

## Chapter 2

# The Nature of Cancer

When I published the results of my experiments on the development of double-fertilized sea-urchin eggs in 1902, I added the suggestion that malignant tumors might be the result of a certain abnormal condition of the chromosomes, which may arise from multipolar mitosis. ... So I have carried on for a long time the kind of experiments I suggested, which are so far without success, but my conviction remains unshaken .

Theodor Boveri, pathologist, 1914

**T**he cellular organization of metazoan tissues has made possible the evolution of an extraordinary diversity of anatomical designs. Much of this plasticity in design can be traced to the fact that the building blocks of tissue and organ construction—individual cells—are endowed with great autonomy and versatility. Most types of cells in the metazoan body carry a complete organismic genome—far more information than any one of these cells will ever require. And many cells retain the ability to grow and divide long after organismic development has been completed. This retained ability to proliferate and to participate in tissue **morphogenesis** (the creation of shape) makes possible the maintenance of adult tissues throughout the life span of an organism. Such maintenance may involve the repair of wounds and the replacement of cells that have suffered attrition after extended periods of service.

At the same time, this versatility and autonomy poses a grave danger, in that individual cells within the organism may gain access to information in their genomes that is normally denied to them. Moreover, their genomic sequences are subject to corruption by various mechanisms that alter the structure and hence information content of the genome. The resulting mutated genes may divert cells into acquiring novel, often highly abnormal phenotypes. Such changes may be incompatible with the normally assigned roles of these cells in

organismic structure and physiology. Among these inappropriate changes may be alterations in cellular growth programs, and these in turn can lead to the appearance of large populations of cells that no longer obey the rules governing normal tissue construction and maintenance.

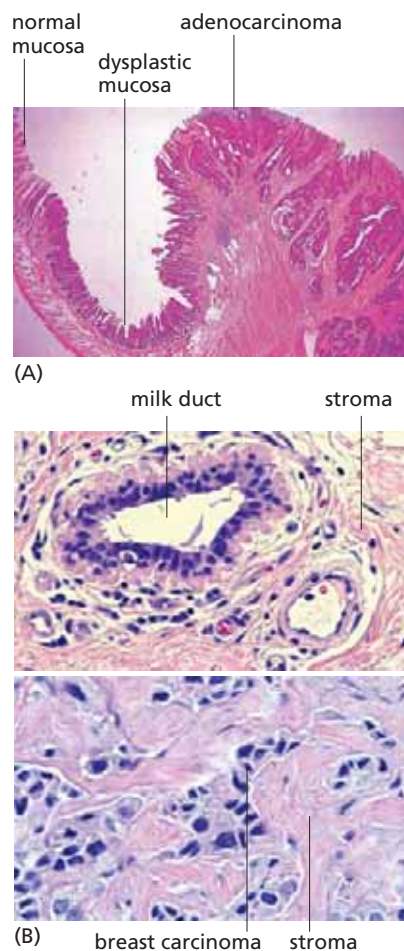
When portrayed in this way, the renegade cells that form a tumor are the result of normal development gone awry. In spite of extraordinary safeguards taken by the organism to prevent their appearance, cancer cells somehow learn to thrive. Normal cells are carefully programmed to participate in constructing the diverse tissues that make possible organismic survival. Cancer cells have a quite different and more focused agenda. They appear to be motivated by only one consideration: making more copies of themselves.

## 2.1 Tumors arise from normal tissues

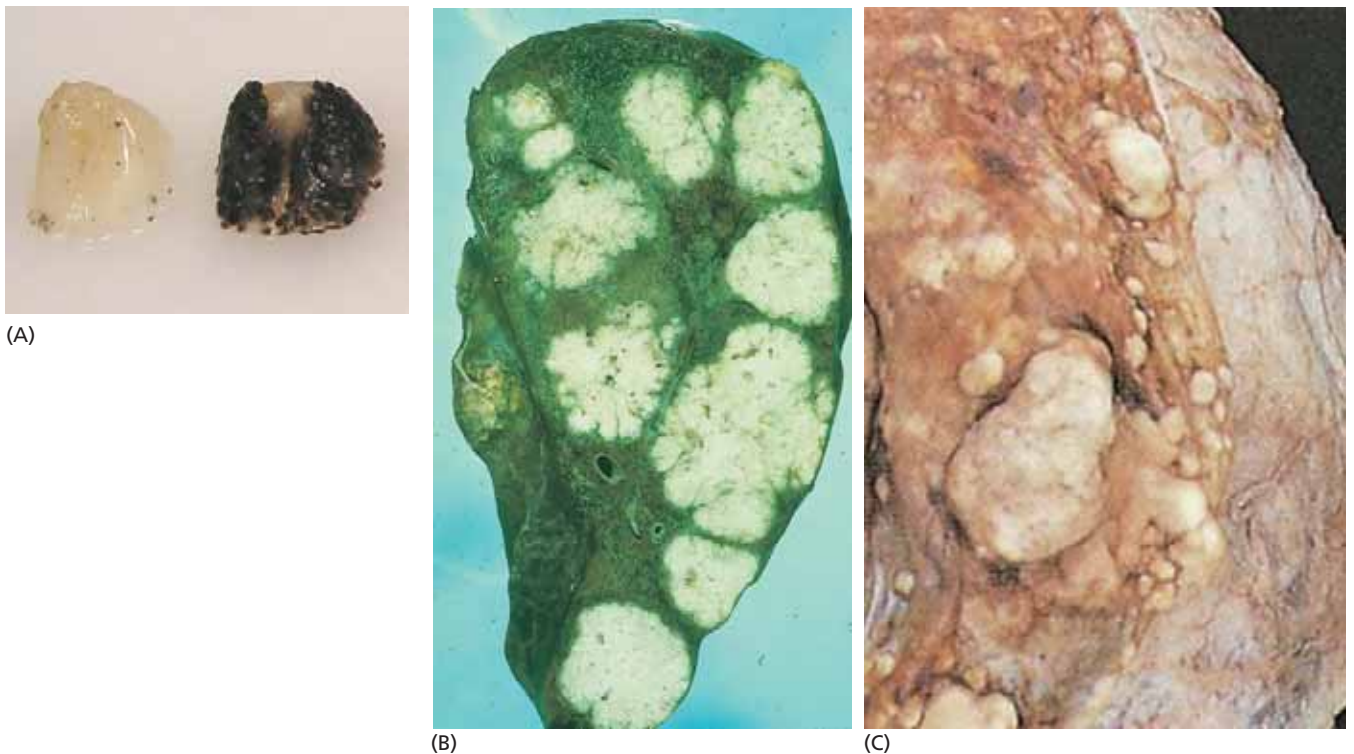
A confluence of discoveries in the mid- and late nineteenth century led to our current understanding of how tissues and complex organisms arise from fertilized eggs. The most fundamental of these was the discovery that all tissues are composed of cells and cell products, and that all cells arise through the division of pre-existing cells. Taken together, these two revelations led to the deduction, so obvious to us now, that all the cells in the body of a complex organism are members of cell lineages that can be traced back to the fertilized egg. Conversely, the fertilized egg is able to spawn all the cells in the body, doing so through repeated cycles of cell growth and division.

These realizations had a profound impact on how tumors were perceived. Previously, many had portrayed tumors as foreign bodies that had somehow taken root in the body of an afflicted patient. Now, tumors, like normal tissues, could be examined under the microscope by researchers in the then-new science of **histology** (or **histopathology**). These examinations, first of normal tissue **sections** (thin slices) and later of sections made from tumor masses, revealed that tumors, like normal tissues, were composed of masses of cells (Figure 2.1).

In addition, evidence accumulated that tumors of various types, rather than invading the body from the outside world, often derive directly from the normal tissues in which they are first discovered. However, tumors did seem to be capable of moving within the confines of the human body: In many patients, multiple tumors were discovered at anatomical sites quite distant from where their dis-



**Figure 2.1 Normal versus neoplastic tissue** (A) This histological section of the lining of the ileum in the small intestine, viewed at low magnification, reveals the continuity between normal and cancerous tissue. To the left is the normal epithelial lining, termed the mucosa. In the middle is mucosal tissue that has become highly abnormal, being termed “dysplastic.” To the right is a frank tumor—an adenocarcinoma—which has begun to invade underlying tissues. (B) This pair of sections of tissue from the human breast, viewed at high magnification, shows how normal tissue architecture becomes deranged in tumors. In the normal mammary gland (*upper panel*), a milk duct is lined by epithelial cells (*dark purple nuclei*). These ducts are surrounded by mesenchymal tissue termed “stroma,” which consists of connective tissue cells, such as fibroblasts and adipocytes, and collagen matrix (*pink*). In an invasive ductal breast carcinoma (*lower panel*), the cancer cells, which arise from the epithelial cells lining the normal breast, have abnormally large nuclei (*purple*), no longer form well-structured ducts, and have invaded the stroma (*pink*). (A, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003; B, courtesy of A. Orimo.)



**Figure 2.2 Metastasis of cancer cells to distant sites**

Many types of tumors eventually release cancer cells that migrate to distant sites in the body, where they form the secondary tumors known as metastases. (A) Metastasis can be studied with facility in the mouse, in which the location of melanoma cells can be pinpointed because of their distinctive dark pigment. Seen here are the lungs of a mouse in which the formation of metastases has been almost entirely blocked (*left*) and one in which hundreds of metastases (*black spots*) were allowed to form, as observed two weeks after B16 mouse melanoma cells were introduced, via injection, into the tail vein of a mouse (*right*). (B) Metastases (*white*) in the liver often arise in patients with advanced colon carcinomas. The portal vein, which drains blood from the colon into the liver, provides a route for metastasizing colon cancer cells to migrate directly into the liver. (C) Breast cancer often metastasizes to the brain. Here, large metastases are revealed post mortem in the right side of a brain where the dura (membrane covering) of the brain (*right*) has been removed. (A, from F. Nimmerjahn et al. *Immunity* 23:41–51, 2005; B, courtesy of Peter Isaacson; C, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

ease first began, a consequence of the tendency of cancers to spread throughout the body and to found new colonies of cancer cells (Figure 2.2). These new settlements, termed **metastases**, were often traceable directly back to the site where the disease of cancer had begun—the founding or **primary tumor**.

Invariably, detailed examination of the organization of cells within tumor masses gave evidence of a tissue architecture that was less organized and structured than the architecture of nearby normal tissues (see Figure 2.1). These histopathological comparisons provided the first seeds of an idea that would take the greater part of the twentieth century to prove: tumors are created by cells that have lost the ability to assemble and create tissues of normal form and function. Stated more simply, cancer came to be viewed as a disease of malfunctioning cells.

It followed logically that all tumors should, in principle, be traceable back to the specific tissue or organ site in which they first arose, often using the histopathological analyses of tumor sections to provide critical clues. This simple idea led for the first time to a new way of classifying these growths, which depended on their presumed tissues of origin. The resulting classifications often united under one roof cancers that arise in tissues and organs that have radically different functions in the body but share common types of tissue organization.

The science of histopathology also made it possible to understand the relationship between the clinical behavior of a tumor (i.e., the effects that the tumor had on the patient) and its microscopic features. Most important here were the criteria that segregated tumors into two broad categories depending on their degree of aggressive growth. Those that grew locally without invading adjacent tissues were classified as **benign**. Others that invaded nearby tissues and spawned metastases were termed **malignant**.

