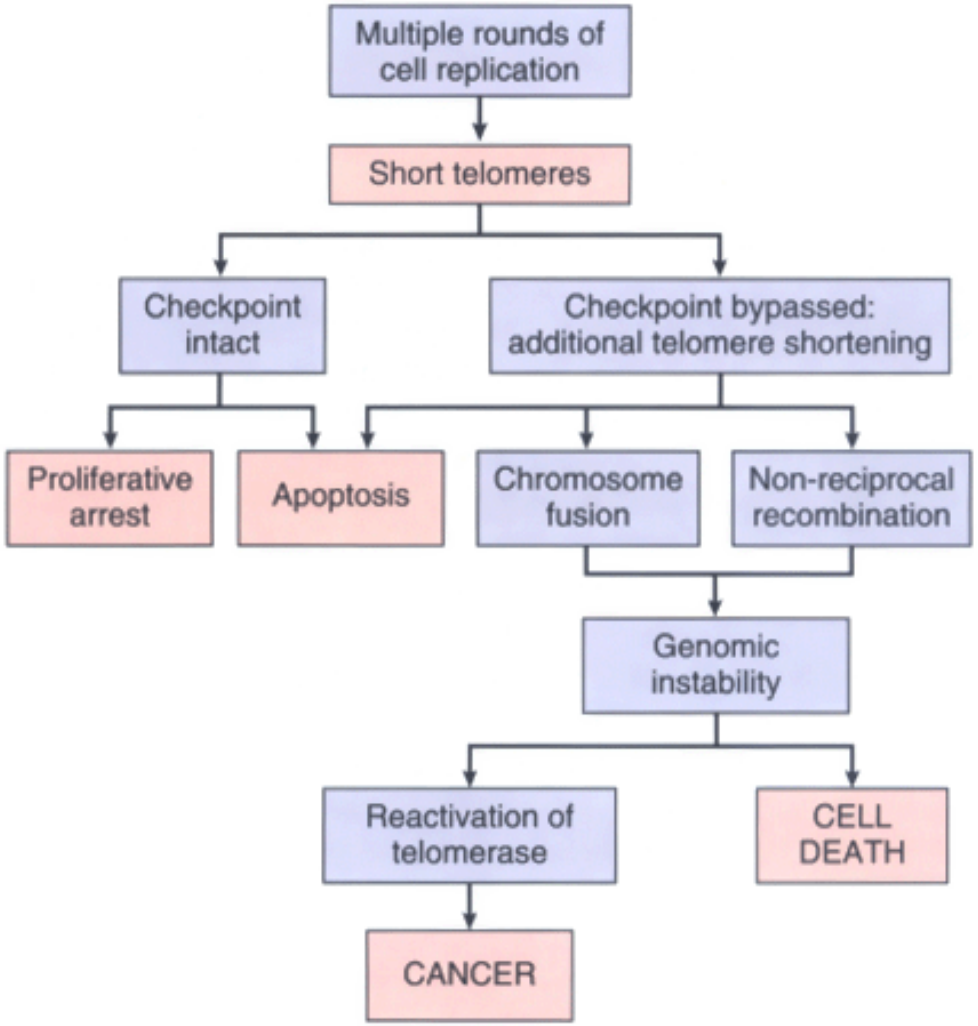
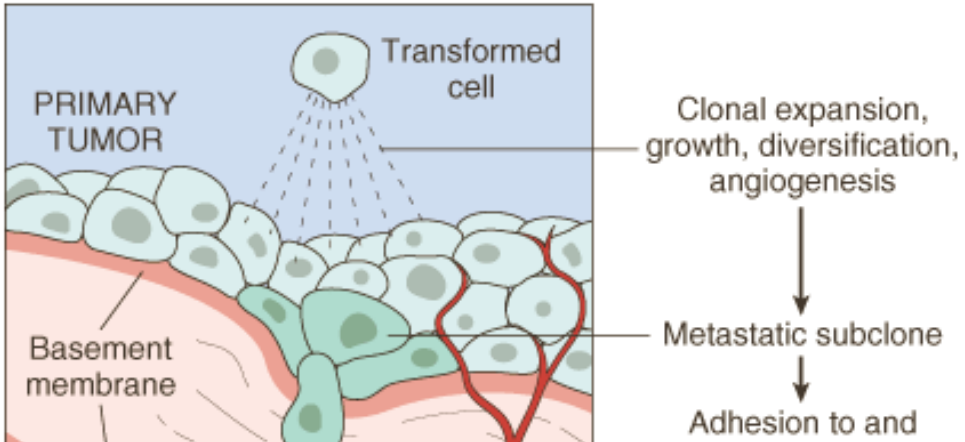
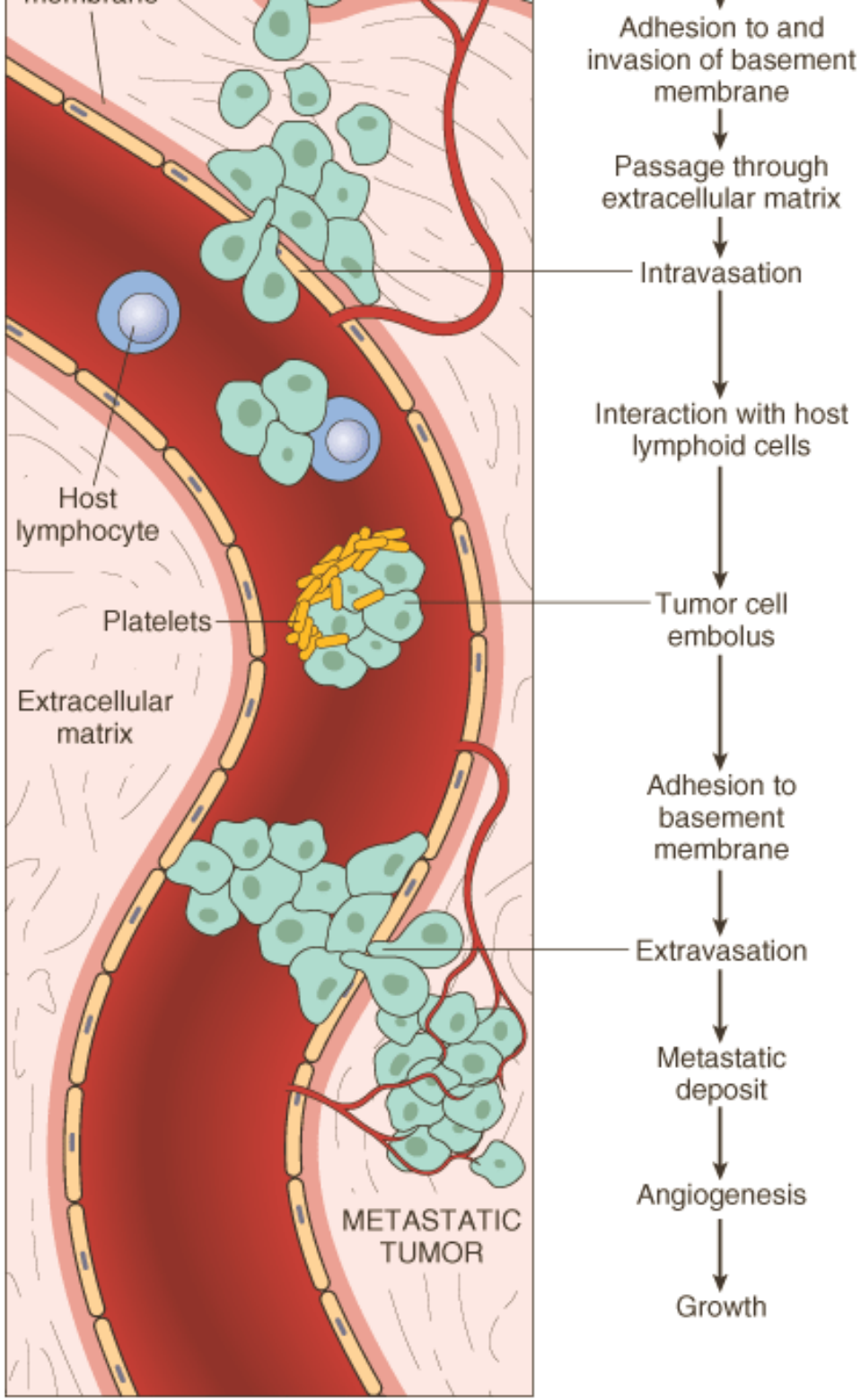


Figure 7-42 The metastatic cascade. Schematic illustration of the sequential steps involved in the hematogenous spread of a tumor.





Adhesion to and invasion of basement membrane

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Passage through extracellular matrix

↓

Intravasation

↓

Interaction with host lymphoid cells

↓

Tumor cell embolus

↓

Adhesion to basement membrane

↓

Extravasation

↓

Metastatic deposit

↓

Angiogenesis

↓

Growth

Figure 7-43 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.

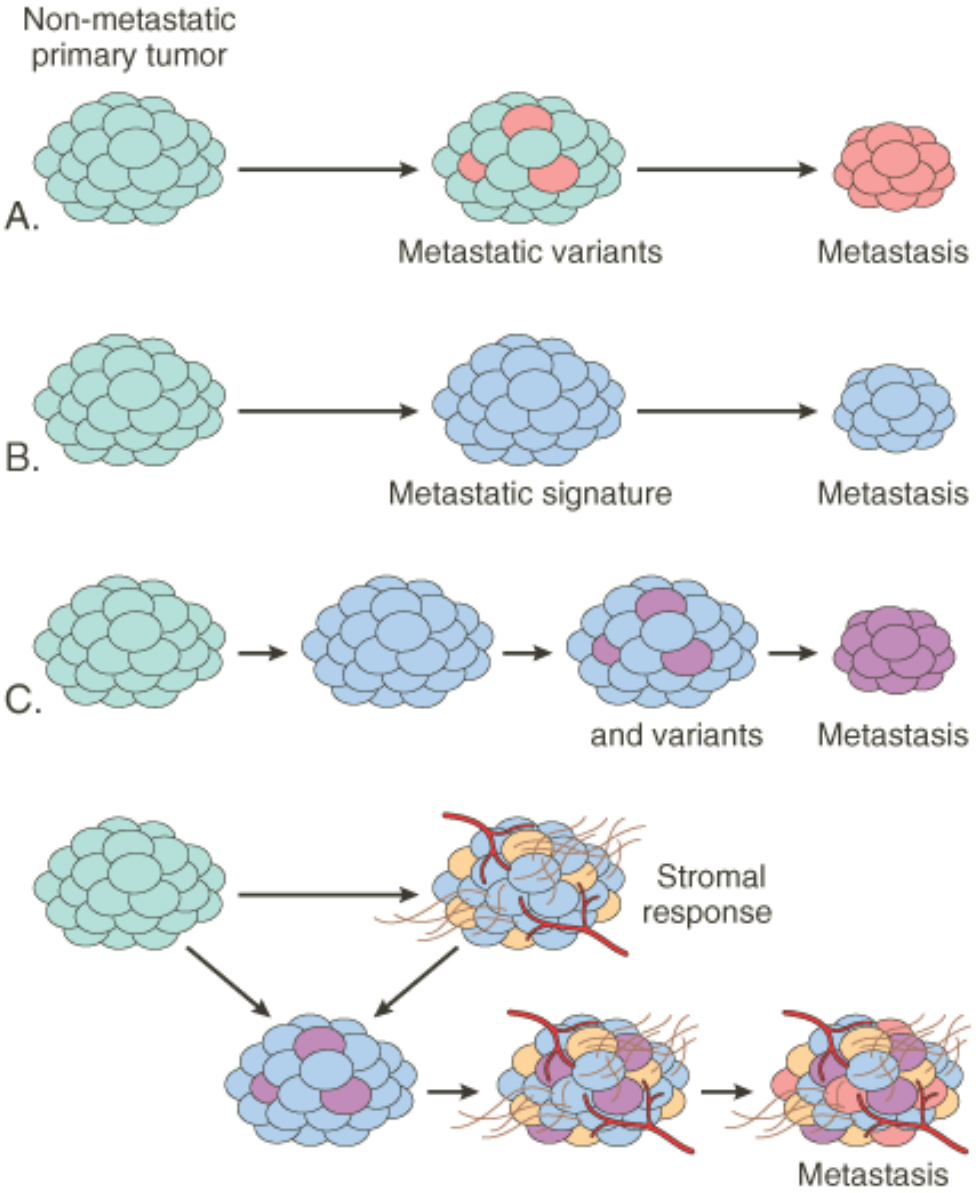
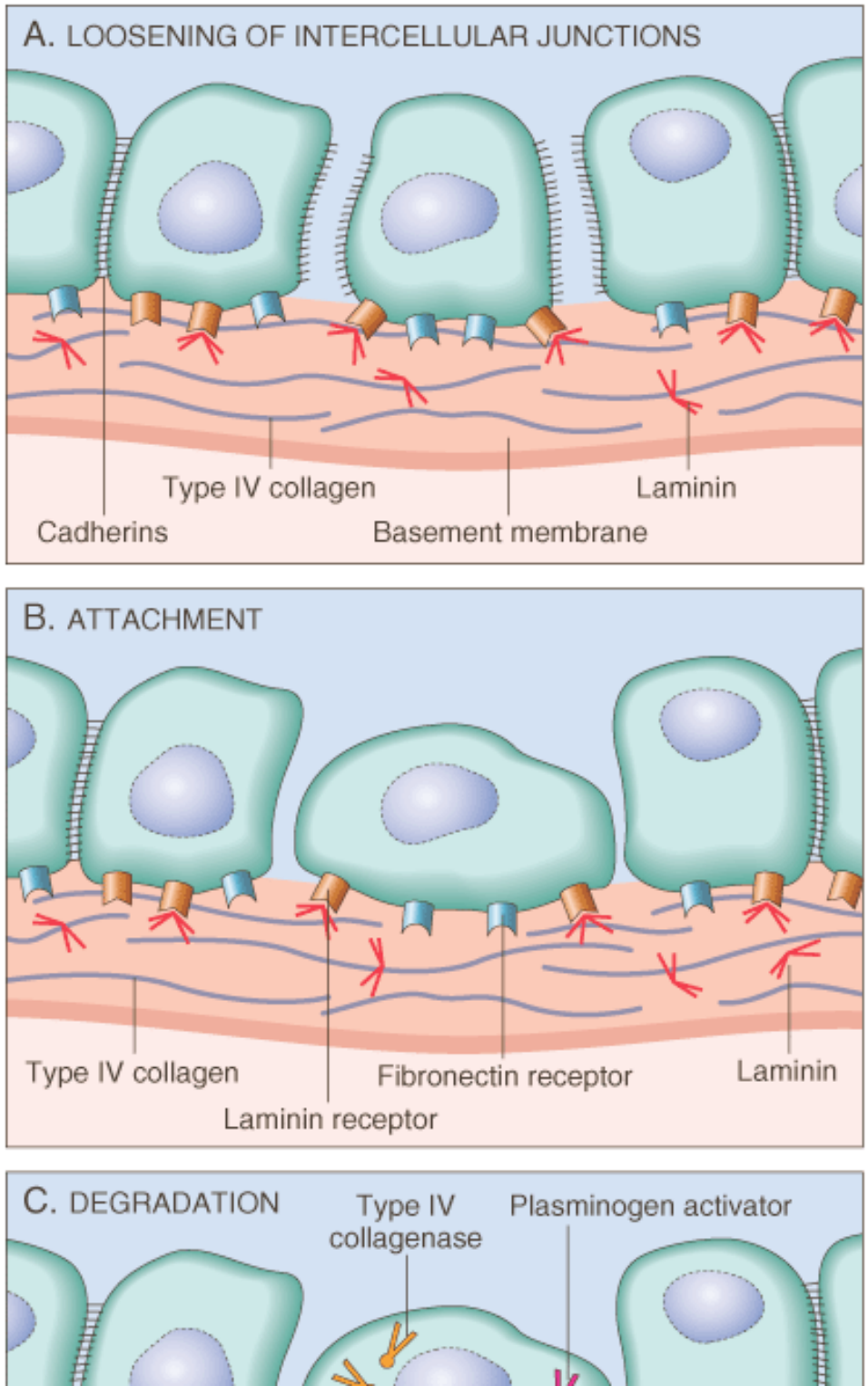
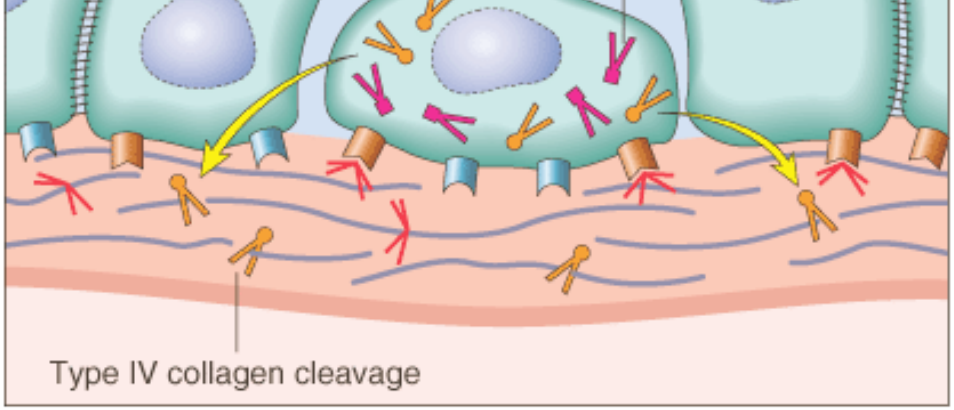