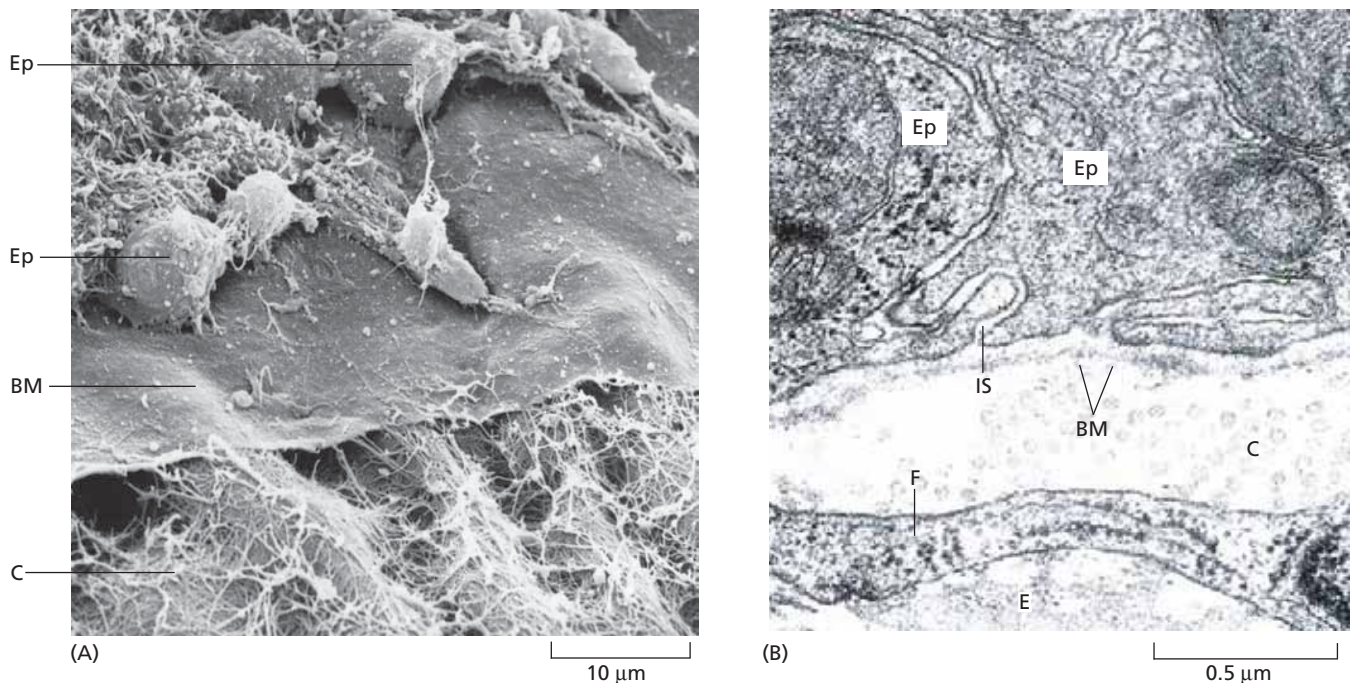
In fact, the great majority of primary tumors arising in humans are benign and are harmless to their hosts, except in the rare cases where the expansion of these localized masses causes them to press on vital organs or tissues. Some benign

tumors, however, may cause clinical problems because they release dangerously high levels of hormones that create physiologic imbalances in the body. For example, thyroid **adenomas** (pre-malignant epithelial growths) may cause excessive release of thyroid hormone into the circulation, leading to hyperthyroidism; pituitary adenomas may release growth hormone into the circulation, causing excessive growth of certain tissues—a condition known as **acromegaly**. Nonetheless, deaths caused by benign tumors are relatively uncommon. The vast majority of cancer-related mortality derives from malignant tumors. More specifically, it is the metastases spawned by these tumors that are responsible for some 90% of deaths from cancer.

## 2.2 Tumors arise from many specialized cell types throughout the body

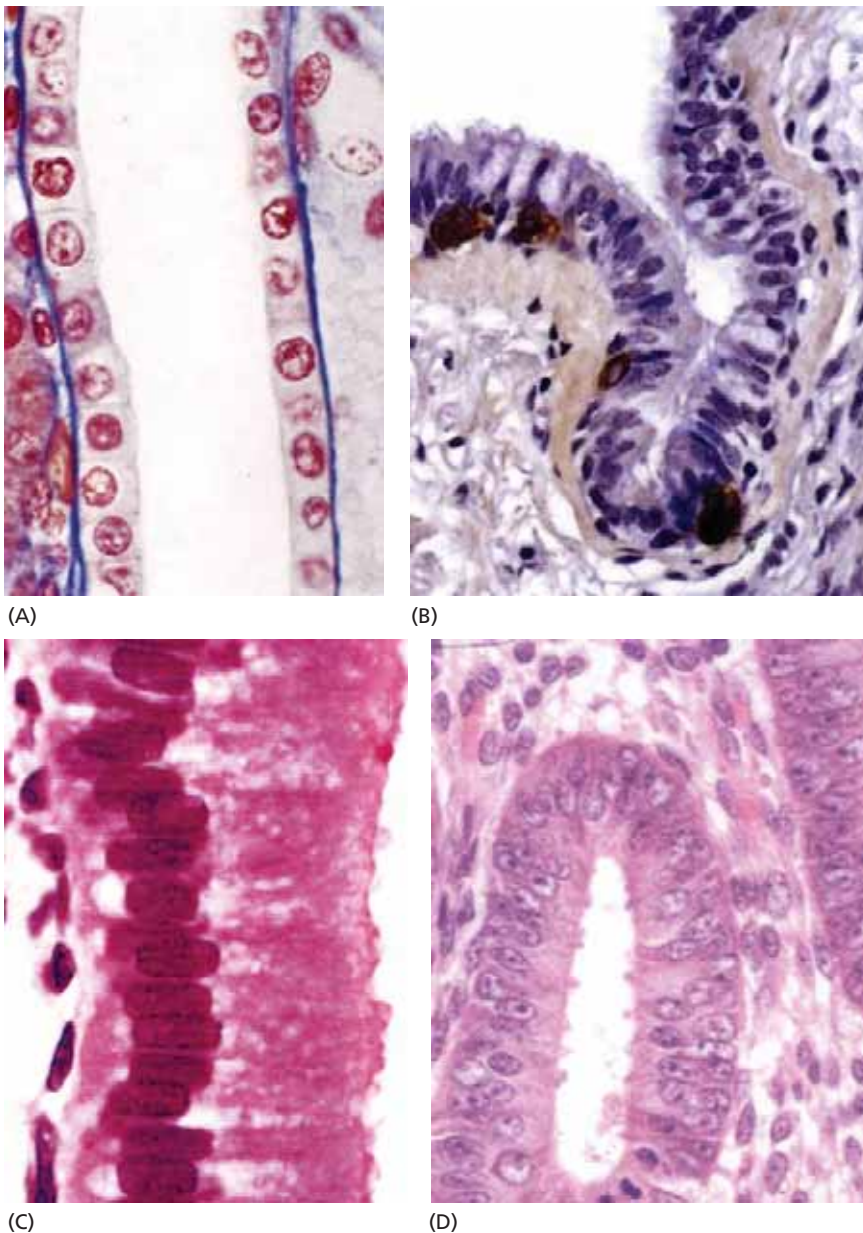
The majority of human tumors arise from epithelial tissues. **Epithelia** are sheets of cells that line the walls of cavities and channels or, in the case of skin, serve as the outside covering of the body. By the first decades of the twentieth century, detailed histological analyses had revealed that normal tissues containing epithelia are all structured similarly. Thus, beneath the epithelial cell layers in each of these tissues lies a **basement membrane** (sometimes called a **basal lamina**); it separates the epithelial cells from the underlying layer of supporting connective tissue cells, termed the **stroma** (Figure 2.3).

The basement membrane is a specialized type of extracellular matrix (ECM) and is assembled from proteins secreted largely by the epithelial cells. Yet other types of basement membrane are present in other kinds of tissue types. For example,



**Figure 2.3 Basement membranes** (A) This scanning electron micrograph of a chick corneal epithelium illustrates the basic plan of epithelial tissues, in which epithelial cells (Ep) are tethered to one side of the basement membrane. The basement membrane (BM), sometimes termed “basal lamina”, seen here as a continuous sheet, is formed as meshwork of extracellular matrix proteins. Below this are seen stromal cells and a network of collagen fibers (C) that anchors the underside of the basement membrane to the ECM of the stroma. (B) When the epithelium of the mouse trachea is

viewed in section at far higher magnification through a transmission electron microscope, the basement membrane (BM) can be visualized. Several epithelial cells (Ep) are seen above the BM, while below are collagen fibrils (C), a fibroblast (F), and elastin fibers (E). Note that the basement membrane is not interrupted at the intercellular space (IS). (A, courtesy of Robert Trelstad; B, from B. Young et al., *Wheater’s Functional Histology*, 4th ed. Edinburgh: Churchill Livingstone, 2003.)



**Figure 2.4 Architecture of epithelial tissues** A common organizational plan describes most of the epithelial tissues in the body: The mature, differentiated epithelial cells are at the exposed surface of an epithelium. In many tissues, underlying these epithelia are less differentiated epithelial cells, not seen in this figure. Beneath the epithelial cell layer lies a basement membrane (see Figure 2.3), which is usually difficult to visualize in the light microscope. Shown here are epithelia of (A) a collecting tubule of the kidney, (B) the bronchiole of the lung, (C) the columnar epithelium of the gallbladder, and (D) the endometrium of the uterus. In each case, the epithelial cells protect the underlying tissue from the contents of the cavity that they are lining. (From B. Young et al., *Wheater's Functional Histology*, 4th ed. Edinburgh: Churchill Livingstone, 2003.)

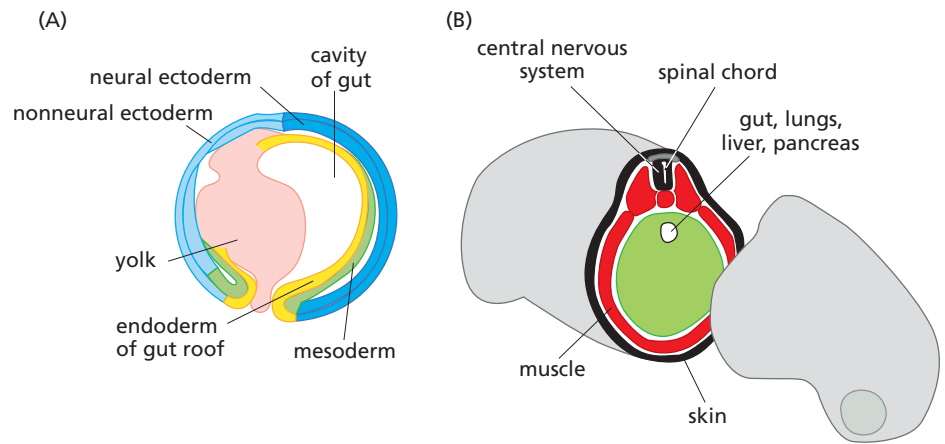
the **endothelial** cells, which form the inner linings of capillaries and larger vessels, rest on a specialized basement membrane that separates them from an outer layer of specialized smooth muscle cells. In all cases, these basement membranes serve as a structural scaffolding of the tissue. In addition, as we will learn later, cells attach a variety of biologically active signaling molecules to basement membranes.

Epithelia are of special interest here, because they spawn the most common human cancers—the **carcinomas**. These tumors are responsible for more than 80% of the cancer-related deaths in the Western world. Included among the carcinomas are tumors arising from the epithelial cell layers of the gastrointestinal tract—which includes mouth, esophagus, stomach, and small and large intestines—as well as the skin, mammary gland, pancreas, lung, liver, ovary, gallbladder, and urinary bladder. Examples of normal epithelial tissues are presented in Figure 2.4.

This group of tissues embraces cell types that arise from all three of the primitive cell layers in the early vertebrate embryo. Thus, the epithelia of the lungs,

**Figure 2.5 Embryonic cell layers**

(A) The tissues of more complex metazoa develop from three embryonic cell compartments—ectoderm (*blue*), mesoderm (*green*), and endoderm (*yellow*). Each of the three embryonic cell layers is precursor to distinct types of differentiated cells. (B) In an early-stage tadpole, the skin and nervous system develop from the ectoderm (*gray, black*), while the connective tissue, including bone, muscle, and blood-forming cells, develops from the mesoderm (*red*). The gut and derived outpouchings, including lung, pancreas, and liver, develop from the endoderm (*white*). The development of all vertebrates follows this plan. (Adapted from T. Mohun et al., *Cell* 22:9–15, 1980.)



liver, gallbladder, pancreas, esophagus, stomach, and intestines all derive from the inner cell layer, the **endoderm**. Skin arises from the outer embryonic cell layer, termed the **ectoderm**, while the ovaries originate embryologically from the middle layer, the **mesoderm** (Figure 2.5). Therefore, in the case of carcinomas, histopathological classification is not informed by the developmental history of the tissue of origin.

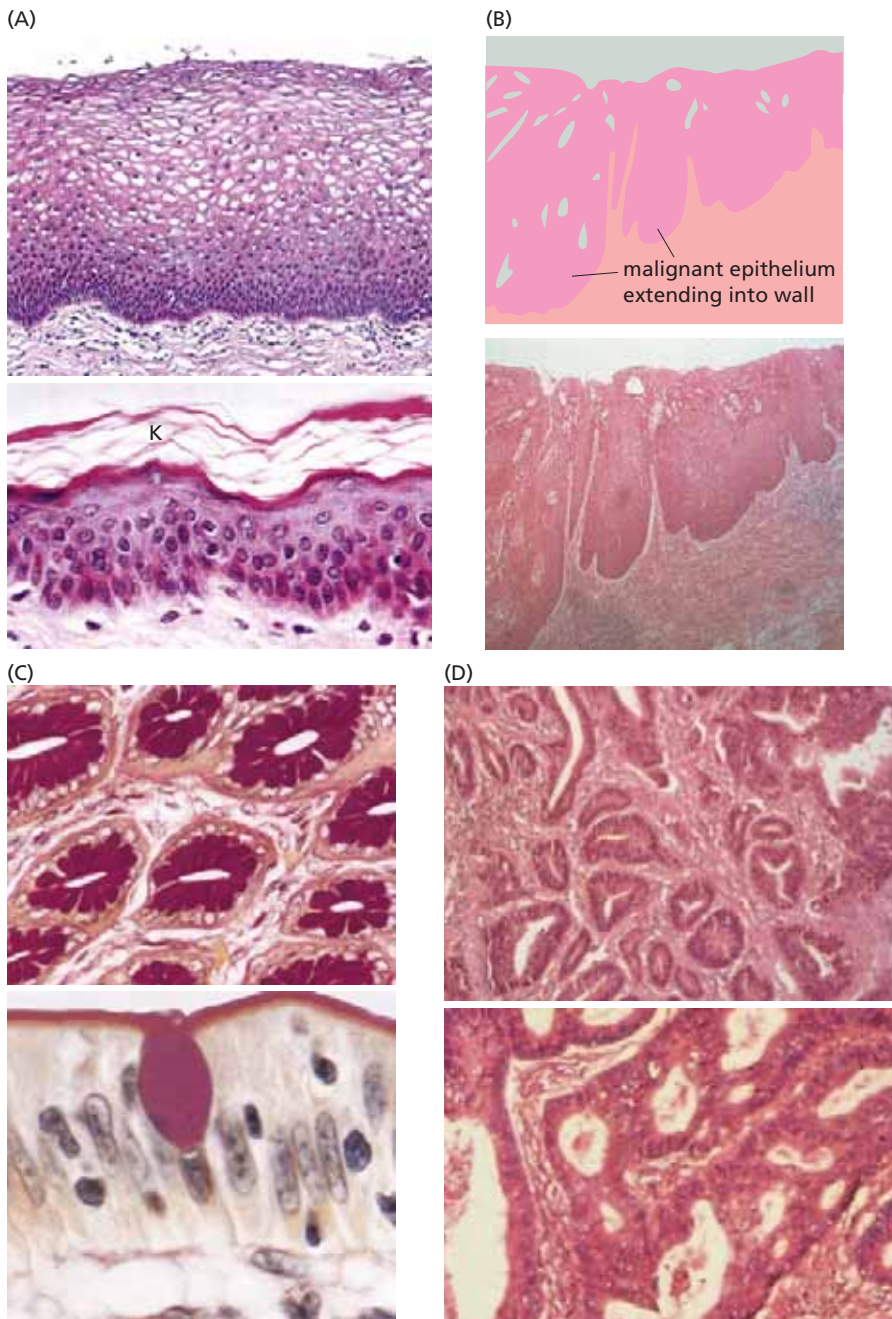
The epithelial and stromal cells of these various tissues collaborate in forming and maintaining the epithelial sheets. When viewed from the perspective of evolution, it now seems that the embryologic mechanisms for organizing and structuring epithelial tissues were invented early in metazoan evolution, likely more than 600 million years ago, and that these mechanistic principles have been exploited time and again during metazoan evolution to construct tissues and organs having a wide array of physiologic functions.

Most of the carcinomas fall into two major categories that reflect the two major biological functions associated with epithelia (Table 2.1). Some epithelial sheets serve largely to seal the cavity or channel that they line and to protect the underlying cell populations (Figure 2.6). Tumors that arise from epithelial cells forming these protective cell layers are said to be **squamous cell carcinomas**. For example, the epithelial cells lining the skin and the esophagus spawn tumors of this type.

Many epithelia also contain specialized cells that secrete substances into the ducts or cavities that they line. This class of epithelial cells generates **adenocarcinomas**. Often these secreted products are used to protect the epithelial cell layers from the contents of the cavities that they surround (see Figure 2.6). Thus, some epithelial cells lining the lung and stomach secrete mucus layers that protect them, respectively, from the air (and airborne particles) and from the corrosive effects of high concentrations of acid. The epithelia in some organs such as the lung, uterus, and cervix have the capacity to give rise to pure adenocarcino-

**Table 2.1 Carcinomas**

(A) Tissue sites of more common types of adenocarcinoma	(B) Tissue sites of more common types of squamous cell carcinoma	(C) Other types of carcinoma
lung colon breast pancreas stomach esophagus prostate endometrium ovary	skin nasal cavity oropharynx larynx lung esophagus cervix	small-cell lung carcinoma large-cell lung carcinoma hepatocellular carcinoma renal cell carcinoma transitional-cell carcinoma (of urinary bladder)



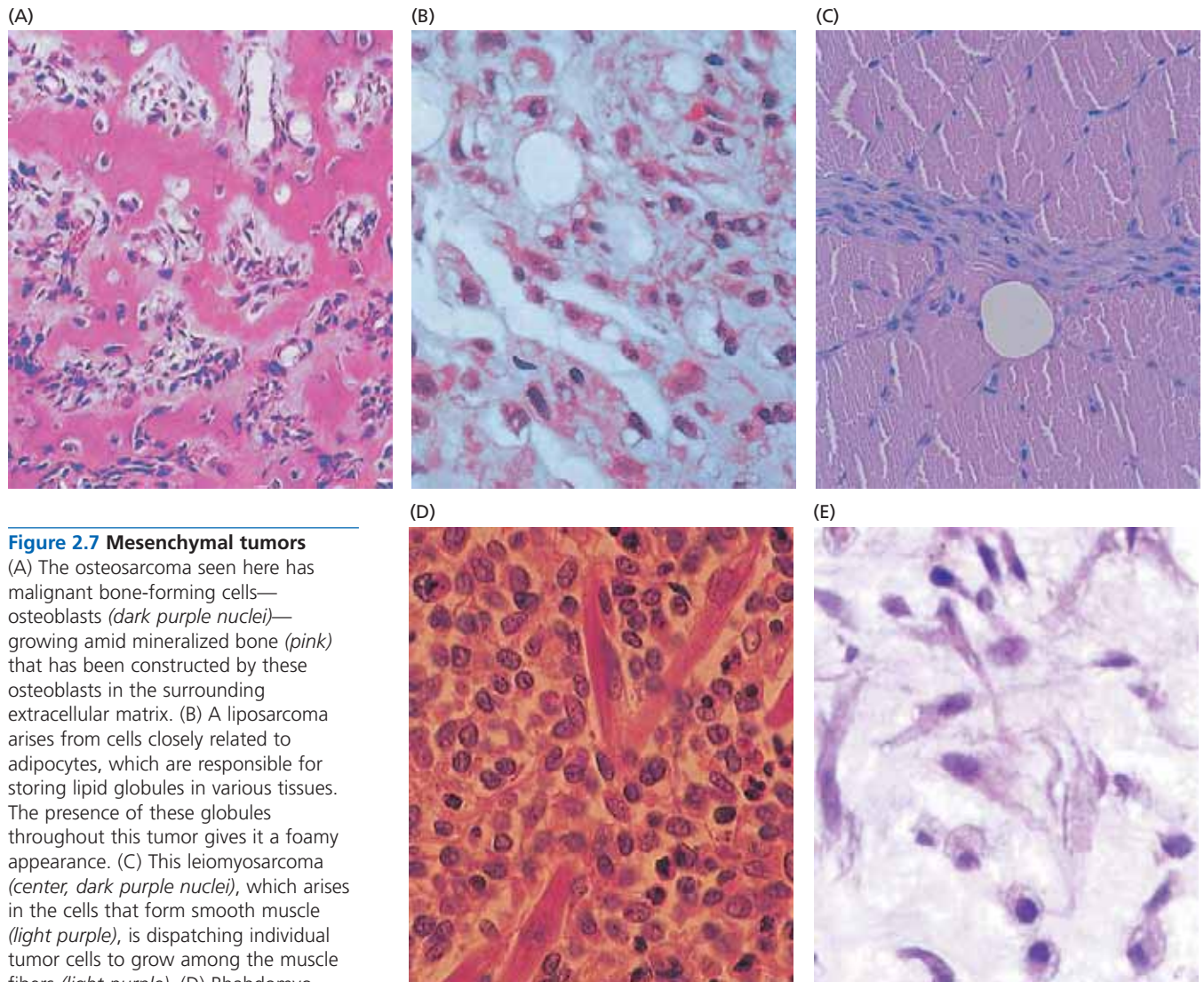
**Figure 2.6 Epithelia and derived carcinomas** Epithelia can be classified into subtypes depending on the shape and function of the normal epithelial cells and the carcinomas arising from them. The origins of squamous cell carcinomas and adenocarcinomas are seen here. (A) Normal squamous cells are often flattened and function to protect the epithelium and underlying tissue from the contents of the lumen or, in the case of skin, from the outside world. The squamous epithelia of the cervix of the uterus (*above*) and the skin (*below*) are organized quite similarly, with mature flattened cells at the surface being continually shed [e.g., the dead keratinocytes (K) of the skin] and replaced by less mature cells produced below. (B) In this carcinoma of the esophagus, large tongues of malignant squamous epithelial cells are invading the underlying stromal/mesenchymal tissue. (C) In some tissues, the glandular cells within epithelia secrete mucopolysaccharides to protect the epithelium; in other tissues, they secrete proteins that function within the *lumina* (cavities) of ducts or are distributed to distant sites in the body. Pits in the stomach wall are lined by mucus-secreting cells (*dark red, upper panel*). In the epithelium of the small intestine (*lower panel*) a single mucus-secreting goblet cell (*purple*) is surrounded by epithelial cells of a third type—columnar cells, which are involved in the absorption of water. (D) These adenocarcinomas of the stomach (*upper panel*) and colon (*lower panel*) show multiple ductal elements, which are clear indications of their derivation from secretory epithelia such as those in panel C. (A and C, from B. Young et al., *Wheater's Functional Histology*, 4th ed. Edinburgh: Churchill Livingstone, 2003; B and D, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

mas or pure squamous cell carcinomas; quite frequently, however, tumors in these organs are found in which both types of carcinoma cell coexist.