Figure 7-44 *A–D*, Schematic illustration of the sequence of events in the invasion of epithelial basement membranes by tumor cells. Tumor cells detach from each other because of reduced adhesiveness, and cells then attach to the basement membrane via the laminin receptors and secrete proteolytic enzymes, including type IV collagenase and plasminogen activator.

Degradation of the basement membrane and tumor cell migration follow.





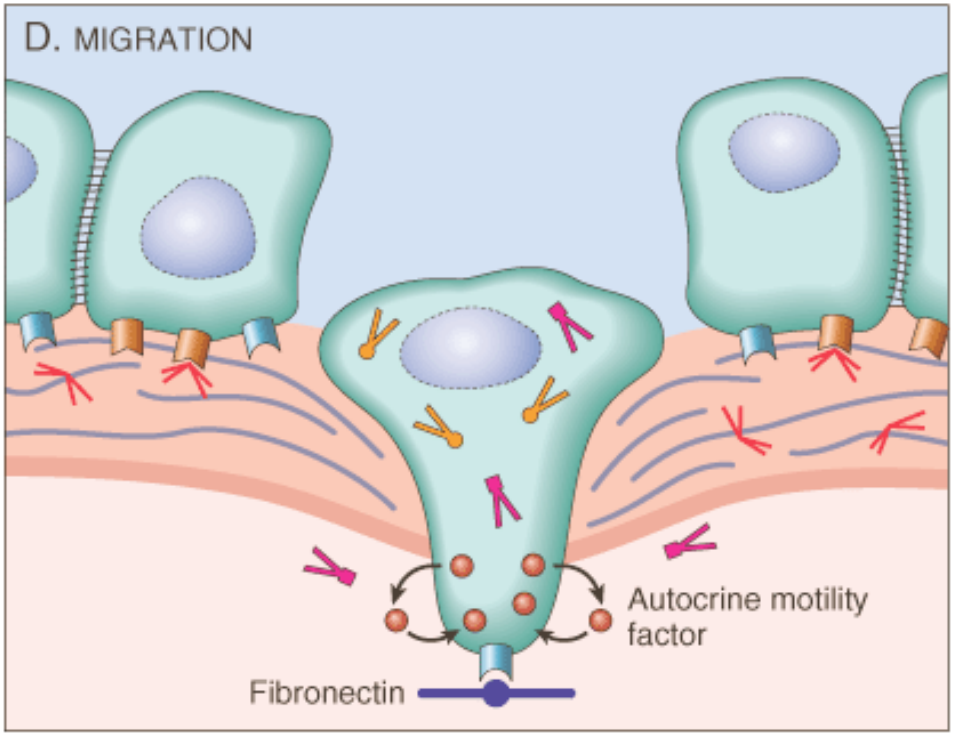


TABLE 7-10 -- Selected Examples of Oncogenes Activated by Translocation

Malignancy	Translocation	Affected Genes
Chronic myeloid leukemia	(9;22)(q34;q11)	Ab1 9q34
		bcr 22q11
Acute leukemias (AML and ALL)	(4;11)(q21;q23)	AF4 4q21
		MLL 11q23

	(6;11)(q27;q23)	AF6 6q27
		MLL 11q23
Burkitt lymphoma	(8;14)(q24;q32)	c-*myc* 8q24
		IgH 14q32
Mantle cell lymphoma	(11;14)(q13;q32)	Cyclin D 11q13
		IgH 14q32
Follicular lymphoma	(14;18)(q32;q21)	IgH 14q32
		bcl-2 18q21
T-cell acute lymphoblastic leukemia	(8;14)(q24;q11)	*c-myc* 8q24
		TCR-α 14q11
	(10;14)(q24;q11)	*Hox* 11 10q24
		TCR-α 14q11
Ewing sarcoma	(11;22)(q24;q12)	Fl-1 11q24
		EWS 22q12
Underlined genes are involved in multiple translocations.		
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia.		

course. The translocations associated with the *ABL* oncogene in chronic myeloid leukemia and with c-*MYC* in Burkitt lymphoma have been mentioned earlier, in conjunction with the discussion of molecular defects in cancer cells (see Fig. 7-32). Several other karyotype alterations in cancer cells are presented in the discussion of specific forms of neoplasia.

Two types of chromosomal rearrangements can activate protooncogenes—translocations and inversions. Chromosomal translocations are much more common (Table 7-10) and are discussed here. Translocations can activate protooncogenes in two ways:

- In lymphoid tumors, specific translocations result in overexpression of protooncogenes by removing them from their regulatory elements.
- In many hematopoietic tumors, the translocations allow normally unrelated sequences from two different chromosomes to recombine and form hybrid genes that encode growth-promoting chimeric proteins.

Overexpression of a protooncogene 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, the expression of the *MYC* gene is tightly controlled; it is expressed only during certain stages of the cell cycle. In Burkitt lymphoma, the most common form of translocation results in the movement of the *MYC*-containing segment of chromosome 8 to chromosome 14q band 32 (Fig. 7-33), placing it close to the immunoglobulin heavy-chain (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 mutations or loss of the regulatory sequences of the *MYC* gene. As the coding sequences remain intact, the gene is constitutively expressed at high levels. The gene may be translocated to the antigen receptor loci simply because these loci are accessible (i.e. in "open" chromatin) and active in developing lymphocytes. The invariable presence of the translocated *MYC* gene in Burkitt lymphomas attests to the importance of *MYC* overexpression 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 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 *BCL-2* 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 protooncogenes 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 chronic myeloid leukemia 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

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a truncated portion of the protooncogene c-*ABL* (from chromosome 9) to the *BCR (b*reak point *c*luster *r*egion) on chromosome 22 (Fig. 7-33). 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 chronic myeloid leukemia and acute lymphoblastic leukemias, they differ at the molecular level. In chronic myeloid leukemia, 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 formed.[62] [63] The molecular pathways activated by the BCR-ABL protein are complex and not completely understood. It inhibits apoptosis, decreases the requirement for growth factors, binds to cytoskeleton components, decreases cell adhesion, and activates multiple pathways, including those of RAS, PI-3 kinase, and STATs (Chapter 3). BCR-ABL also acts on DNA repair and may cause genomic instability that contributes to the progression of the disease.

Transcription factors are often the partners in gene fusions occurring in cancer cells. For instance, the *MLL* (myeloid, lymphoid leukemia) gene on 11q23 is known to be involved in 25 different translocations with several different partner genes, some of which encode transcription factors (see Table 7-10). The *Ew*ing *S*arcoma (*EWS*) gene at 22q12 was first described in the t(11;22)(q24;12) reciprocal translocation present in Ewing sarcoma (a highly malignant tumor of children; Chapter 26) but may be translocated in other types of sarcomas. *EWS* is itself a transcription factor, and all of its partner genes analyzed so far also encode a transcription factor. In Ewing tumor, for example, the *EWS* gene fuses with the *FLI 1* gene; the resultant chimeric EWS-FLI 1 protein is a member of the ETS transcription factor family, which has transforming ability.

Gene Amplification

Activation of protooncogenes associated with overexpression of their products may result from reduplication and *amplification* of their DNA sequences. Such amplification may produce several hundred copies of the protooncogene in the tumor cell.[137] 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, chromosome-like structures called *double minutes* (dms), and *homogeneous staining regions* (HSRs). The latter derive from the assembly of amplified genes into new chromosomes; because the regions containing amplified genes lack a normal banding pattern, they appear homogeneous in a G-banded karyotype (see Fig. 7-34). The most interesting cases of amplification involve N-*MYC* in neuroblastoma and *ERB B2* 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 dms and HSRs. *ERB B2* amplification occurs in about 20% of breast cancers and may represent a distinct tumor phenotype. Amplification of C-*MYC*, L-*MYC*, and N-*MYC* correlates with disease progression in small cell cancer of the lung. Another gene frequently amplified is *CYCLIN D1* (breast carcinomas, head and neck carcinomas, and other squamous cell carcinomas).

Epigenetic Changes

It has become evident during the past few years that certain tumor suppressor genes may be inactivated not because of structural changes but because the gene *is silenced by hypermethylation of promoter sequences without a change in DNA base sequence*.[138] Such changes appear to be stably maintained through multiple rounds of cell division. Methylation

takes place in CpG islands in DNA, but de novo methylation rarely occurs in normal tissues. However, methylation has been detected in various tumor suppressor genes in human cancers. They include *p14ARF* in colon and stomach cancers, *p16INK4a* in various types of cancers, *BRCA1* in breast cancer, *VHL* in renal cell carcinomas, and the *MLH1* mismatch repair gene in colorectal cancer.[139] 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.[140] Although the discussion of whether methylation of tumor suppressor genes has a causal role in cancer development continues, 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 strengthens the notion that epigenetic changes may directly contribute to tumor development.[141]

Molecular Profiles of Cancer Cells

A new era in cancer research was initiated with the development of methods to measure the expression of thousands of genes in tumors and normal tissues. Among these new methods, the determination of RNA levels by microarray analysis has found wide application (Box 7-1). Currently, this method can measure RNA expression from virtually all known genes (Fig. 7-45). The expression profiles obtained from DNA microarray analysis are known as *gene expression signatures or molecular profiles*. The application of this technique to the study of breast cancers and leukemias has been particularly rewarding (see Box 7-1). It was recently found that there are breast cancer subtypes that can be identified by their molecular profiles and that the molecular signatures of some of these subtypes can help predict the course of the disease (see Box 23-1 , Chapter 23).[142] Analysis of acute lymphoblastic leukemia by DNA microarrays has established the molecular signatures of prognostic subtypes and uncovered novel markers associated with these subtypes.[143]

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. Many eons ago, before

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Box 7-1. Gene Expression Profiles of Human Cancers. Microarrays and Proteomics

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 thousands of genes simultaneously. [208] [209] The most common method for large-scale analysis of gene expression in use today is based on DNA microarray technology. In this method, DNA fragments, either cDNAs or oligonucleotides, are spotted on a glass slide or on some other solid support. As the techniques used for the spotting are similar to those employed to produce semiconductor chips for electronic products, the arrays are known as "*gene chips*." Chips can be purchased from commercial suppliers or produced in-house, and can contain more than 20,000 gene fragments. The fragments are typically obtained from complementary DNA (cDNA) libraries or sets of nucleotides from known and uncharacterized genes. The gene chip is then hybridized to "probes" prepared from tumor and control samples (the probes are usually cDNA copies of RNAs extracted from tumor and uninvolved tissues). Before hybridization to the chip, the probes are labeled with fluorochromes that emit different colors (e.g. red color for tumor RNA and green color for control RNA). After hybridization the chip is read using a laser scanner (Fig. 7-45); each spot on the array will be red (increased expression of a gene in the tumor), green (decreased expression in the tumor) or, if there is no difference in gene expression between the tumor and control sample, the spots will be either black or yellow (depending on the type of fluorescent scanning). Sophisticated software has been developed to measure the intensity of the fluorescence for each spot and produce data sets in which genes with similar expression patterns are clustered. [210] This method of analysis, called *hierarchical clustering,* groups together genes according to the similarity of their gene expression patterns. The software can be linked to large sequencing and array databases available through the Web. This allows appropriate gene identification and comparison between expression profiles from various

sources. A major problem in the analysis of gene expression in tumors is the heterogeneity of the tissue. In addition to the heterogeneity between 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 tissues using *laser capture microdissection*. In this technique, the dissection of the tumor or cells is made under a microscope through a focused laser. The dissected material is then captured or "catapulted" into a small cap and processed for RNA and DNA isolation.

Gene expression profiling of tumors has multiple uses, and the number of publications using this technique has grown enormously during the past few years. Much of the work performed is not directed toward proving or disproving a proposed hypothesis. Gene expression analysis can be used to classify tumors; to predict metastatic potential, prognosis, and response to therapy; to reveal gene expression patterns that are dependent on the mutation of a single oncogene; and to analyze the effects of hormones and environmental agents on cancer development. [209] The applications of this technology keep expanding and being refined, but much has already been accomplished.[141] [209] [210] [211] We mention only a few interesting examples. Profiling of cells from adult and pediatric T-cell acute lymphoblastic leukemia has identified the patterns of gene expression in leukemic blast cells and has accurately classified each prognostic subtype.[211] The work that has received the highest publicity involves gene expression profiling of breast cancers. In addition to identifying new subtypes of breast cancers, a 70-gene prognosis profile was established. Using this type of profile, it has been reported that: (1) the profile was a powerful predictor of disease prognosis for young patients; (2) it was particularly accurate for predicting metastasis during the first 5 years after diagnosis; and (3) prognosis determined by gene expression profiles correlated highly with histologic grade and estrogen receptor status but not with lymphatic spread of the tumor.[211] A more recent analysis has pooled together data gathered by different laboratories and has confirmed the identification of distinct subtypes of breast cancer.[142] Given all of these remarkable results, it is time to ask whether this technology is "ready for prime time"; that is, ready for day to day clinical applications. Things are moving very fast in this area, but before clinical applications are considered, many issues need to settled. Not only do larger trials need to be conducted to prove the reliability and accuracy of the analysis but also, just as important, the procedures for handling samples, performing the analyses, and reporting the data need to be standardized, so that data obtained in various laboratories can be compared.

Next on the horizon of molecular techniques for the global analysis of gene expression in cancers is *proteomics*, a technique used to obtain expression profiles of proteins contained in tissues, serum, or other body fluids. The original method consisted of the separation of proteins by 2-dimensional gel electrophoresis, followed by identification of individual proteins by mass spectrometry. A more recent technique, called *ICAT* (*i*sotope-*c*oding *a*ffinity *t*ags) does not rely on electrophoresis for protein separation. In ICAT, proteins in the test and control samples are labeled with light or heavy isotopes. The differentially labeled proteins are then identified and quantified by mass spectrometry. A variation of proteomic analysis has been used to obtain protein profiles in the blood of cancer patients without identification of individual proteins.[215]

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. [208]

Figure 7-45 Schematic representation of the steps required for the analysis of global gene expression by DNA microarray. RNA is extracted from tumor and normal tissue. 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 square*, 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 ventrical row corresponds to single samples.

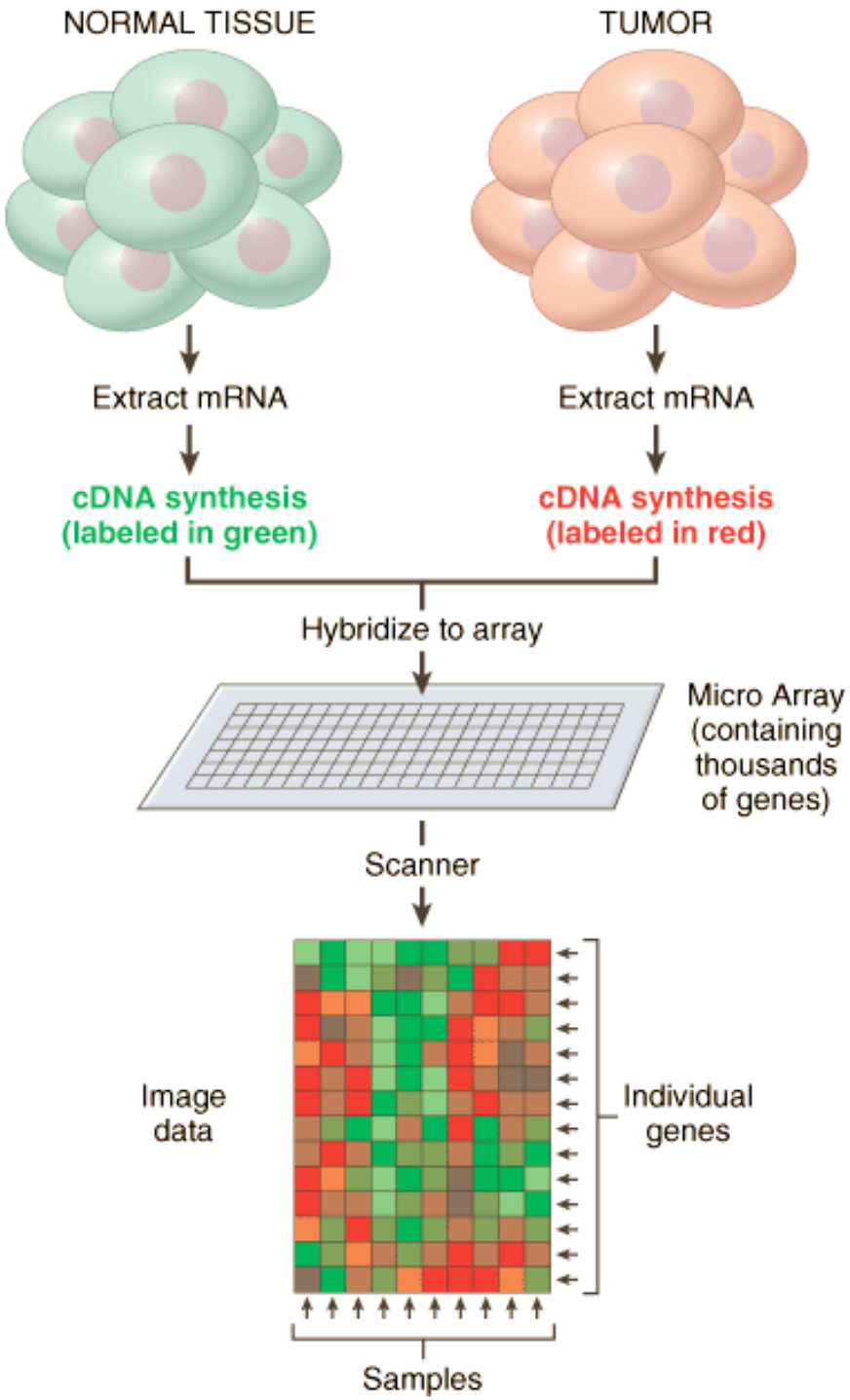

Figure 7-47 Schematic illustration of the pathways of malignancy initated by mutation of the gatekeeper genes (e.g., *APC*, *NF-1*, *RB*) or caretaker genes (e.g., *hMSH2*, *BRCA-1*, *BRCA-2*).

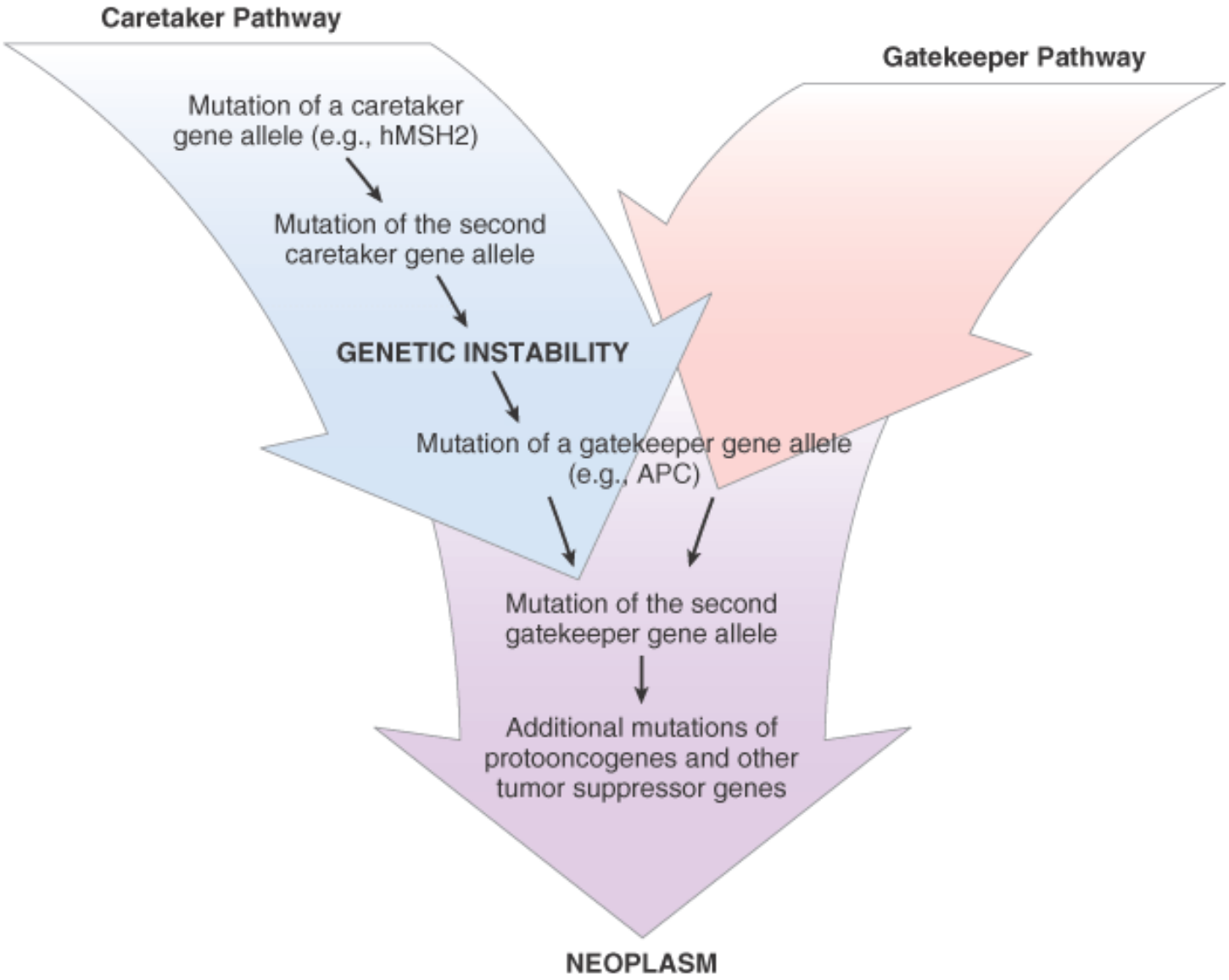

Figure 7-48 Experiments demonstrating the initiation and promotion phases of carcinogenesis in mice. Group 2: application of promoter repeated at twice-weekly intervals for several months. Group 3: application of promoter delayed for several months and then applied twice weekly. Group 6: promoter applied at monthly intervals.