The remainder of malignant tumors arise from nonepithelial tissues throughout the body. The first major class of nonepithelial cancers derive from the various connective tissues throughout the body, which do share a common origin in the mesoderm of the embryo (Table 2.2). These tumors, the **sarcomas**, constitute only about 1% of the tumors encountered in the oncology clinic. Sarcomas derive from a variety of **mesenchymal** cell types. Included among these are **fibroblasts** and related connective tissue cell types that secrete collagen, the major structural component of the extracellular matrix of tendons and skin; **adipocytes**, which store fat in their cytoplasm; **osteoblasts**, which assemble calcium phosphate crystals within matrices of collagen to form bone; and **myocytes**, which assemble to form muscle (Figure 2.7). A relatively unusual tumor, an **angiosarcoma**,

**Table 2.2 Various types of more common sarcomas**

- osteosarcoma
- liposarcoma
- leiomyosarcoma
- rhabdomyosarcoma
- malignant fibrous histiocytoma
- fibrosarcoma
- synovial sarcoma
- angiosarcoma
- chondrosarcoma



**Figure 2.7 Mesenchymal tumors**

(A) The osteosarcoma seen here has malignant bone-forming cells—osteoblasts (*dark purple nuclei*)—growing amid mineralized bone (*pink*) that has been constructed by these osteoblasts in the surrounding extracellular matrix. (B) A liposarcoma arises from cells closely related to adipocytes, which are responsible for storing lipid globules in various tissues. The presence of these globules throughout this tumor gives it a foamy appearance. (C) This leiomyosarcoma (*center, dark purple nuclei*), which arises in the cells that form smooth muscle (*light purple*), is dispatching individual tumor cells to grow among the muscle fibers (*light purple*). (D) Rhabdomyosarcomas arise from the cells forming striated skeletal muscles; the cancer cells (*dark red nuclei*) are seen here amid several normal muscle cells (*red*). (E) This particular sarcoma arose in an unusual anatomic location—the meninges, which form the protective covering of the brain. (A to D, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003; E, from B. Young et al., *Wheater’s Functional Histology*, 4th ed. Edinburgh: Churchill Livingstone, 2003.)

arises from precursors of the endothelial cells. The stromal layers of epithelial tissues include many of these mesenchymal cell types.

The second group of nonepithelial cancers arise from the various cell types that constitute the blood-forming (**hematopoietic**) tissues, including the cells of the immune system (Table 2.3 and Figure 2.8). Among these are cells destined to form **erythrocytes** (red blood cells), antibody-secreting (**plasma**) cells, as well as T and B **lymphocytes**. The term **leukemia** (literally “white blood”) refers to malignant derivatives of several of these hematopoietic cell lineages that move freely through the circulation and, unlike the red blood cells, are nonpigmented. **Lymphomas** include tumors of the **lymphoid** lineages (B and T lymphocytes) that aggregate to form solid tumor masses, most frequently found in lymph nodes, rather than the dispersed, single-cell populations of tumor cells seen in leukemias.

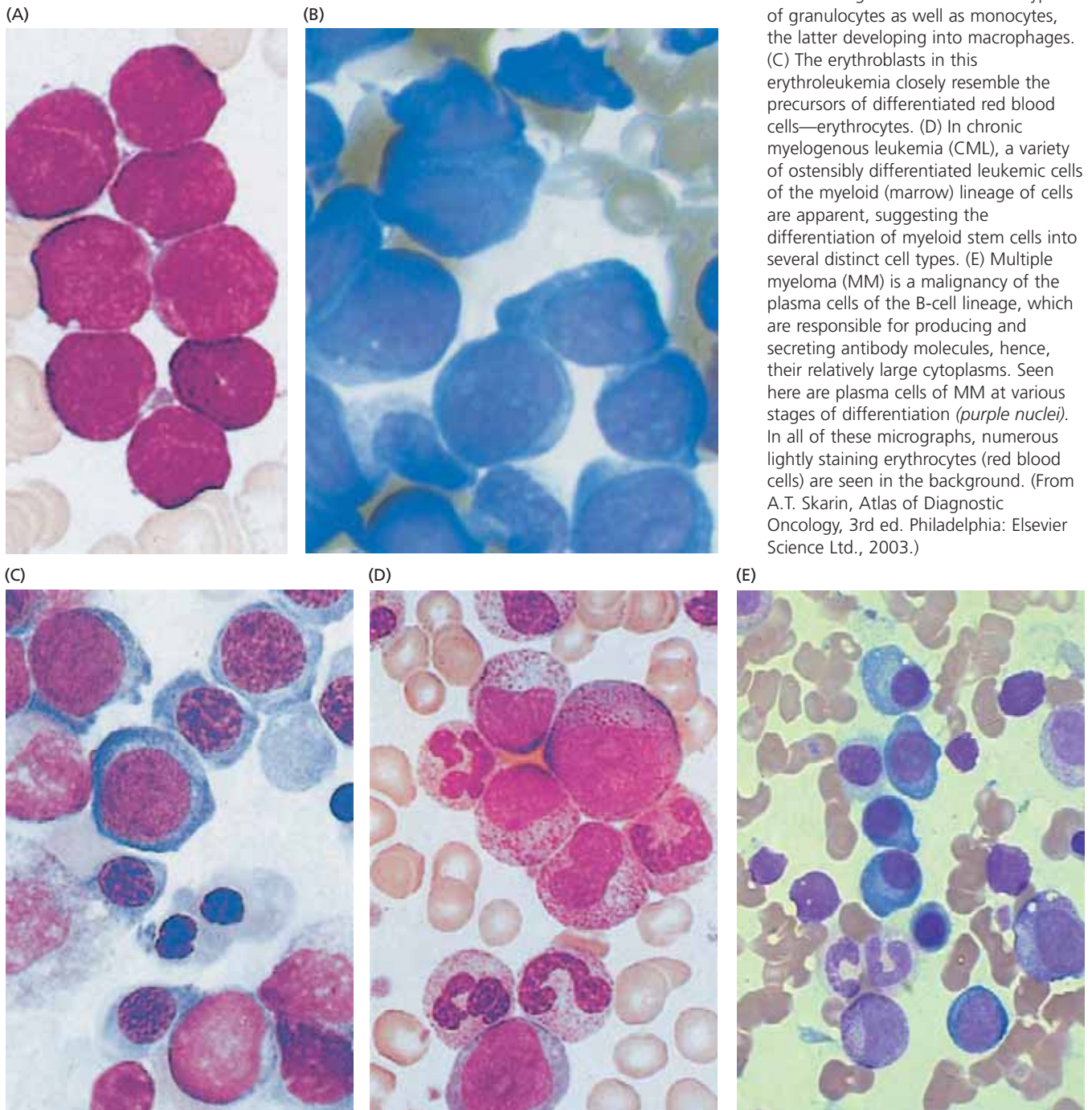
The third and last major grouping of nonepithelial tumors arises from cells that form various components of the central and peripheral nervous system (Table 2.4). These are often termed **neuroectodermal** tumors to reflect their origins in the outer cell layer of the early embryo. Included here are **gliomas**, **glioblastomas**, **neuroblastomas**, **schwannomas**, and **medulloblastomas** (Figure 2.9). While comprising only 1.3% of all diagnosed cancers, these are responsible for about 2.5% of cancer-related deaths.

**Table 2.3** Various types of more common hematopoietic malignancies

acute lymphocytic leukemia  
 acute myelogenous leukemia  
 chronic myelogenous leukemia  
 chronic lymphocytic leukemia  
 multiple myeloma  
 non-Hodgkin's lymphoma<sup>a</sup>  
 Hodgkin's disease

<sup>a</sup>The non-Hodgkin's lymphoma types, also known as lymphocytic lymphomas, can be placed in as many as 15–20 distinct subcategories, depending upon classification system.

**Figure 2.8 Hematopoietic malignancies** Hematopoietic malignancies take a variety of forms. (A) Acute lymphocytic leukemias (ALLs) arise from both the B-cell (80%) and T-cell (20%) lineages of lymphocytes (see Section 15.1). The cells forming this particular tumor exhibited the antigenic markers indicating origin from pre-B cells. (B) As in many hematopoietic malignancies, these acute myelogenous leukemia (AML) cells have only a small rim of cytoplasm around their large nuclei. They derive from precursor cells of the lineage that forms various types of granulocytes as well as monocytes, the latter developing into macrophages. (C) The erythroblasts in this erythroleukemia closely resemble the precursors of differentiated red blood cells—erythrocytes. (D) In chronic myelogenous leukemia (CML), a variety of ostensibly differentiated leukemic cells of the myeloid (marrow) lineage of cells are apparent, suggesting the differentiation of myeloid stem cells into several distinct cell types. (E) Multiple myeloma (MM) is a malignancy of the plasma cells of the B-cell lineage, which are responsible for producing and secreting antibody molecules, hence, their relatively large cytoplasm. Seen here are plasma cells of MM at various stages of differentiation (*purple nuclei*). In all of these micrographs, numerous lightly staining erythrocytes (red blood cells) are seen in the background. (From A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)



**Table 2.4** Various types of neuroectodermal malignancies

glioblastoma multiforme  
astrocytoma  
meningioma  
neurinoma  
retinoblastoma  
neuroblastoma  
ependymoma  
oligodendroglioma  
medulloblastoma

### 2.3 Some types of tumors do not fit into the major classifications

Not all tumors fall neatly into one of these four large groups. For example, **melanomas** derive from melanocytes, the pigmented cells of the skin and the retina. The melanocytes, in turn, arise from a primitive embryonic structure termed the **neural crest**. While having an embryonic origin close to that of the neuroectodermal cells, the melanocytes end up during development as wanderers that settle in the skin and the eye, provide pigment to these tissues, and acquire no direct connections with the nervous system (Figure 2.10).

**Small-cell lung carcinomas** (SCLCs) contain cells having many attributes of **neurosecretory** cells, such as those of neural crest origin in the **adrenal** glands that sit above the kidneys. Such cells, often in response to neuronal signaling, secrete biologically active peptides. It remains unclear whether the SCLCs, frequently seen in tobacco users, arise from neuroectodermal cells that have insinuated themselves during normal development into the developing lung. According to a more likely alternative, these tumors originate in endodermal cell populations of the lung that have shed some of their epithelial characteristics and taken on those of the neuroectodermal lineage.