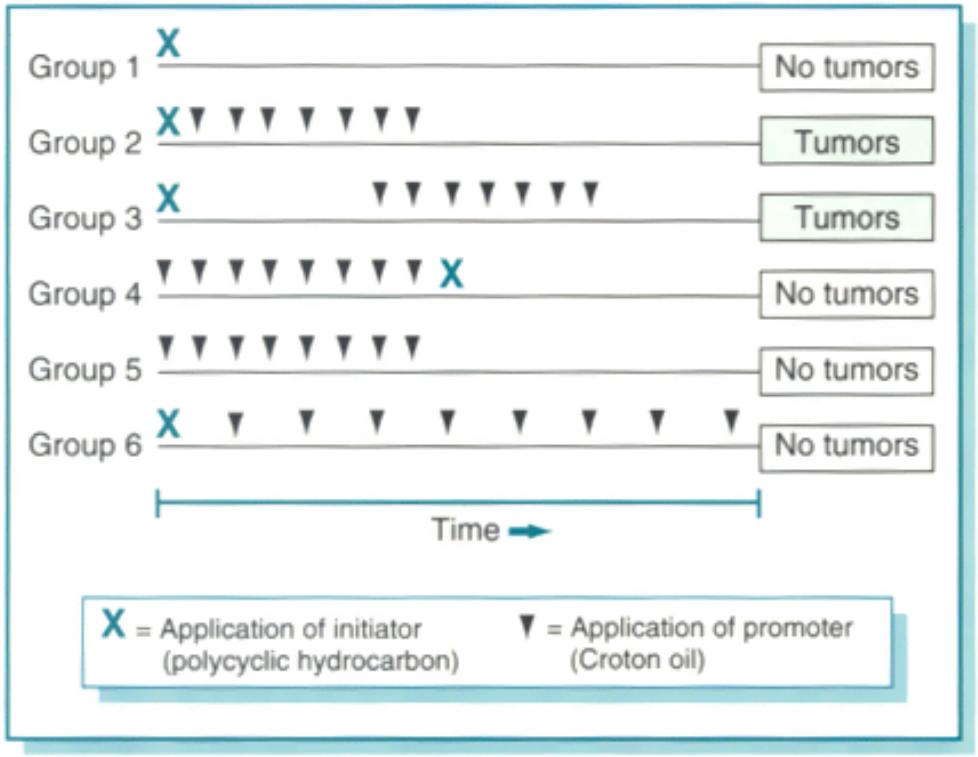

Figure 7-49 General schema of events in chemical carcinogenesis. Note that promoters cause clonal expansion of the initiated cell, thus producing a preneoplastic clone. Further proliferation induced by the promoter or other factors causes accumulation of additional mutations and emergence of a malignant tumor.

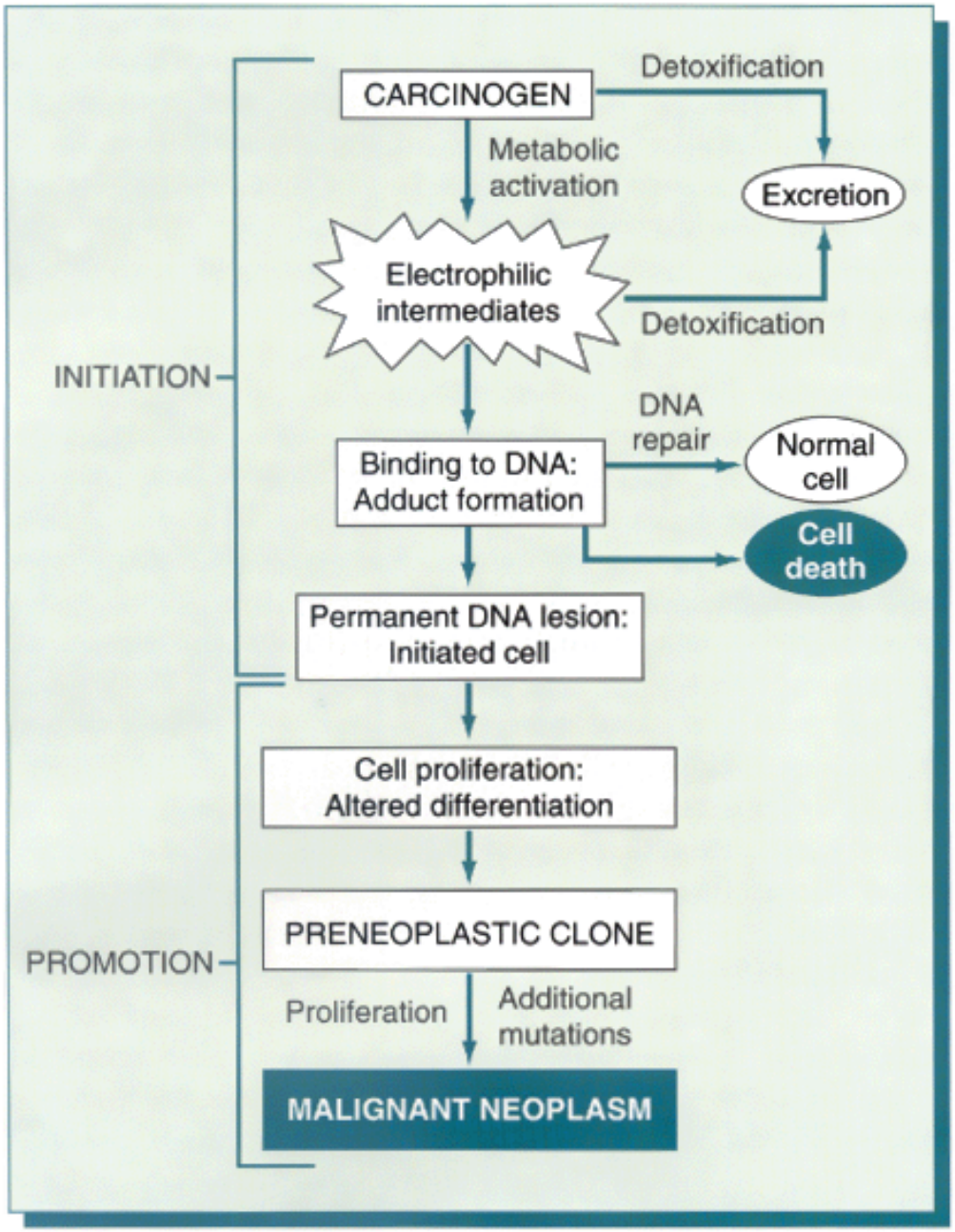

TABLE 7-11 -- Major Chemical Carcinogens

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

of promoters leads to proliferation and clonal expansion of initiated (mutated) cells. Initiated cells respond differently to promoters than do normal cells and hence expand selectively. Such cells (especially after *RAS* activation) have reduced growth factor requirements and may also be less responsive to growth inhibitory signals in their extracellular milieu. Forced 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 initiation-promotion sequence of chemical carcinogenesis raises an important question: *Since promoters are not mutagenic, how do they contribute to tumorigenesis?* Although the effects of tumor promoters are pleiotropic, induction of cell proliferation is a sine qua non of tumor promotion. TPA (tetradecanoyl phorbol-13 acetate), a phorbol ester tumor promoter, is a powerful activator of protein kinase C (see Chapter 3), an enzyme that phosphorylates several substrates involved in signal transduction pathways, including those activated by growth factors. The promoting effect of phenobarbital in liver carcinogenesis has been linked to stimulation of cell proliferation associated with blockage of the TGF-β pathway.[154]

The concept that sustained cell proliferation increases the risk of mutagenesis and hence neoplastic transformation is also applicable to human carcinogenesis. For example, pathologic hyperplasia of the endometrium (Chapter 22) and increased regenerative activity that accompanies chronic liver cell injury (Chapter 18) are associated with the development of cancer in these organs.

Carcinogenic Chemicals

Before closing this discussion of chemical carcinogenesis, we briefly describe some initiators (see Table 7-11) and promoters of chemical carcinogenesis, with special emphasis on those that have been linked to cancer development in humans.[155]

Direct-Acting Alkylating Agents.

These agents are activation independent, and in general they are weak carcinogens. Nonetheless, they are important because many therapeutic agents (e.g., cyclophosphamide, chlorambucil, busulfan, and melphalan) fall into this category. These are used as anticancer drugs but have been documented to induce lymphoid neoplasms, leukemia, and other forms of cancer. Some alkylating agents, such as cyclophosphamide, are also powerful immunosuppressive agents and are therefore used in treatment of immunologic disorders, including rheumatoid arthritis and Wegener granulomatosis. Although the risk of induced cancer with these agents is low, judicious use of them is indicated. Alkylating agents appear to exert their therapeutic effects by interacting with and damaging DNA, but it is precisely these actions that render them carcinogenic.

Polycyclic Aromatic Hydrocarbons.

These agents represent some of the most potent carcinogens known. They require metabolic activation and can induce tumors in a wide variety of tissues and species. Painted on the skin, they cause skin cancers; injected subcutaneously, they induce sarcomas; introduced into a specific organ, they cause cancers locally. The polycyclic hydrocarbons are of particular interest as carcinogens because they are produced in the combustion of tobacco, particularly with cigarette smoking, and are thought to contribute to the causation of lung and bladder cancers.[156] The various components of cigarette smoke that may be associated with carcinogenicity are listed in Chapter 9 . Polycyclic aromatic hydrocarbons are also produced from animal fats in the process of broiling meats and are present in smoked meats and fish.

Aromatic Amines and Azo Dyes.

The carcinogenicity of most aromatic amines and azo dyes is exerted mainly in the liver, where the "ultimate carcinogen" is formed by the action of the cytochrome P-450 oxygenase systems. Thus, fed to rats, acetylaminofluorene and the azo dyes induce hepatocellular carcinomas (but not cancers of the gastrointestinal tract). An agent implicated in human cancers, β-naphthylamine, is an exception. In the past, it was responsible for a 50-fold increased incidence of bladder cancer in heavily exposed workers in aniline dye and rubber industries.[157] After absorption, it is hydroxylated into an active form, then detoxified by conjugation with glucuronic acid. When excreted in the urine, the nontoxic conjugate is split by the urinary enzyme glucuronidase to release the electrophilic reactant again, thus inducing bladder cancer. Regrettably, humans are one of the few species to possess urinary glucuronidase. Some of the azo dyes were developed as food coloring (e.g., butter yellow to give margarine the appearance of butter and scarlet red to impart the seductive coloration of certain foods such as maraschino cherries). These dyes are now federally regulated in the United States because of the fear that they may be dangerous to humans.

Naturally Occurring Carcinogens.

Among the several known chemical carcinogens produced by plants and microorganisms, the potent hepatic carcinogen aflatoxin B1 is particularly important. This mycotoxin is produced by some strains of the fungus *Aspergillus flavus* that thrive on improperly stored corn, rice, and peanuts. A strong correlation has been found between the dietary level of this hepatocarcinogen and the incidence of hepatocellular carcinoma in some parts of Africa and China. As discussed earlier, the aflatoxin and HBV collaborate in the production of this form of neoplasia.

Nitrosamines and Amides.

These carcinogens are of interest because of the possibility that they are formed in the gastrointestinal tract of humans and so may contribute to the induction of some forms of cancer, particularly gastric carcinoma. They are derived in the stomach from the reaction of nitrostable amines and nitrate used as a preservative, which is converted to nitrites by bacteria. Concerns about these agents have led many to shun processed food containing nitrate preservatives.

Miscellaneous Agents.

Scores of other chemicals have been indicted as carcinogens. Only a few that represent important industrial hazards are listed in Table 7-3 and are briefly mentioned here. Occupational exposure to *asbestos* has been associated with an increased incidence of bronchogenic carcinomas, mesotheliomas, and gastrointestinal cancers, as discussed in Chapter 15 . Concomitant cigarette smoking heightens the risk of bronchogenic carcinoma manyfold. *Vinyl chloride,* the monomer from which the polymer polyvinyl chloride is fabricated, was first identified as a carcinogen in animals, but investigations soon disclosed a scattered incidence

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of the extremely rare hemangiosarcoma of the liver among workers exposed to this chemical. *Chromium, nickel,* and other metals, when volatilized and inhaled in industrial environments, have caused cancer of the lung. Skin cancer associated with arsenic is also well established. Similarly, there is reasonable evidence that many insecticides, such as aldrin, dieldrin, and chlordane and the polychlorinated biphenyls, are carcinogenic in animals (Chapter 9).

Promoters of Chemical Carcinogenesis.

Certain promoters may contribute to cancers in humans. It has been argued that promoters are at least as important as initiating chemicals in carcinogenesis because cells initiated by exposure to environmental carcinogens are innocuous unless subjected to repeated assault by promoters. Tumor promotion may occur after exposure to exogenous agents, such as cigarette smoke or viral infections, that cause tissue damage and reactive hyperplasia. Perhaps more serious, because they are difficult to control, are endogenous promoters such as hormones and bile salts. Hormones such as estrogens serve in animals as promoters of liver tumors. The prolonged use of diethylstilbestrol is implicated in the production of postmenopausal endometrial

carcinoma and in the causation of vaginal cancer in offspring exposed in utero (Chapter 22). Intake of high levels of dietary fat has been associated with increased risk of colon cancer. This may be related to an increase in synthesis of bile acids, which have been shown to act as promoters in experimental models of colon cancer. Alcohol consumption increases the risk of development of cancers of the mouth, pharynx, and larynx by more than tenfold, probably by acting as a promoting agent (Chapter 9).

RADIATION CARCINOGENESIS

Radiant energy, whether in the form of the UV rays of sunlight or as ionizing electromagnetic and particulate radiation, can transform virtually all cell types in vitro and induce neoplasms in vivo in both humans and experimental animals. 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 have produced a variety of forms of malignant neoplasia. Although the contribution of radiation to the total human burden of cancer is probably small, the well-known latency of 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 the atomic bomb. The incidence peaked during 1988–1992 and then declined during the period 1993–1997.[158] Moreover, radiation's possible additive or synergistic effects with other potential carcinogenic influences add another dimension to the picture. The effects of UV light on DNA differ from those of ionizing radiation. The cellular and molecular effects of ionizing radiation are discussed in Chapter 9 .

Ultraviolet Rays

There is ample evidence from epidemiologic studies that *UV rays* derived from the sun induce an increased incidence of squamous cell carcinoma, basal cell carcinoma, and possibly malignant melanoma of the skin.[159] The degree of risk depends on the type of UV rays, the intensity of exposure, and the quantity of light-absorbing "protective mantle" of melanin in the skin. Persons of European origin who have fair skin that repeatedly gets 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 in the world. The UV portion of the solar spectrum can be divided into three wavelength ranges: UVA (320 to 400 nm), UVB (280 to 320 nm), and UVC (200 to 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).

UV rays have a number of effects on cells, including inhibition of cell division, inactivation of enzymes, induction of mutations and, in sufficient dosage, death of cells. *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 (NER) pathway. There are five steps in NER: (1) recognition of the DNA lesion, (2) incision of the damaged strand on both sites of the lesion, (3) removal of the damaged nucleotide, (4) synthesis of a nucleotide patch, and (5) its ligation. In mammalian cells, the process may involve 30 or more proteins. It is postulated that with excessive sun exposure, the capacity of the NER pathway is overwhelmed; hence, some DNA damage remains unrepaired. This leads to large transcriptional errors and, in some instances, cancer. The importance of the NER pathway of DNA repair is most graphically illustrated by a study of patients with the hereditary disorder *xeroderma pigmentosum*. This autosomal recessive disorder is characterized by extreme photosensitivity, a 2000-fold increased risk of skin cancer in sun-exposed skin and, in some cases, neurologic abnormalities. The molecular basis of the degenerative changes in sun-exposed skin and occurrence of cutaneous tumors rests on an inherited inability to repair UV-induced DNA damage. Xeroderma pigmentosum is a genetically heterogeneous condition, with at least seven different variants. Each of these is caused by a mutation in one of several genes involved in NER.[160]

As with other carcinogens, UVB also causes mutations in oncogenes and tumor suppressor genes. In particular, mutant forms of the *RAS* and *p53* genes have been detected both in human skin cancers and in UVB-induced cancers in mice. These mutations occur mainly at dipyrimidine sequences within the DNA, thus implicating UVB-induced genetic damage in the causation of skin cancers. In animal models, *p53* mutations occur early after exposure to UVB, before the appearance of tumors.

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. Many of the pioneers in the development 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. 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 myelocytic 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.

Residents of the Marshall Islands were exposed on one occasion to accidental fallout from a hydrogen bomb test that contained thyroid-seeking radioactive iodines. As many as 90% of the children under age 10 years on Rongelap Island developed thyroid nodules within 15 years, and about 5% of these nodules proved to be thyroid carcinomas. A marked increase in the incidence of thyroid cancer has also been noted in areas exposed to the fallout from the nuclear power plant accident in Chernobyl in 1986. In addition to approximately 30 deaths that occurred at the time of the accident, more than 2000 cases of thyroid cancers have been recorded in children living in the area.[161] Cytogenetic studies have detected an elevated frequency of chromosomal alterations in persons who did cleanup work at the power plant after the accident.[162] It is evident that radiant energy—whether absorbed in the pleasant form of sunlight, through the best intentions of a physician, or by tragic exposure to an atomic bomb blast or radiation released by nuclear plant accidents—has awesome carcinogenic potential. Even therapeutic irradiation has been documented to be carcinogenic. Thyroid cancers have developed in approximately 9% of those exposed during infancy and childhood to head and neck radiation. The previous practice of treating ankylosing spondylitis with therapeutic irradiation yielded a 10- to 12-fold increase in the incidence of leukemia years later.

In humans, there is a hierarchy of vulnerability of different tissues to radiation-induced cancers. Most frequent are the leukemias, except for chronic lymphocytic leukemia, which, for unknown reasons, almost never develops after radiation. 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

A large number of DNA and RNA viruses have proved to be oncogenic in a wide variety of animals, ranging from amphibia to primates, and the evidence grows stronger that certain forms of human cancer are of viral origin. In the following discussion, the better-characterized and most intensively studied human oncogenic viruses are presented first. This is followed by a brief account of the association between infection by the bacterium *Helicobacter pylori* and gastric tumors.

Oncogenic DNA Viruses

Several DNA viruses have been associated with the causation of cancer in animals.[163] Some, such as adenoviruses, cause tumors only in laboratory animals, whereas others, such as the bovine papillomaviruses, cause benign as well as malignant neoplasms in their natural hosts. Of the various human DNA viruses, four (papillomaviruses [HPV], Epstein-Barr virus [EBV], hepatitis B virus [HBV], and Kaposi sarcoma herpesvirus [KSHV]) are of particular interest because they have been implicated in the causation of human cancer. KSHV is discussed in Chapter 6 and Chapter 11 . Although not a DNA virus, hepatitis C virus (HCV) is also associated with cancer. Before we discuss the role of these viruses in carcinogenesis, a few general comments relating to transformation by DNA viruses are offered:

- The genomes of oncogenic DNA viruses integrate into and form stable associations with the host cell genome. The virus is unable to complete its replicative cycle because the viral genes essential for completion of replication are interrupted during integration of viral DNA. Thus, the virus can remain in a latent state for years.
- Those viral genes that are transcribed early in the viral life cycle (early genes) are important for transformation, and are expressed in transformed cells.

Human Papillomavirus.

Approximately 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. Human papillomaviruses

have been implicated in the genesis of several cancers, particularly squamous cell carcinoma of the cervix and anogenital region, and in some cases, to the causation of oral and laryngeal cancers (Chapter 16).[164]

Epidemiologic studies suggest that carcinoma of the cervix is caused by a sexually transmitted agent, and HPV is the culprit. DNA sequences of HPV 16 and 18 and, less commonly, HPV 31, 33, 35, and 51 are found in approximately 85% of invasive squamous cell cancers and their presumed precursors (severe dysplasias and carcinoma in situ). In contrast to cervical cancers, genital warts with low malignant potential are associated with distinct HPV types, predominantly HPV 6 and HPV 11 ("low-risk" types).

Molecular analyses of HPV-associated carcinomas and benign genital warts reveal differences that may be pertinent to the transforming activity of these viruses. In benign warts and in preneoplastic lesions, the HPV genome is maintained in an episomal (nonintegrated) form, whereas in cancers, the viral DNA is usually integrated into the host cell genome. This suggests that integration of viral DNA is important in malignant transformation. Although the site of viral integration in host chromosomes is random (the viral DNA is found at different locations in different cancers), the pattern of integration is clonal; that is, the site of integration is identical within all cells of a given cancer. This would not occur if HPV were merely a passenger that infects cells after transformation. Furthermore, the viral DNA is interrupted at a fairly constant site in the process of integration: It is almost always within the E1/E2 open reading frame of the viral genome. Because the E2 region of the viral DNA normally represses the transcription of the E6 and E7 early viral genes, its interruption causes over-expression of the E6 and E7 proteins of HPV 16 and HPV 18. The oncogenic potential of HPV 16 and HPV 18 can be related to these two early viral gene products, which act in conjunction to immortalize and transform cells.[162] [165] The replication of DNA viruses is dependent on the replication machinery of

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the host cells, and E6 and E7 act to overcome the activity of cell-cycle inhibitors (Fig. 7-50).[166] E6 binds to p53 and E7 binds to RB, inducing the degradation of these proteins. In addition, E7 can interfere with *p53* transcriptional activity and also inactivate *p21*. Thus, E6 and E7 block *p53* and *RB* cell cycle suppression pathways. The affinity of these viral proteins for the products of tumor suppressor genes differs depending on the oncogenic potential of HPV. E6 proteins derived from high-risk HPV (HPV 16, 18, and 31) inactivate p53 by enhancing its degradation through ubiquitin-dependent proteolysis.[167] E6 proteins of low-risk HPV (HPV 6 and 11) bind p53 with low affinity and have no effect on p53 stability. E7 proteins from high-risk HPV strongly bind to RB, disrupting the E2F/RB complex and promoting the degradation of RB. By contrast, E7 proteins from low-risk HPV have lower affinity for RB and have a weak capacity to transform cells. Thus, *E6 and E7 proteins of high-risk HPV disable two important tumor suppressor proteins that regulate the cell cycle*. In HPV-induced tumors, *p53* mutations are extremely uncommon, presumably because loss of *p53* function is accomplished by binding to the E6 oncoprotein. This binding not only blocks the inhibitory effect of p53 on the cell cycle but also interferes with p53 activation after DNA damage, a mechanism that allows DNA repair or elimination of cells with genomic damage. Moreover, E6 may have other effects independent of its binding of p53, such as the activation of telomerase and tyrosine kinases.[164]

The E6-p53 interaction may also offer some clues regarding risk factors for cervical cancer development in infected persons. Human *p53* is polymorphic at amino acid 72, encoding either a proline or arginine residue at that position. It turns out that the arginine-containing p53 at position 72 is much more susceptible to degradation by E6. The arginine form is found more frequently in infected individuals with cervical

Figure 7-50 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 restrains to cell proliferation (see Fig. 7-29). *(Modified from Münger K, Howley PM: Human papillomavirus immortalization and transformation functions. Virus Research 89:213–228, 2002.)*

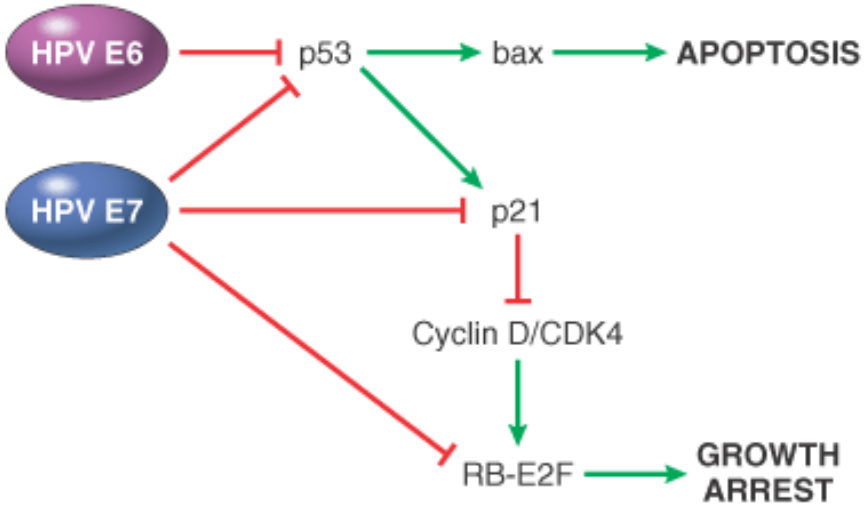


Figure 7-51 Schema depicting the possible evolution of Epstein-Barr virus (EBV)-induced Burkitt lymphoma.

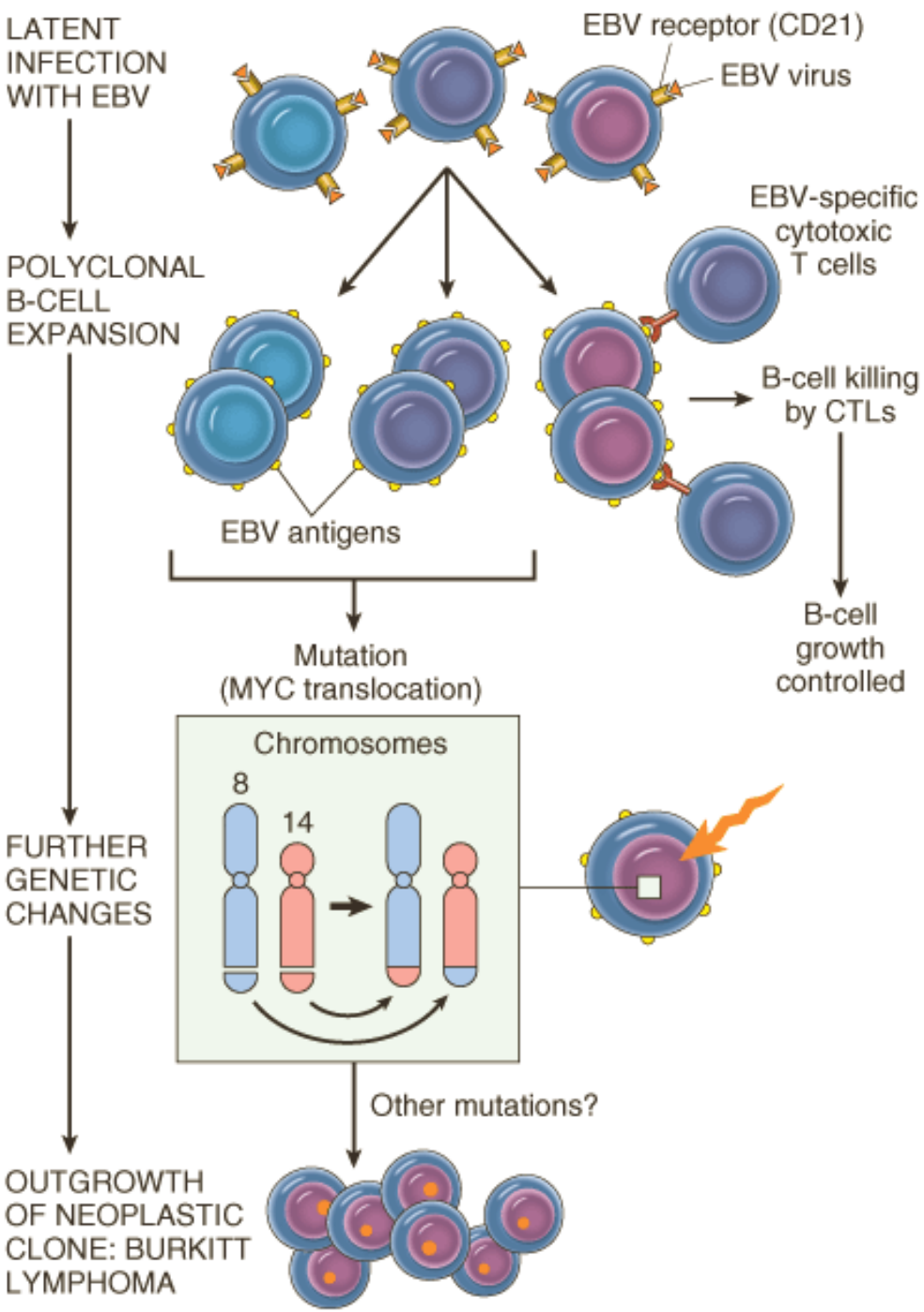


Figure 7-52 Tumor antigens recognized by CD8+ T cells. *(Modified from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003.)*