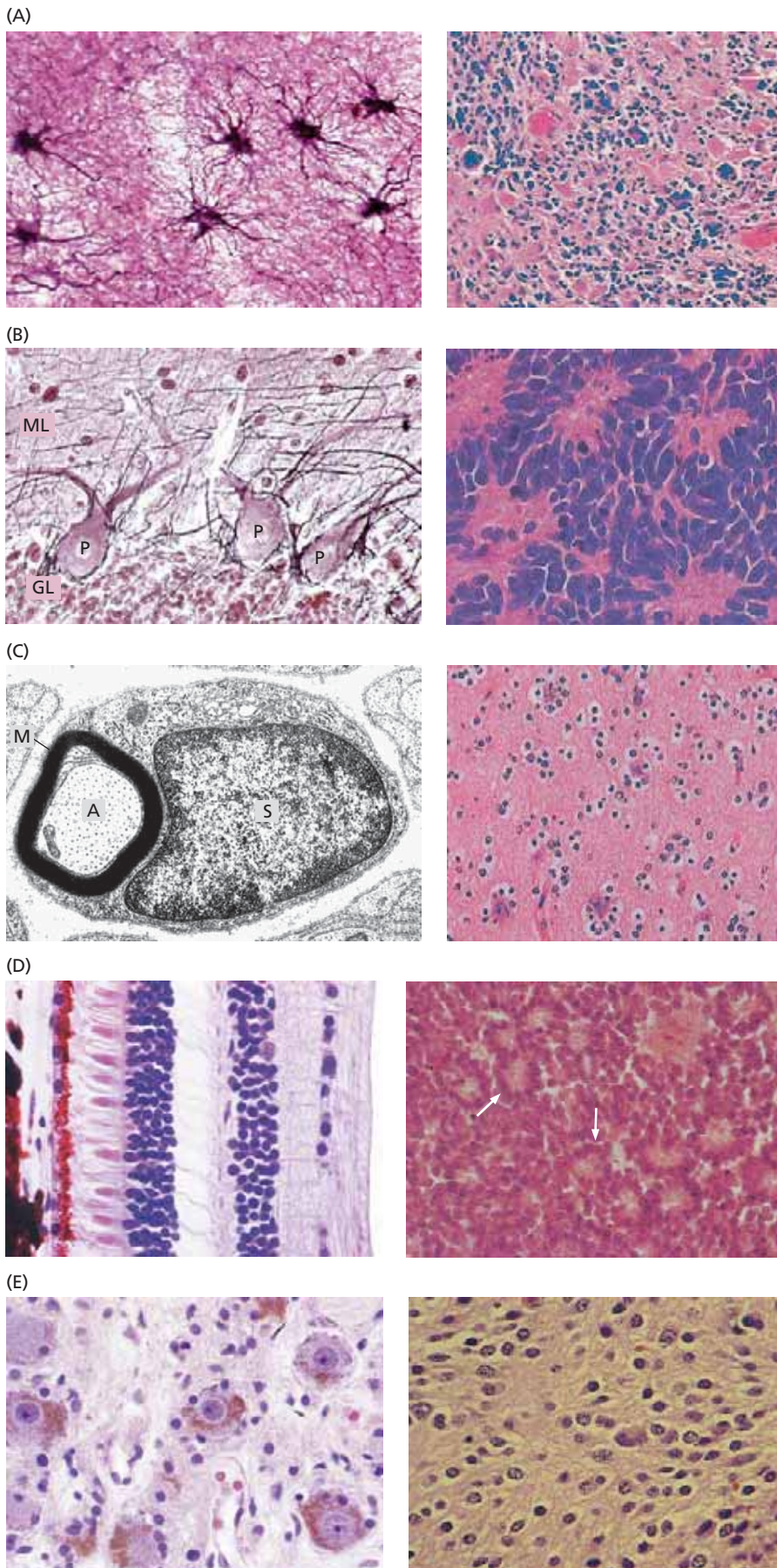
This switching of tissue lineage and resulting acquisition of an entirely new set of differentiated characteristics is often termed **transdifferentiation**. The term implies that the commitments cells have made during embryogenesis to enter into one or another tissue and cell lineage are not irreversible, and that under certain conditions, cells can move from one differentiation lineage to another. Such a change in phenotype may affect both normal and cancer cells. For example, at the borders of many carcinomas, epithelial cancer cells often change shape and gene expression programs and take on attributes of the nearby stromal cells of mesenchymal origin. This dramatic shift in cell phenotype, termed the **epithelial-mesenchymal transition** or simply EMT, implies great plasticity on the part of cells that normally seem to be fully committed to behaving like epithelial cells. As described later (Chapters 13 and 14), this transition may often accompany and enable the invasion by carcinoma cells into adjacent normal tissues.

Still, even with these occasional rule-breaking exceptions in mind, one major biological principle seems to govern the vast majority of cancers. While cancer cells deviate substantially in behavior from their normal cellular precursors, they almost always retain some of the distinctive attributes of the normal cell types from which they have arisen. These attributes provide critical clues about the origins of most tumors; they enable pathologists to examine tumor biopsies under the microscope and assign a tissue of origin and tumor classification, even without prior knowledge of the anatomical site from which these biopsies were prepared.

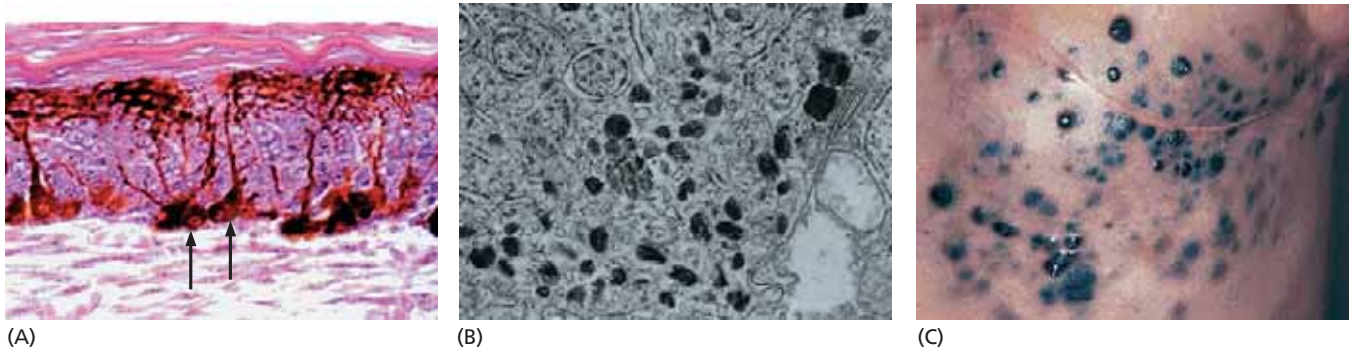
In a small minority of cases, (1–2%), the tumors given to pathologists for analysis have shed virtually all of the tissue-specific, differentiated traits of their normal precursor tissues. The cells in such tumors are said to have **dedifferentiated**, and the tumors as a whole are **anaplastic**, in that it is no longer possible to use histopathological criteria to identify the tissues from which they have arisen (Figure 2.11).

### 2.4 Cancers seem to develop progressively

Between the two extremes of fully normal and highly malignant tissue architectures lies a broad spectrum of tissues of intermediate appearance. The different gradations of abnormality may well reflect cell populations that are evolving



**Figure 2.9 Neuroectodermal tumors**  
 Various cellular components of the central and peripheral nervous systems can become malignant. (A) Astrocytes—nonneuronal, supporting cells of the brain (*dark purple, left panel*)—are the presumed precursors of astrocytomas and glioblastomas (*right panel*). Glioblastoma multiforme takes its name from the multiple distinct neuroectodermal cell types that constitute the tumor. The tumor cells are seen to have nuclei of various sizes (*purple*). (B) Cells of the granular layer (GL) of the cerebellum (*left panel*) reside below Purkinje cells (P) and cells of the molecular layer (ML) in the cortex of the cerebellum. The precursors of granular cells yield medulloblastomas (*right panel*), the cells of which are notable for their ability to differentiate into neurons, glial cells, and pigmented neuroepithelial cells (*purple nuclei, pink cytoplasm*). About one-third of these tumors show the rosettes of cells seen here. (C) The Schwann cells (S, *left panel*), as seen in this electron micrograph, normally wrap multiple membrane layers (M) around axons (A) to provide the latter with a sheathing that aids in conductance. They are precursors of the oligodendrogliomas (*right panel*). Each of the neoplastic cell nuclei here has a halo around it, which is characteristic of this tumor. (D) Rods, cones and other neuronal cell types (*left panel*) constitute important components of the normal retina. Retinoblastomas (*right panel*) arise from the cells that are the common precursors of the cells forming the normal retina. Retinoblastomas often show the characteristic rosettes, indicated here with arrowheads. (E) Cells of the sympathetic ganglia of the peripheral nervous system (*larger cells, left panel*) give rise to neuroblastomas (*right panel*), which are usually seen in children. The individual tumor cells here are surrounded by dense fibrillary webs, which are derived from neurites—cytoplasmic processes used by neurons to communicate with one another. (A to E *left panels*, from B. Young et al., *Wheater's Functional Histology*, 4th ed. Edinburgh: Churchill Livingstone, 2003; A to E *right panels*, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)



**Figure 2.10 Melanocytes and melanomas** (A) Melanocytes (arrows), which form pigment granules, are normally scattered among the basal keratinocytes of the skin (see also Figure 2.6A). They extend long, thin cytoplasmic processes through which they deposit these granules in the cytoplasm of the keratinocytes, which form the bulk of the epithelium. Layers of dead keratinocytes at the surface of the skin (*above*) and stroma cells (*below*) are also apparent. (B) The pigment granules, visualized here by electron microscopy, have made melanomas favored objects of research because of the readily detected metastases that they often form

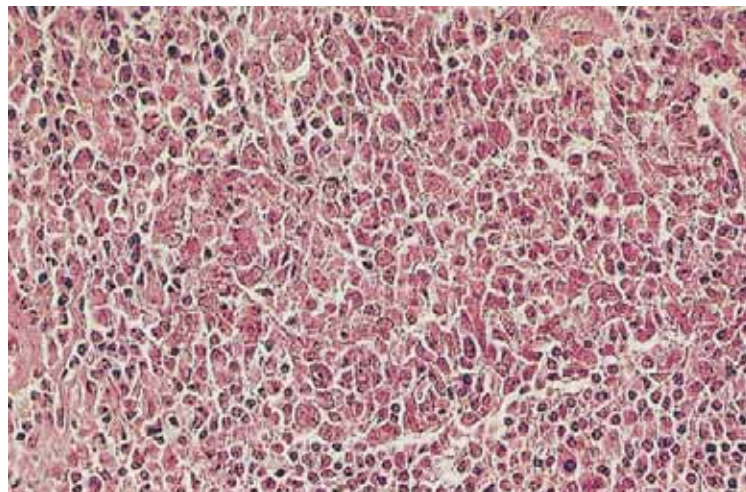
(e.g., see Figure 2.2A). Once melanomas have begun to invade vertically from the superficial layers of the skin into the underlying stroma, they have a high tendency to metastasize to distant tissues sites. (C) This case of cutaneous melanoma dramatizes the metastatic nature of the disease and the readily observed, pigmented metastases. (A, from W.J. Bacha Jr. et al., *Color Atlas of Veterinary Histology*, 2nd ed. Philadelphia: Lippincott, Williams and Wilkins, 2000; B and C, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