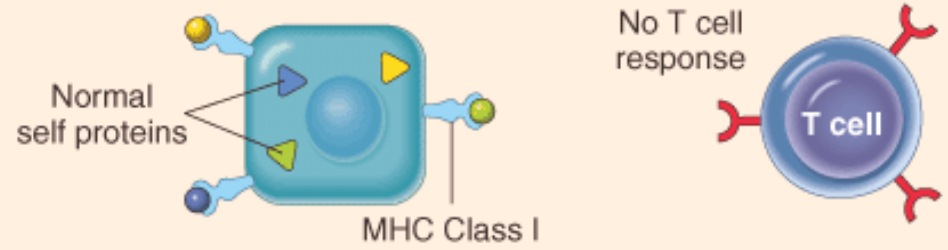
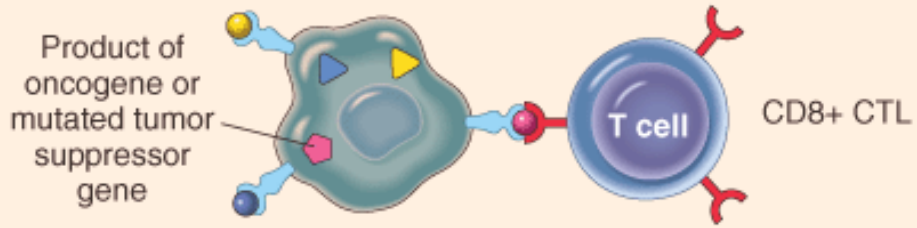
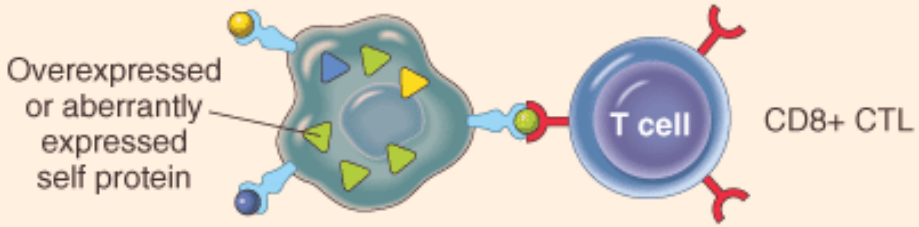
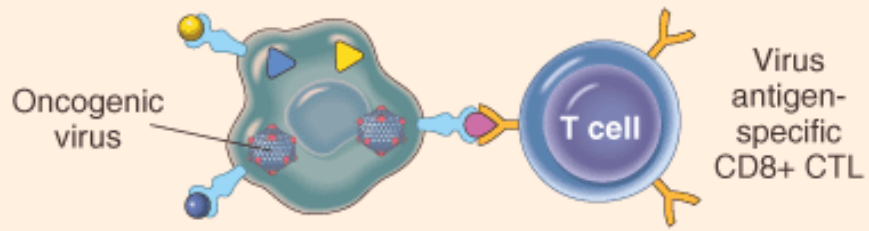
Normal host cell displaying multiple MHC-associated self antigens		EXAMPLES
Tumor cells expressing different types of tumor antigens		Oncogene products: mutated RAS, Bcr/Abl fusion proteins Tumor suppressor gene products: mutated p53 protein
		Various mutant proteins in carcinogen, or radiation, induced animal tumors; various mutated proteins in melanomas
		Overexpressed: tyrosinase, gp100, MART in melanomas Aberrantly expressed: cancer-testis antigens (MAGE, BAGE)
		Human papilloma virus E6, E7 proteins in cervical carcinoma; EBNA proteins in EBV induced lymphoma

Figure 7-53 Mechanisms by which tumors evade the immune system. *(Reprinted from Abbas AK, Lichtman AH: Cellular and Molecular Immunology, 5th ed. Philadelphia, WB Saunders, 2003.)*

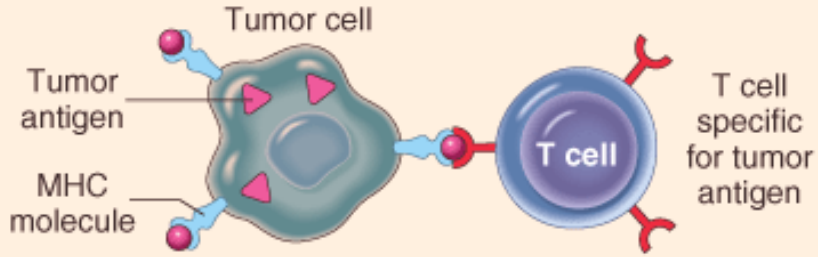
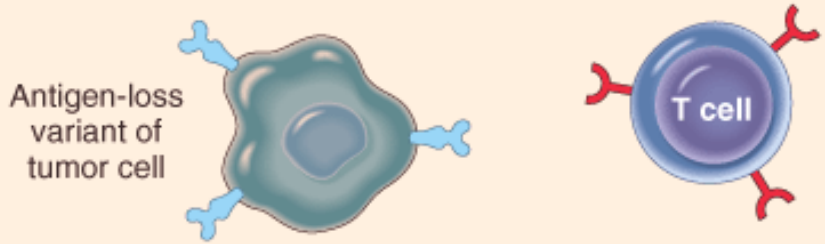
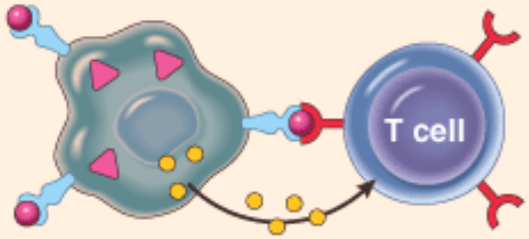
Anti-tumor immunity		T cell recognition of tumor antigen leading to T cell activation
Immune evasion by tumors	**Failure to produce tumor antigen** 	Lack of T cell recognition of tumor
	Mutations in MHC genes or genes needed for antigen processing 	Lack of T cell recognition of tumor
	Production of immuno-suppressive proteins 	Inhibition of T cell activation

TABLE 7-12 -- Paraneoplastic Syndromes

Clinical Syndromes	Major Forms of Underlying Cancer	Causal Mechanism
Endocrinopathies		
Cushing syndrome	Small cell carcinoma of lung	ACTH or ACTH-like substance
	Pancreatic carcinoma	
	Neural tumors	
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	Parathyroid hormone-related protein (PTHRP), TGF-α, TNF, IL-1
	Breast carcinoma	
	Renal carcinoma	
	Adult T-cell leukemia/lymphoma	
	Ovarian carcinoma	
Hypoglycemia	Fibrosarcoma	Insulin or insulin-like substance
	Other mesenchymal sarcomas	
	Hepatocellular carcinoma	
Carcinoid syndrome	Bronchial adenoma (carcinoid)	Serotonin, bradykinin
	Pancreatic carcinoma	
	Gastric carcinoma	
Polycythemia	Renal carcinoma	Erythropoietin
	Cerebellar hemangioma	
	Hepatocellular carcinoma	
Nerve and Muscle Syndromes		
Myasthenia	Bronchogenic carcinoma	Immunologic
Disorders of the central and peripheral nervous systems	Breast carcinoma	
Dermatologic Disorders		
Acanthosis nigricans	Gastric carcinoma	Immunologic; secretion of epidermal growth factor
	Lung carcinoma	
	Uterine carcinoma	
Dermatomyositis	Bronchogenic, breast carcinoma	Immunologic
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	Tumor products (mucins that activate clotting)
	Bronchogenic carcinoma	
	Other cancers	
Nonbacterial thrombotic endocarditis	Advanced cancers	Hypercoagulability
Anemia	Thymic neoplasms	Unknown
Others		
Nephrotic syndrome	Various cancers	Tumor antigens, immune complexes

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

are small. It is thought that PTHRP regulates calcium transport in the lactating breast and across the placenta. 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 the squamous cell bronchogenic carcinoma, rather than small cell cancer of the lung (more often associated with endocrinopathies). 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*. The cause of these syndromes is poorly understood. In some cases, antibodies,

presumably induced against tumor cells that cross-react with neuronal cells, 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 13). 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 patients 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

Prognosis of the course of the disease and the determination of efficacy of various forms of cancer treatment require a high degree of similarity among the tumors being considered. 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 the number of mitoses within the tumor as presumed correlates of the neoplasm's aggressiveness. Thus, cancers are classified as grades I to IV with increasing anaplasia. 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 highly undifferentiated, retroperitoneal malignant tumor—probably sarcoma. 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. Two major staging systems are currently in use, one developed by the Union Internationale Contre Cancer (UICC) and the other by the American Joint Committee (AJC) on Cancer Staging. The UICC employs 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 added 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 blood-borne metastases and some judgment as to their number.

The AJC employs a somewhat different nomenclature and divides all cancers into stages 0 to IV, incorporating within each of these stages the size of the primary lesions as well as the presence of nodal spread and distant metastases. The staging systems and additional details are mentioned in appropriate chapters, in conjunction with the discussion of specific tumors. It merits emphasis here, however, that staging of neoplastic disease has assumed great importance in the selection of the best form of therapy for the patient. It bears repeating that *staging has proved to be of greater clinical value than grading*. In some cases, such as for lung cancers, staging has been greatly aided by imaging techniques such as *positron emission tomography*.[55]

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 a number of 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 where one should 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 tend to underestimate the value of the clinical data. 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

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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 margins 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 enable the method to be extended 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. In experienced hands, it is an extremely reliable, rapid, and useful technique.

Cytologic (Pap) smears provide yet another method for the detection of cancer (Chapter 22). This approach is widely used

Figure 7-54 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.)*

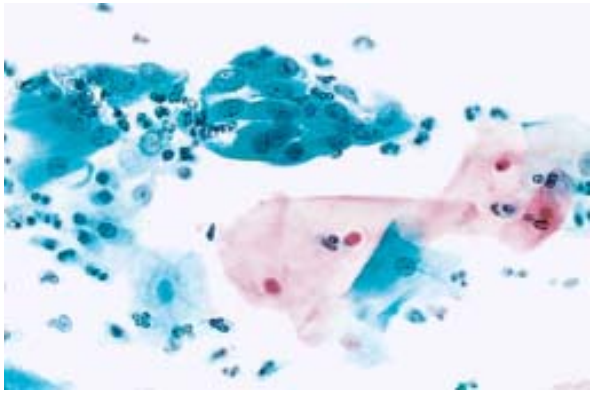

Figure 7-55 An abnormal cervicovaginal smear shows numerous malignant cells that have pleomorphic, hyperchromatic nuclei; interspersed are some normal polymorphonuclear leukocytes. *(Courtesy of Dr. P.K. Gupta, University of Pennsylvania, Philadelphia, PA.)*

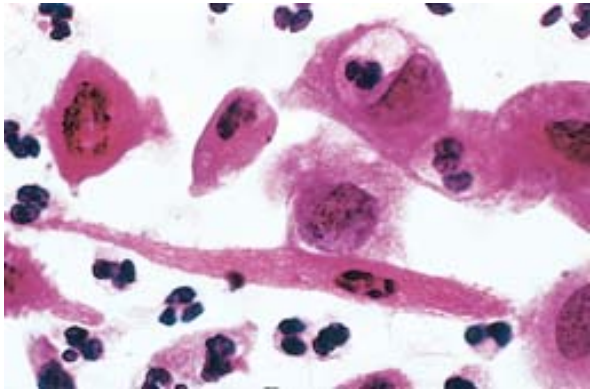

Figure 7-56 Anticytokeratin immunoperoxidase stain of a tumor of epithelial origin (carcinoma). *(Courtesy of Dr. Melissa Upton, University of Washington, Seattle, WA.)*

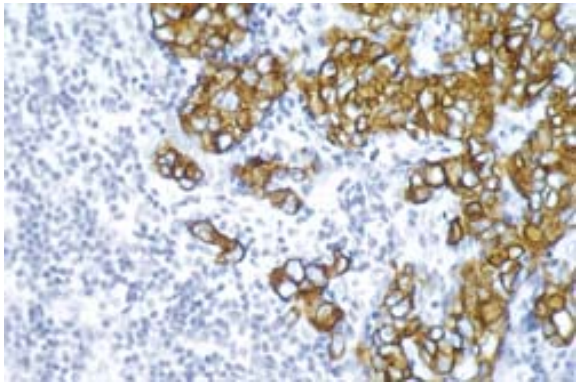

TABLE 7-13 -- Selected Tumor Markers

Markers	**Associated Cancers**
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 in Table 7-12
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* mutations in stool and serum	Colon cancer
p53 and *RAS* mutations in stool and serum	Pancreatic cancer
p53 and *RAS* mutations in sputum and serum	Lung cancer
p53 mutations in urine	Bladder cancer

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 patients with colorectal carcinomas; the presence of mutated *p53* and of hypermethylated genes in the sputum of patients with lung cancer and in the saliva of patients with head and neck cancers; and the detection of mutated *p53* in the urine of patients with bladder cancer.[207]

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Chapter 8 - Infectious Diseases

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General Principles of Microbial Pathogenesis

Despite the availability and use of effective vaccines and antibiotics, infectious diseases remain an important cause of death in the United States and worldwide. In the United States, two of the top 10 leading causes of death are infectious diseases (pneumonia and influenza, and septicemia).[1] Infectious diseases are particularly important causes of death among the elderly and people with acquired immunodeficiency syndrome (AIDS), those with chronic diseases, and those receiving immunosuppressive drugs. In developing countries, unsanitary living conditions and malnutrition contribute to a massive burden of infectious diseases that kills more than 10 million people each year. Most of these deaths are among children, especially from respiratory and diarrheal infections.[2]

HISTORY

The history of infectious disease pathology is intertwined with that of microbiology. Some of the major historical events in these fields are briefly described here to provide a perspective for the concepts of pathogenesis to be discussed later. Some important experiments that were performed in the past would not be ethically acceptable today.

Louis Pasteur and Robert Koch were pioneers in establishing the microbiologic etiology of infectious diseases. Pasteur is credited with proving that microorganisms can cause disease (the germ theory of disease). Pasteur also created the first attenuated vaccines, including a rabies vaccine for humans in 1885. In 1882, Koch championed criteria for linking a specific microorganism to a disease. Koch's postulates require that (1) the organism is found in the lesions of the disease, (2) the organism can be isolated as single colonies on solid media, (3) inoculation of the organism causes lesions in experimental animals, and (4) the organism can be recovered from the experimental animal. Koch also isolated the bacteria that cause tuberculosis *(Mycobacterium tuberculosis)* and anthrax *(Bacillus anthracis)*.

Ronald Ross, an English military physician posted in India, demonstrated in 1897 that mosquitoes carry malaria. At the time, it was believed that malaria was caused by breathing the air near swamps ("malaria" comes from the Italian for "bad air"). Ross's demonstration that *Anopheles* mosquitoes transmit malaria led to public health efforts to reduce malaria through control of mosquitoes. This was successful in the United States, but malaria continues to be a major health problem in many parts of the world.

Walter Reed, an American military physician, led a team of investigators in Cuba in 1900 who demonstrated that yellow fever, like malaria, is transmitted by the bite of mosquitoes. Military volunteers allowed themselves to be bitten by mosquitoes that had previously bitten people sick with yellow fever. Following Reed's result, Dr. James Carroll showed in 1901 that yellow fever was caused by a virus. This was the first demonstration that a virus causes disease in humans.

F. Peyton Rous found the first evidence for an infectious cause of cancer in 1909. In 1911, Rous demonstrated that a virus causes sarcoma in chickens. Although a viral cause has not been found for most human cancers, we now know that viruses can contribute to the development of some; such associations include human papillomaviruses and cervical cancer.

The dawn of modern microbiology, which is based on molecular genetics, came in 1944, when Oswald Avery demonstrated that transfer of DNA from virulent to avirulent *Streptococcus pneumoniae* transformed the latter into a virulent phenotype. This showed that DNA is the genetic material, leading to an explosion of research in molecular genetics. Today, the entire genomic sequences of many species, including microbes and humans, are known, and this holds great promise for future research into the pathogenesis, diagnosis, and treatment of infectious diseases. Knowledge of the genomes of the host and pathogens promise to produce a much richer description of the host response to infectious

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agents than the morphologic descriptions of antimicrobial responses in this chapter.

NEW AND EMERGING INFECTIOUS DISEASES

Although infectious diseases such as leprosy have been known since biblical times and parasitic schistosomes and mycobacteria have been demonstrated in Egyptian mummies, a surprising number of new infectious agents continue to be discovered (Table 8-1). The infectious causes of some diseases with significant morbidity and mortality (e.g., *Helicobacter pylori* gastritis, hepatitis B and hepatitis C, human metapneumovirus respiratory disease, and Legionnaire's pneumonia) were previously unrecognized because the infectious agents are difficult to culture. Some infectious agents are genuinely new to humans, e.g., human immunodeficiency virus (HIV), which causes the acquired immunodeficiency syndrome (AIDS); *Borrelia burgdorferi*, which causes Lyme disease; and the coronavirus that may cause severe acute respiratory syndrome (SARS) (Chapter 15). Other infections are much more

TABLE 8-1 -- Some Recently Recognized Infectious Agents and Manifestations

1977	Ebola virus	Epidemic hemorrhagic fever
	Hantaan virus	Hemorrhagic fever with renal disease
	Legionella pneumophila	Legionnaire's disease
	Campylobacter jejuni	Enteritis
1980	HTLV-I	T-cell lymphoma or leukemia
1981	*Staphylococcus aureus*	Toxic shock syndrome
1982	HTLV-II	Hairy cell leukemia
	Escherichia coli O 157:H7	Hemolytic-uremic syndrome
	Borrelia burgdorferi	Lyme disease
1983	HIV	AIDS
	Helicobacter pylori	Gastric ulcers
1985	*Enterocytozoon bieneusi*	Chronic diarrhea
1988	HHV-6	Roseola subitum

	Hepatitis E	Enterically transmitted hepatitis
1989	Hepatitis C	Hepatitis C
	Ehrlichia chaffeensis	Human monocytic ehrlichiosis
1992	*Vibrio cholerae* O 139	New epidemic cholera strain
	Bartonella henselae	Cat-scratch disease
1993	*Encephalitozoon cuniculi*	Opportunistic infections
1994	*Anaplasma phagocytophilium*	Human granulocytic ehrlichiosis (anaplasmosis)
1995	KSHV (HHV-8)	Kaposi sarcoma in AIDS
2001	Human metapneumovirus	Respiratory infections
2002	West Nile virus	Acute flaccid paralysis
2003	SARS coronavirus	Severe acute respiratory syndrome

Adapted from Lederberg J: Infectious disease as an evolutionary paradigm. Emerg Infect Dis 3:417, 1997.

commonly seen because of immunosuppression caused by AIDS (e.g., cytomegalovirus [CMV], Kaposi sarcoma herpesvirus, *Mycobacterium avium-intracellulare*, *Pneumocystis jiroveci* (*carinii*), and *Cryptosporidium parvum*).[3] [4] Finally, infectious diseases that are common in one area may be introduced into a new area. West Nile virus was common in Europe, Asia, and Africa when it was first described in the United States in 1999.

Human demographics and behavior are among the many factors that contribute to the emergence of infectious diseases. AIDS has been predominantly (but not exclusively) a disease of homosexuals and drug abusers in the United States and Western countries, while in Africa, AIDS is predominantly a heterosexual disease that is much more frequent in areas where men remain uncircumcised.[5] Changes in the environment occasionally drive rates of infectious diseases. Reforestation of the eastern United States has led to massive increases in the populations of deer and mice, which carry the ticks that transmit Lyme disease, babesiosis, and ehrlichiosis. [6] Failure of DDT to control the mosquitoes that transmit malaria and the development of drug-resistant parasites have dramatically increased the morbidity and mortality of *Plasmodium falciparum* in Asia, Africa, and Latin America. Microbial adaptation to widespread antibiotic use contributed to the development of new drug-resistant strains of *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, and *Enterococcus faecium*.

AGENTS OF BIOTERRORISM

Sadly, the anthrax attacks in the United States in 2001 transformed the theoretical threat of bioterrorism into reality. The Centers for Disease Control and Prevention (CDC) have evaluated the microorganisms that pose the greatest danger as weapons on the basis of how efficiently disease can be transmitted, how hard the microorganisms are to produce and distribute, how well they can be defended against, and how likely they are to alarm the public and produce widespread fear. The CDC has ranked bioweapons into three categories, A, B, and C, based on these criteria. These agents are listed in Table 8-2 . [7]

Category A agents are the highest-risk agents and can be readily disseminated or transmitted from person to person, can cause high mortality with potential for major public health impact, might cause public panic and social disruption, and require special action for public health preparedness. For example, smallpox is a category A agent owing to its high transmissibility in any climate or season, case mortality rate of 30% or greater, and lack of effective antiviral therapy. This agent can be easily disseminated because of the stability of the virus in aerosol form and the very small dose needed for infection. Smallpox naturally spreads from person to person mainly by respiratory aerosol or by direct contact with virus in skin lesions or contaminated clothing or bedding. Symptoms appear after 7 to 17 days. Initially, there is high fever, headache, and backache, followed by the appearance of the rash, which first appears on

the mucosa of the mouth and pharynx, face, and forearms and later spreads to the trunk and legs and becomes vesicular and later pustular. Because people are infectious during the incubation period, this virus has the potential to continue to spread throughout an unprotected population. Since vaccination ended in the United States in 1972 and vaccination immunity has waned, the population is

TABLE 8-2 -- Potential Agents of Bioterrorism *

Category A Diseases/Agents	Category B Diseases/Agents	Category C Diseases/Agents
• Anthrax *(Bacillus anthracis)*	• Brucellosis (*Brucella* species)	• Emerging infectious disease threats such as Nipah virus and Hantavirus
• Botulism *(Clostridium botulinum* toxin*)*	• Epsilon toxin of *Clostridium perfringens*	
• Plague *(Yersinia pestis)*	• Food safety threats (e.g., *Salmonella* species, *Escherichia coli* 0157: H7, *Shigella*)	
• Smallpox *(Variola major virus)*		
• Tularemia *(Francisella tularensis)*	• Glanders *(Burkholderia mallei)*	
• Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg], arenaviruses [Lassa fever virus and New World arenaviruses], bunyaviruses [e.g. Crimean-Congo hemorrhagic fever and Rift Valley Fever viruses]	• Melioidosis *(Burkholderia pseudomallei)*	
	• Psittacosis *(Chlamydia psittaci)*	
	• Q fever *(Coxiella burnetti)*	
	• Ricin toxin from *Ricinus communis* (castor beans)	
	• Staphylococcal enterotoxin B	
	• Typhus fever *(Rickettsia prowazekii)*	
	• Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])	
	• Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)	

**Adapted from Centers for Disease Control Information.*

highly susceptible to smallpox. Recent concern that smallpox could be used for bioterrorism has led to a return of vaccination for selected groups in the U.S. and Israel.

Category B agents are moderately easy to disseminate, produce moderate morbidity but low mortality, and require specific diagnostic and disease surveillance. Many of these agents are foodborne or waterborne. Category C agents include emerging pathogens that could be engineered for mass dissemination because of availability, ease of production and dissemination, potential for high morbidity and mortality, and great impact on health.

CATEGORIES OF INFECTIOUS AGENTS

Infectious agents belong to a wide range of classes and vary in size from the ~27-kD nucleic acid-free prion to 20-nm poliovirus to 10-m tapeworms (Table 8-3).

TABLE 8-3 -- Classes of Human Pathogens and Their Habitats

Taxonomic	Size	Site of Propagation	Sample Species	Disease
Viruses	20–300 nm	Obligate intracellular	Poliovirus	Poliomyelitis
Chlamydiae	200–1000 nm	Obligate intracellular	*Chlamydia trachomatis*	Trachoma, urethritis
Rickettsiae	300–1200 nm	Obligate intracellular	*Rickettsia prowazekii*	Typhus fever
Mycoplasmas	125–350 nm	Extracellular	*Mycoplasma pneumoniae*	Atypical pneumonia
Bacteria	0.8–15 µm	Cutaneous	*Staphylococcus aureus*	Wound
		Mucosal	*Vibrio cholerae*	Cholera
		Extracellular	*Streptococcus pneumoniae*	Pneumonia
		Facultative intracellular	*Mycobacterium tuberculosis*	Tuberculosis
Fungi	2–200 µm	Cutaneous	*Trichophyton* sp.	Tinea pedis (athlete's foot)
		Mucosal	*Candida albicans*	Thrush
		Extracellular	*Sporothrix schenckii*	Sporotrichosis
		Facultative intracellular	*Histoplasma capsulatum*	Histoplasmosis
Protozoa	1–50 µm	Mucosal	*Giardia lamblia*	Giardiasis
		Extracellular	*Trypanosoma gambiense*	Sleeping sickness
		Facultative intracellular	*Trypanosoma cruzi*	Chagas disease
		Obligate intracellular	*Leishmania donovani*	Kala-azar
Helminths	3 mm–10 m	Mucosal	*Enterobius vermicularis*	Enterobiasis
		Extracellular	*Wuchereria bancrofti*	Filariasis
		Intracellular	*Trichinella spiralis*	Trichinosis

Prions

Prions are apparently composed of abnormal forms of a host protein, termed prion protein (PrP).[8] These agents cause transmissible spongiform encephalopathies, including kuru (associated with human cannibalism), Creutzfeldt-Jakob disease (CJD; associated with corneal transplants), bovine spongiform encephalopathy (BSE; better known as mad cow disease), and variant Creutzfeldt-Jakob disease (vCJD; likely transmitted to humans from BSE-infected cattle). [9] PrP is normally found in neurons. Diseases occur when the prion protein undergoes a conformational change that confers resistance to proteases. The protease-resistant PrP promotes conversion of the normal protease-sensitive PrP to the abnormal form, explaining the infectious nature of these diseases. Accumulation of abnormal PrP leads to neuronal damage and distinctive spongiform pathologic changes in the brain.