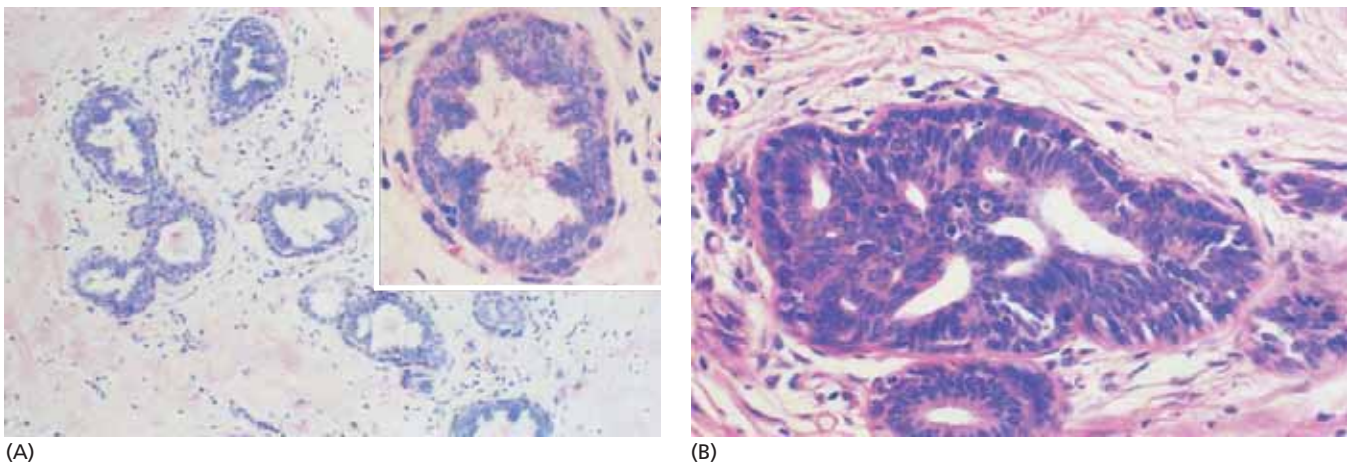
progressively away from normal and toward greater degrees of aggressive and invasive behavior. Thus, each type of growth may represent a distinct step along this evolutionary pathway. If so, these architectures suggest, but hardly prove, that the development of tumors is a complex, multi-step process, a subject that is discussed in great detail in Chapter 11.

Some growths contain cells that deviate only minimally from those of normal tissues but may nevertheless be abnormal in that they contain excessive *numbers* of cells. Such growths are termed **hyperplastic** (Figure 2.12). In spite of their apparently deregulated proliferation, the cells forming hyperplastic growths have retained the ability to assemble into tissues that appear reasonably normal.

An equally minimal deviation from normal is seen in **metaplasia**, where one type of normal cell layer is displaced by cells of another type that are not normally encountered in this site within a tissue. These invaders, although present in the wrong location, often appear completely normal under the microscope. Metaplasia is most frequent in epithelial transition zones where one type of epithelium meets another. Transition zones like these are found at the junction of the cervix with the uterus and the junction of the esophagus and the stomach.

**Figure 2.11 Anaplastic tumors of obscure origin** The histological appearance of an anaplastic tumor, such as that shown here, gives little indication of its tissue of origin. Attempts to determine the origin of these cells with an antibody stain that specifically recognizes one or another tissue-specific protein marker may also prove uninformative. (From A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)





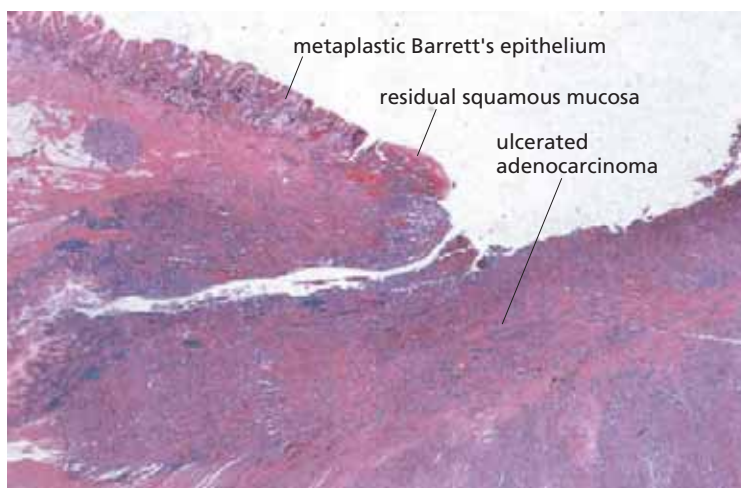
In both locations, a squamous epithelium normally undergoes an abrupt transition into a mucus-secreting epithelium. For example, an early indication of premalignant change in the esophagus is a metaplastic condition termed **Barrett's esophagus**, in which the normally present squamous epithelium is replaced by secretory epithelial cells of a type usually found within the stomach (Figure 2.13). Even though these gastric cells have a quite normal appearance, this metaplasia is considered an early step in the development of esophageal carcinomas. Indeed, patients suffering from Barrett's esophagus have a thirty-fold increased risk of developing these highly malignant tumors.

A slightly more abnormal tissue is said to be **dysplastic**. Cells within a dysplasia are usually abnormal **cytologically**; that is, the appearance of individual cells is no longer normal. The cytological changes include variability in nuclear size and shape, increased nuclear staining by dyes, increased ratio of nuclear versus cytoplasmic size, increased mitotic activity, and lack of the cytoplasmic features associated with the normal differentiated cells of the tissue (Figure 2.14). In dysplastic growths, the relative numbers of the various cell types seen in the normal tissue are no longer observed. Together, these changes in individual cells and in cell numbers have major effects on the overall tissue architecture. Dysplasia is considered to be a transitional state between completely benign growths and those that are premalignant.

Even more abnormal are the growths that are seen in epithelial tissues and termed variously adenomas, **polyps**, adenomatous polyps, **papillomas**, and, in skin, warts (Figure 2.15). These are often large growths that can be readily detected with the naked eye. They contain all the cell types found in the normal

#### Figure 2.12 Normal versus hyperplastic epithelium

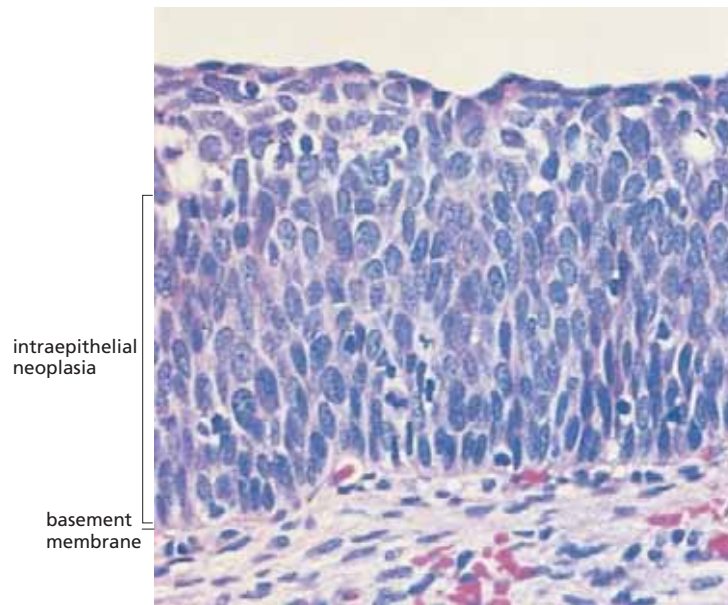
The morphology of the normal ductal epithelium of the mammary gland (see Figure 2.1B) can be compared with different degrees of hyperplasia. (A) In these mildly hyperplastic milk ducts, shown at low magnification and high magnification (*inset*), mammary epithelial cells have begun to pile up and to protrude into the lumina. (B) A more advanced hyperplastic mammary duct shows epithelial cells that are crowded together and almost completely fill the lumen. However, they have not penetrated the basement membrane (*not visible*) and invaded the surrounding stroma. (From A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)



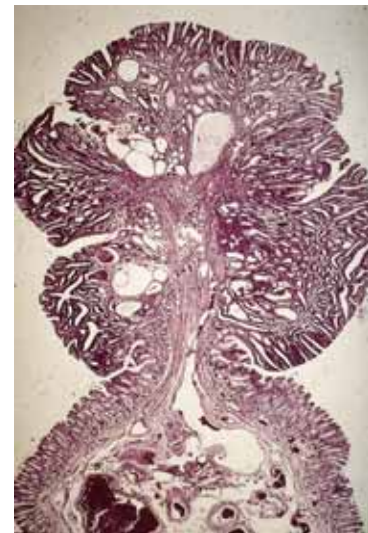
#### Figure 2.13 Metaplastic conversion of epithelia

In certain precancerous conditions, the normally present epithelium is replaced by an epithelium from a nearby tissue—the process of metaplasia. For example, in Barrett's esophagus (sometimes termed Barrett's esophagitis), the squamous cells that normally line the wall of the esophagus (*residual squamous mucosa*) are replaced by secretory cells that migrate from the lining of the stomach (*metaplastic Barrett's epithelium*). This particular metaplasia, which is provoked by chronic acid reflux from the stomach, can become a precursor lesion to an esophageal carcinoma, which has developed here from cells of gastric origin (*ulcerated adenocarcinoma*). (Adapted from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

**Figure 2.14 Formation of dysplastic epithelium** In this high-grade intraepithelial squamous neoplasia of the cervix, the epithelial cells have not broken through the basement membrane (*not visible*) and invaded the underlying stroma. However, only the top layer of cells retains the flattened appearance of squamous cells, while the cell layers below (*purple*) have lost the differentiated, flattened appearance (see Figure 2.6A), have slightly enlarged nuclei, and have accumulated in extra cell layers. (From A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

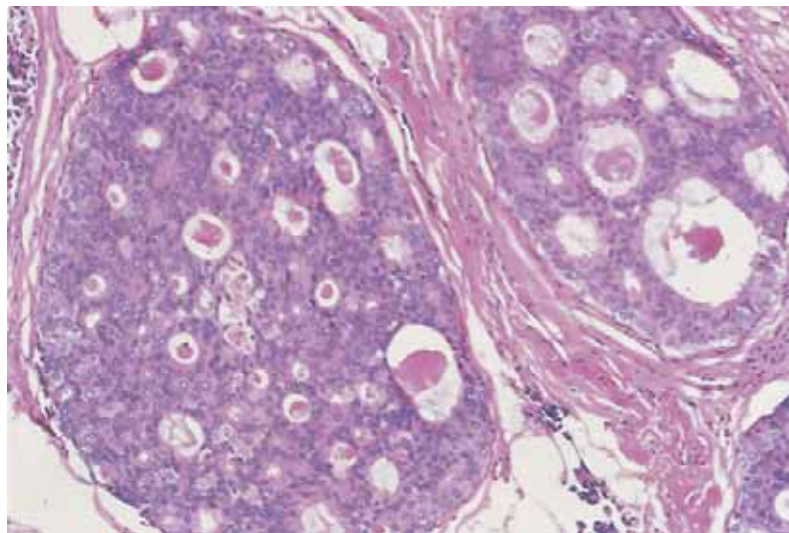


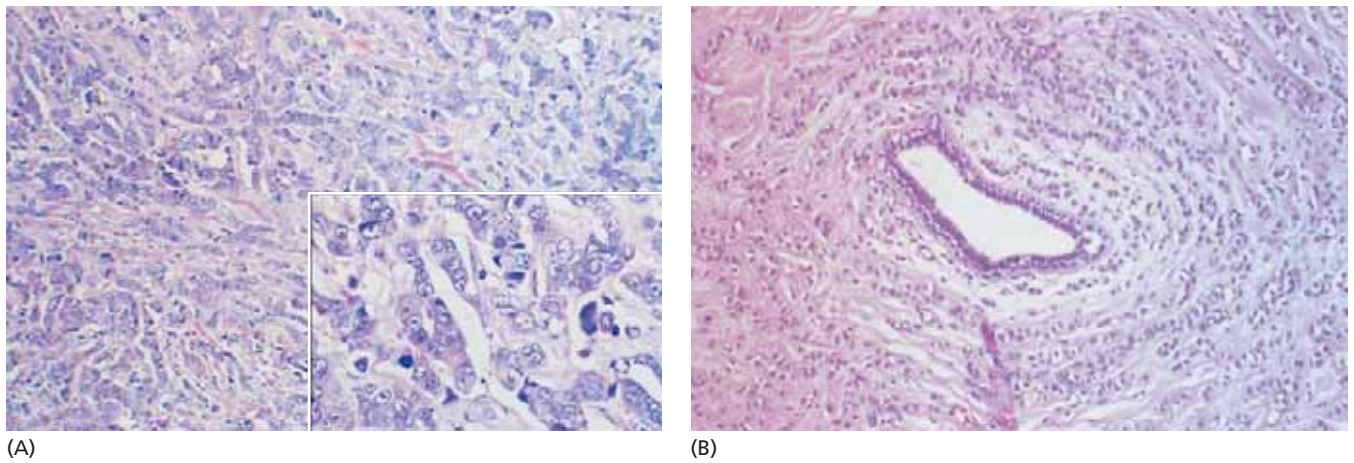
(A)



**Figure 2.15 Pre-invasive adenomas and carcinomas** Adenomatous growths, termed polyps in certain organs, have a morphology that sets them clearly apart from normal and dysplastic epithelium. (A) In the colon, pre-invasive growths appear as either flat thickenings of the colonic wall (sessile polyps, *not shown*) or as the stalk-like growths (pedunculated polyps) shown here. These growths, also termed "adenomas," have not penetrated the basement membrane and invaded the underlying stroma. Polyps are seen here in a photograph (*left*) and a micrograph section (*right*). (B) In this intraductal carcinoma of the breast, the epithelial cancer cells have almost completely filled two ducts (*left, right*) and have expanded them to great size but have not yet broken through the surrounding basement membrane and invaded the stroma. (A, *left*, courtesy of John Northover and Cancer Research, UK; *right*, courtesy of Anne Campbell; B, from A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

(B)





**Figure 2.16 Invasive carcinomas**

Tumors are considered malignant only after they have breached the basement membrane and invaded the nearby surrounding stroma. (A) In this invasive ductal carcinoma of the breast, islands of epithelial cancer cells are intermingled with stromal cells. The ductal nature of this carcinoma is revealed in the inset, where the carcinoma cells are seen to form rudimentary ducts (*e.g.*, *middle of inset*) with lumina. (B) In this invasive lobular carcinoma of the breast, individual carcinoma cells have ventured out into the stroma, often doing so in single-file formation (*numerous small purple nuclei*). They surround a single duct—a carcinoma *in situ*—in which the epithelial cancer cells are growing facing the lumen of the duct and have not penetrated the basement membrane and invaded the surrounding stroma. (From A.T. Skarin, *Atlas of Diagnostic Oncology*, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)

epithelial tissue, but this mass of cells has launched a program of substantial expansion, creating a macroscopic mass. Under the microscope, the tissue within these adenomatous growths is seen to be dysplastic. They usually grow to a certain size and then stop growing, and they respect the boundary created by the basement membrane, which continues to separate them from underlying epithelium. Since adenomatous growths do not penetrate the basement membrane and invade underlying tissues, they are considered to be benign.

A further degree of abnormality is represented by growths that do invade underlying tissues (Figure 2.16). Here, for the first time, we encounter malignant tumors that have a substantial potential of threatening the life of the individual who carries them. Clinical oncologists and surgeons often reserve the word “cancer” for these and even more abnormal growths. However, in this book, as in much of contemporary cancer research, the word cancer is used more loosely to include all types of abnormal growths. (In the case of epithelial tissues, the term “carcinoma” is usually applied to growths that have acquired this degree of invasiveness.) This disparate collection of growths—both benign and malignant—are called collectively **neoplasias**, *i.e.*, new types of tissue. (Some reserve the term “neoplasia” for malignant tumors.)

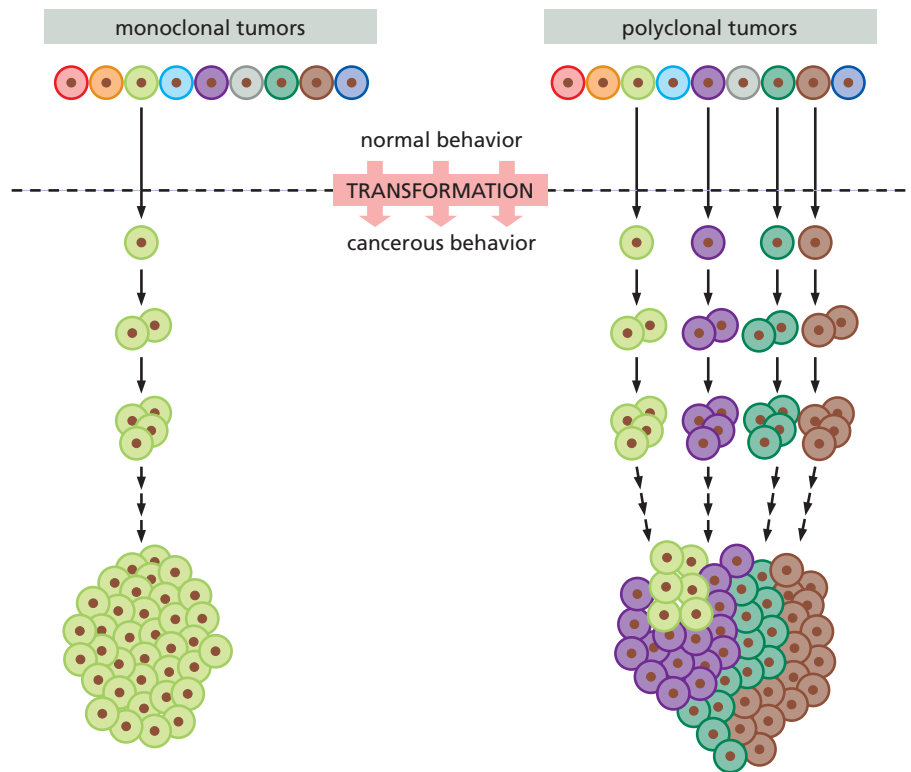
As mentioned above, cells in an initially formed primary tumor may seed new tumor colonies at distant sites in the body through the process of metastasis. This process is itself extraordinarily complex, and it depends upon the ability of cancer cells to invade adjacent tissues, to enter into blood and lymph vessels, to migrate through these vessels to distant anatomical sites, to leave the vessels and invade underlying tissue, and to found a new tumor cell colony at the distant site. These steps are the subject of detailed discussion in Chapter 14.

Because the various growths cataloged here represent increasing degrees of tissue abnormality, it would seem likely that they are distinct stopping points along the road of **tumor progression**, in which a normal tissue evolves progressively into one that is highly malignant. However, the precursor–product relationships of these various growths (*i.e.*, normal → hyperplastic → dysplastic → neoplastic → metastatic) is only suggested by the above descriptions but by no means proven.

## 2.5 Tumors are monoclonal growths

Even if we accept the notion that tumors arise through the progressive alteration of normal cells, another question remains unanswered: how many normal cells are the ancestors of those that congregate to form a tumor (Figure 2.17)? Do the tumor cells descend from a single ancestral cell that crossed over the boundary

**Figure 2.17 Monoclonality versus polyclonality of tumors** In theory, tumors may be polyclonal or monoclonal in origin. In a polyclonal tumor (*right*), multiple cells cross over the border from normalcy to malignancy to become the ancestors of several, genetically distinct subpopulations of cells within a tumor mass. In a monoclonal tumor (*left*), only a single cell is transformed from normal to cancerous behavior to become the ancestor of the cells in a tumor mass.



from normal to abnormal growth? Or did a large cohort of normal cells undergo this change, each becoming the ancestor of a distinct subpopulation of cells within a tumor mass?

The most effective way of addressing this issue is to determine whether all the cells in a tumor mass share a common, highly unique genetic or biochemical marker. For example, a randomly occurring somatic mutation might mark a cell in a very unusual way. If this particular genetic marker were present in all cells within a tumor, this would suggest that they all descend from the initially mutated cell and that they have all inherited the marker from this common progenitor. Such a population of cells, all of which derive from a common ancestral cell, is said to be **monoclonal**. Alternatively, if the tumor mass is composed of a series of genetically distinct subpopulations of cells that give no indication of a common origin, it can be considered to be **polyclonal**.

The first experiments designed to measure the clonality of tumor cell populations actually relied on a naturally occurring, nongenetic (**epigenetic**) marking event. As described earlier (Sidebar 1.2; Figure 1.10), in the somatic cells of early embryos of female placental mammals, one of the two X chromosomes in each cell is selected randomly for silencing. This silencing causes all genes on one X chromosome in a cell to be repressed transcriptionally and is manifested karyotypically through the condensation of the silenced X chromosome into a small particle termed the Barr body. Once the decision to inactivate an X chromosome (of maternal or paternal origin) has occurred in an embryonic cell, all descendant cells in adult tissues appear to respect the decision made by their ancestor in the embryo and thus continue to inactivate the same X chromosome.

The gene for glucose-6-phosphate dehydrogenase (G6PD) is located on the X chromosome, and more than 30% of African American women are heterozygous at this locus. Thus, they carry two alleles specifying forms of this enzyme that can be distinguished either by starch gel electrophoresis or by the fact that one form is more resistant to heat inactivation than the other. Because of X-chromosome silencing, each of the cells in these heterozygous women will

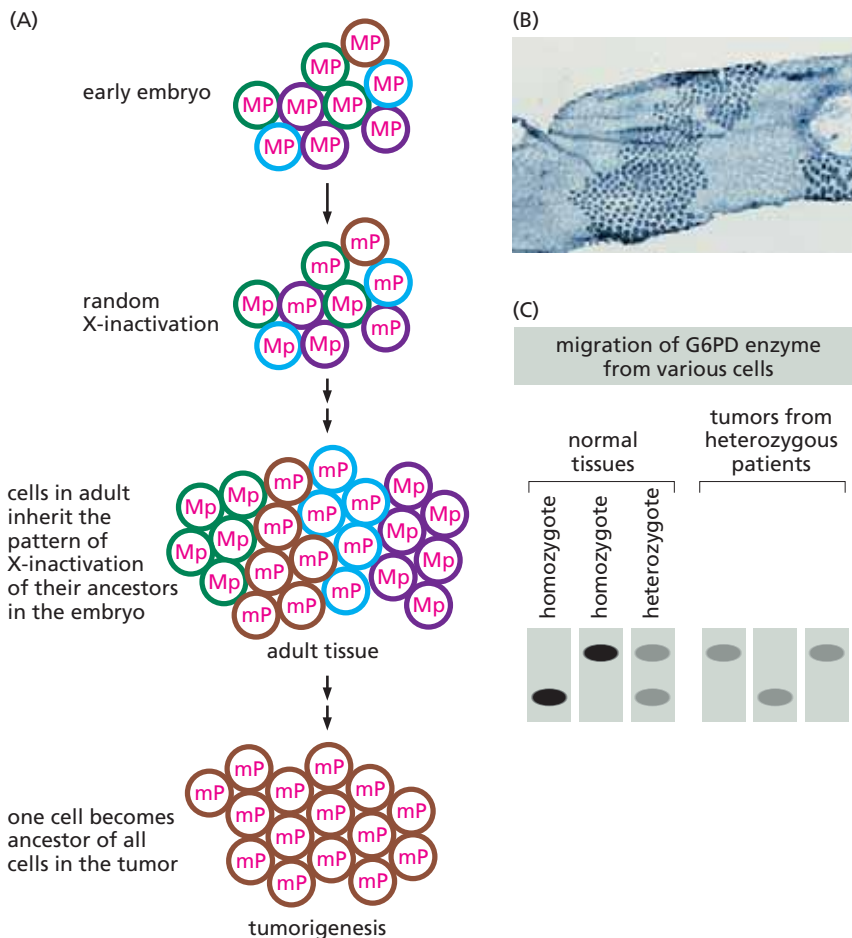
express only one or the other allele of the *G6PD* gene, which is manifested in turn in the variant of the G6PD protein that these cells synthesize (Figure 2.18). In most of their tissues, half of the cells make one variant enzyme, while the other half make the other variant. In 1965, observations were reported on a number of **leiomyomas** (benign tumors of the uterine wall) in African American heterozygotes. Each leiomyoma invariably expressed either one or the other variant form of the G6PD enzyme. This meant that, with great likelihood, its component cells all descended from a single common progenitor that expressed only that particular allele.