Spontaneous or inherited mutations in PrP, which make PrP protease resistant, have been observed in the sporadic and familial forms of CJD, respectively. These diseases are discussed in detail in Chapter 28 .

Viruses

Viruses are obligate intracellular parasites that depend on the host cell's metabolic machinery for their replication. They consist of a nucleic acid genome surrounded by a protein coat (called a capsid) that is sometimes encased in a lipid membrane. Viruses are classified by their nucleic acid genome (DNA or RNA but not both), the shape of the capsid (icosahedral or helical), the presence or absence of a lipid

TABLE 8-4 -- Selected Human Viral Diseases and Their Pathogens

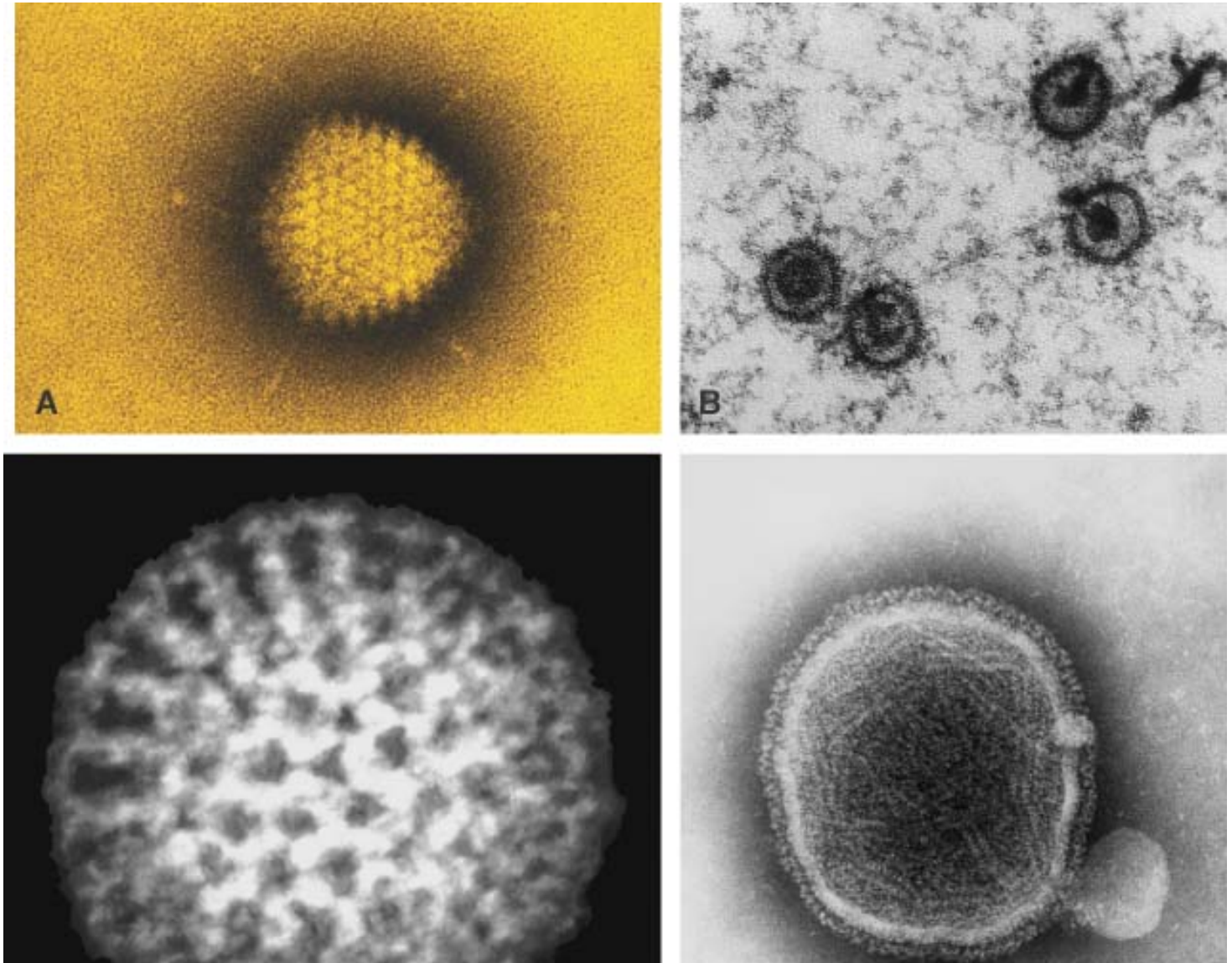
Viral Pathogen	Virus Family	Genomic Type	Disease Expression
Respiratory			
Adenovirus	Adenoviridae	DS DNA	Upper and lower respiratory tract infections, conjunctivitis, diarrhea
Rhinovirus	Picornaviridae	SS RNA	Upper respiratory tract infection
Coxsackievirus	Picornaviridae	SS RNA	Pleurodynia, herpangina, hand-foot-and-mouth disease, SARS
Coronavirus	Coronaviridae	SS RNA	Upper respiratory tract infection
Influenza viruses A, B	Orthomyxoviridae	SS RNA	Influenza
Respiratory syncytial virus	Paramyxoviridae	SS RNA	Bronchiolitis, pneumonia
Digestive			
Mumps virus	Paramyxoviridae	SS RNA	Mumps, pancreatitis, orchitis
Rotavirus	Reoviridae	DS RNA	Childhood diarrhea
Norwalk agent	Caliciviridae	SS RNA	Gastroenteritis
Hepatitis A virus	Picornaviridae	SS RNA	Acute viral hepatitis
Hepatitis B virus	Hepadnaviridae	DS DNA	Acute or chronic hepatitis

Hepatitis D virus	Viroid-like	SS RNA	With HBV, acute or chronic hepatitis
Hepatitis C virus	Flaviviridae	SS RNA	Acute or chronic hepatitis
Hepatitis E virus	Norwalk-like	SS RNA	Enterically transmitted hepatitis
Systemic with Skin Eruptions			
Measles virus	Paramyxoviridae	SS RNA	Measles (rubeola)
Rubella virus	Togaviridae	SS RNA	German measles (rubella)
Parvovirus	Parvoviridae	SS DNA	Erythema infectiosum, aplastic anemia
Vaccinia virus	Poxviridae	DS DNA	Smallpox vaccine
Varicella-zoster virus	Herpesviridae	DS DNA	Chickenpox, shingles
Herpes simplex virus 1	Herpesviridae	DS DNA	"Cold sore"
Herpes simplex virus 2	Herpesviridae	DS DNA	Genital herpes
Systemic with Hematopoietic Disorders			
Cytomegalovirus	Herpesviridae	DS DNA	Cytomegalic inclusion disease
Epstein-Barr virus	Herpesviridae	DS DNA	Infectious mononucleosis
HTLV-I	Retroviridae	SS RNA	Adult T-cell leukemia; tropical spastic paraparesis
HIV-1 and HIV-2	Retroviridae	SS RNA	AIDS
Arboviral and Hemorrhagic Fevers			
Dengue virus 1–4	Togaviridae	SS RNA	Dengue, hemorrhagic fever
Yellow fever virus	Togaviridae	SS RNA	Yellow fever
Regional hemorrhagic fever viruses	Filoviridae	SS RNA	Ebola, Marburg disease
	Hantavirus	SS RNA	Korean, U.S. pneumonia
Warty Growths			
Papillomavirus	Papovaviridae	DS DNA	Condyloma; cervical carcinoma
Central Nervous System			
Poliovirus	Picornaviridae	SS RNA	Poliomyelitis
JC virus	Papovaviridae	DS DNA	Progressive multifocal leukoencephalopathy (opportunistic)
Arboviral encephalitis viruses	Togaviridae	SS RNA	Eastern, Western, Venezuelan, St. Louis,
DS, double-stranded; SS, single-stranded.			

envelope, their mode of replication, the preferred cell type for replication (called tropism), or the type of pathology (Table 8-4). Because viruses are only 20 to 300 nm in size, they are best visualized with the electron microscope (Fig. 8-1). However, some viral particles aggregate within the cells they infect and form characteristic inclusion bodies, which may be seen

with the light microscope and are useful for diagnosis. For example, cytomegalovirus (CMV)-infected cells are enlarged and show a large eosinophilic nuclear inclusion and smaller basophilic cytoplasmic inclusions; herpesviruses form a large nuclear inclusion surrounded by a clear halo; and both smallpox and rabies viruses form characteristic cytoplasmic inclusions. Many viruses do not give rise to inclusions (e.g., Epstein Barr virus [EBV]).

Figure 8-1 The variety of viral structures, as seen by electron microscopy. A, Adenovirus, an icosahedral nonenveloped DNA virus with fibers. B, Epstein Barr virus, an icosahedral enveloped DNA virus. C, Rotavirus, a nonenveloped, wheel-like, RNA virus. D, Paramyxovirus, a spherical enveloped RNA virus. RNA is seen spilling out of the disrupted virus. *(Photos courtesy of Science Source; © Photo Researchers, Inc., New York, New York.)*



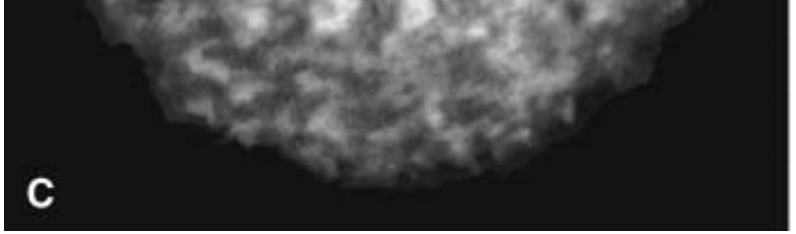


TABLE 8-5 -- Examples of Bacterial, Spirochetal, and Mycobacterial Diseases

Clinical or Microbiologic Category	Species	Frequent Disease Presentations
Infections by pyogenic cocci	*Staphylococcus aureus, S. epidermidis*	Abscess, cellulitis, pneumonia, septicemia
	Streptococcus pyogenes, β-hemolytic	Upper respiratory tract infection, erysipelas, scarlet fever, septicemia
	Streptococcus pneumoniae (pneumoccoccus)	Lobar pneumonia, meningitis
	Neisseria meningitidis (meningococcus)	Cerebrospinal meningitis
	Neisseria gonorrhoeae (gonococcus)	Gonorrhea
Gram-negative infections, common	* *Escherichia coli*	Urinary tract infection, wound infection, abscess, pneumonia, septicemia, endotoxemia, endocarditis
	* *Klebsiella pneumoniae*	
	* *Enterobacter (Aerobacter) aerogenes*	
	* *Proteus* spp. *(P. mirabilis, P. morgagni)*	
	* *Serratia marcescens*	
	* *Pseudomonas* spp. *(P. aeruginosa)*	
	Bacteroides spp. *(B. fragilis)*	Anaerobic infection
	Legionella spp. *(L. pneumophila)*	Legionnaires disease
Contagious childhood bacterial diseases	*Haemophilus influenzae*	Meningitis, upper and lower respiratory tract infections
	Bordetella pertussis	Whooping cough
	Corynebacterium diphtheriae	Diphtheria
Enteropathic infections	Enteropathogenic *E. coli*	Invasive or noninvasive gastroenterocolitis, some with septicemia
	Shigella spp.	
	Vibrio cholerae	
	Campylobacter fetus, C. jejuni	
	Yersinia enterocolitica	

	Salmonella spp. (1000 strains)	
	Salmonella typhi	Typhoid fever
Clostridial infections	*Clostridium tetani*	Tetanus (lockjaw)
	Clostridium botulinum	Botulism (paralytic food poisoning)
	Clostridium perfringens, C. septicum	Gas gangrene, necrotizing cellulitis
	* *Clostridium difficile*	Pseudomembranous colitis
Zoonotic bacterial infections	*Bacillus anthracis*	Anthrax (malignant pustule)
	* *Listeria monocytogenes*	*Listeria* meningitis, listeriosis
	Yersinia pestis	Bubonic plague
	Francisella tularensis	Tularemia
	Brucella melitensis, B. suis, B. abortus	Brucellosis (undulant fever)
	Burkholderia mallei, B. pseudomallei	Glanders, melioidosis
	Leptospira spp. (many groups)	Leptospirosis, Weil disease
	Borrelia recurrentis	Relapsing fever
	Borrelia burgdorferi	Lyme borreliosis
	Bartonella henselae	Cat-scratch disease; bacillary angiomatosis
	Spirillum minus, Streptobacillus moniliformis	Rat-bite fever
Human treponemal infections	*Treponema pallidum*	Venereal, endemic syphilis (bejel)
	Treponema pertenue	Yaws (frambesia)
	Treponema carateum (T. herrejoni)	Pinta (carate, mal del pinto)
Mycobacterial infections	* *Mycobacterium tuberculosis, M. bovis* (Koch bacillus)	Tuberculosis
	M. leprae (Hansen bacillus)	Leprosy
	* *M. kansasii, M. avium, M. intracellulare*	Atypical mycobacterial infections
	M. ulcerans	Buruli ulcer
Actinomycetaceae	* *Nocardia asteroides*	Nocardiosis
	Actinomyces israelii	Actinomycosis

*Important opportunistic infections.