This initial demonstration of the monoclonality of human tumors was followed by many other confirmations of this concept. One proof came from observations of **myelomas**, which derive from the B-cell precursors of antibody-producing plasma cells. Normally, the pool of these B-cell precursors consists of hundreds of thousands, likely millions of distinct subpopulations, each expressing its own specific antibody molecules as a consequence of a particular **immunoglobulin** gene rearrangement. In contrast, all the myeloma cells in a patient produce the identical antibody molecule, indicating their descent from a single, common ancestor that was present years earlier in this complex, heterogeneous cell population (Figure 2.19A).

Perhaps the most vivid demonstrations of tumor monoclonality have come from observations of cancer cells that have a variety of chromosomal aberrations that can be visualized microscopically when chromosomes are condensed during the metaphase of mitosis. Often, a very peculiar chromosomal abnormality—the clear result of a rare genetic accident—is seen in all the cancer cells within a tumor mass (Figure 2.19B). This observation makes it obvious that all

**Figure 2.18 X-chromosome inactivation patterns and the monoclonality of tumors**

(A) While the female embryo begins with both X chromosomes in an equally active state, either the X chromosome inherited from the mother (M) or the X chromosome inherited from the father (P) soon undergoes inactivation at random. Such inactivation silences expression of almost all genes residing on that chromosome. In the adult, all of the lineal descendants of a particular embryonic cell continue to inactivate the same X chromosome. Hence, the adult female body is made of patches (clones) of cells of the type Mp and patches of the type mP, where the *lowercase letter* denotes an inactivated state. (B) The two allelic forms of glucose-6-phosphate dehydrogenase (G6PD), which is encoded by a gene on the X chromosome, have differing sensitivities to heat inactivation. Hence, gentle heating of tissue from a heterozygote—in this case a section of intestine—reveals patches of cells that carry the heat-resistant, still-active enzyme variant (*dark blue spots*) among patches that do not. The cells in each patch are the descendants of an embryonic cell that decided early in embryogenesis to inactivate either its maternal or paternal X chromosome. (C) Use of starch gel electrophoresis to resolve the two forms of G6PD showed that all of the cancer cells in a tumor arising in a *G6PD* heterozygous patient express either one or the other form of this enzyme. This indicated that these cells all descended from a common, founding ancestor that already exhibited this particular pattern of X-inactivation. This finding suggested that the cancer cells within a tumor mass constitute a monoclonal growth. (B, from M. Novelli et al., *Proc. Natl. Acad. Sci. USA* 100:3311–3314, 2003; C, adapted from P.J. Fialkow, *N. Engl. J. Med.* 291:26–35, 1974.)

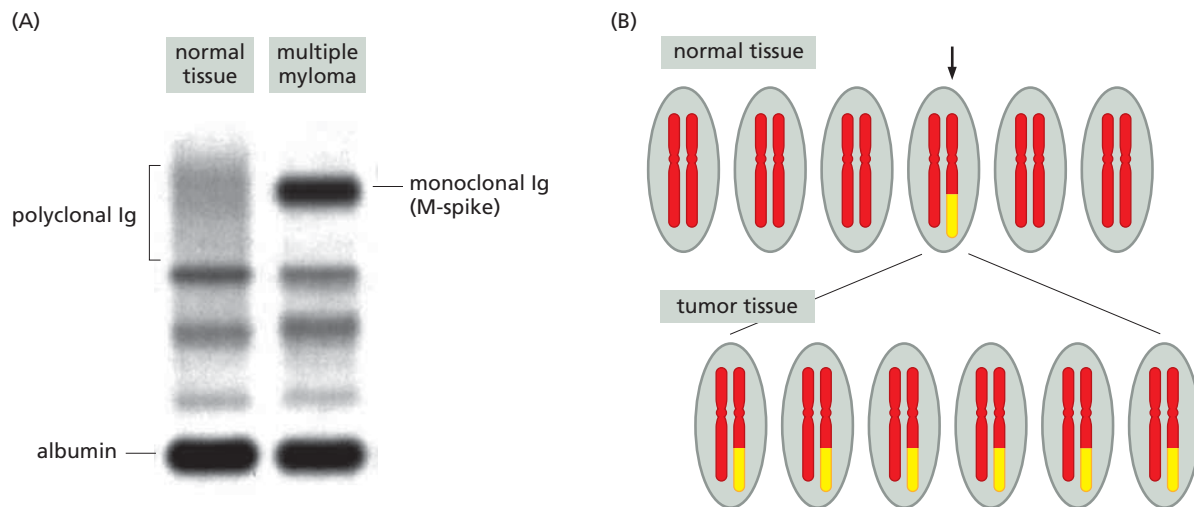


the malignant cells within this tumor descend from the single ancestral cell in which this chromosomal restructuring originally occurred.

While such observations seem to provide compelling proof that tumor populations are monoclonal, tumorigenesis may actually be more complex. Let us imagine, as a counterexample, that ten normal cells in a tissue simultaneously crossed over the border from being normal to being malignant (or at least pre-malignant) and that each of these cells, and its descendants in turn, proliferated uncontrollably (see Figure 2.17). Each of these founding cells would spawn a large monoclonal population, and the tumor mass, as a whole, consisting of a mixture of these ten cell populations, would be polyclonal.

It is highly likely that each of these ten clonal populations varies subtly from the other nine in a number of characteristics, among them the time required for cells in this population to double. Simple mathematics indicates that a cell population that exhibits a slightly shorter doubling time will, sooner or later, outgrow all the others, and that the descendants of these cells will dominate in the tumor mass, creating what will appear to be a monoclonal tumor. In fact, many tumors seem to require decades to develop, which is plenty of time for one clonal subpopulation to dominate in the overall tumor cell population. Hence, the monoclonality of the cells in a large tumor mass hardly proves that this tumor was strictly monoclonal during its early stages of development.

A second confounding factor derives from the genotypic and phenotypic instability of tumor cell populations. As we will discuss in great detail in Chapter 11, the population of cells within a tumor may begin as a relatively homogeneous collection of cells (thus constituting a monoclonal growth), but soon this population may become quite heterogeneous because of the continual acquisition of new mutant alleles by different cells in this population. The resulting genetic



**Figure 2.19 Additional proofs of tumor monoclonality** (A) In normal plasma, the immunoglobulin (Ig) molecules (i.e., antibodies) migrate as a heterogeneous collection of molecules upon gel electrophoresis (*top of left channel*); this heterogeneity is indicative of the participation of a diverse spectrum (a polyclonal population) of plasma cells in antibody production. However, in the disease of multiple myeloma, this heterogeneous population of Ig molecules is replaced by a single antibody species (termed an M-spike) that is produced by a single clonal population of antibody-secreting tumor cells. (B) Illustrated here is an unusual translocation (*arrow*) that involves exchange of segments between two separate

(nonhomologous) chromosomes—a *red* and a *yellow* chromosome; the translocation affects only one of the paired homologous chromosomes. This translocation event, occurring among a population of karyotypically normal cells (*top row*), creates a characteristic “signature” of the particular tumor being studied. (Only one of the two chromosomal products of the translocation is shown here.) Since all of the cancer cells within a given tumor carry the identical, rare translocation (*bottom row*), this indicates their descent from a common progenitor in which this translocation initially occurred. (A, courtesy of S. Chen-Kiang and S. Ely.)

heterogeneity may mask the true monoclonal origin of this cell population, since many of the genetic markers in these descendant cells will be present only in specific subpopulations of cells within the tumor mass.

These caveats complicate our assessment of the monoclonal origins of tumors. Nonetheless, it is a widespread consensus that the vast majority of human tumors are monoclonal growths descended from single progenitor cells that took the first small steps to becoming cancerous.

## 2.6 Cancers occur with vastly different frequencies in different human populations

The nature of cancer suggests that it is a disease of chaos, a breakdown of existing biological order within the body. More specifically, the disorder seen in cancer appears to derive directly from malfunctioning of the controls that are normally responsible for determining when and where cells throughout the body will multiply. In fact, there is ample opportunity for the disorder of cancer to strike a human body. Most of the more than  $10^{13}$  cells in the body continue to carry the genetic information that previously allowed them to come into existence and might, in the future, allow them to multiply once again. This explains why the risk of uncontrolled cell proliferation in countless sites throughout the body is substantial throughout the lives of mammals like ourselves.

To be more accurate, the risk of cancer is far greater than the  $>10^{13}$  population size would suggest, since this number represents the average, steady-state population of cells in the body at any point in time during adulthood. The aggregate number of cells that are formed during an average human lifetime is about  $10^{16}$ , a number that testifies to the enormous amount of cell turnover—involving cell death and replacement (almost  $10^7$  events per second)—that occurs continuously in many tissues in the body. As discussed in Chapters 9 and 12, each time a new cell is formed by the complex process of cell growth and division, there are many ways for things to go awry. Hence, the chance for disaster to strike, including the inadvertent formation of cancer cells, is great.

Since a normal biological process (incessant cell division) is likely to create a substantial risk of cancer, it would seem logical that human populations throughout the world would experience similar frequencies of cancer. However, when cancer **incidence** rates (that is, the rates with which the disease is diagnosed) are examined in various countries, we learn that the risks of many types of cancer vary dramatically (Table 2.5), while other cancers (not indicated in Table 2.5) do indeed show comparable incidence rates across the globe. So, our speculation that all cancers should strike different human populations at comparable rates is simply wrong. Some do and some don't. This realization forces us to reconsider our thinking about how cancers are formed.

Some of the more than 100 types of human cancers do seem to have a high proportion of tumors that are caused by random, unavoidable accidents of nature and thus occur with comparable frequencies in various human populations. This seems to be true for certain pediatric tumors. In addition to this relatively constant “background rate” of some specific cancers, yet other factors appear to intervene in certain populations to increase dramatically the total number of cancer cases. The two obvious contributory factors here are heredity and environment. Different human populations may carry cancer-susceptibility alleles at greatly different frequencies. Alternatively, the environments in which people live may contribute enormously to disease incidence rates. The environment, in the broadest sense, includes both the air and the water that enter into their bodies, as well as aspects of lifestyle, such as dietary choices, reproductive habits, and tobacco usage.

**Table 2.5** Geographic variation in cancer incidence and death rates

Countries showing highest and lowest incidence of specific types of cancer <sup>a</sup>			
Cancer site	Country of highest risk	Country of lowest risk	Relative risk H/L <sup>b</sup>
Skin (melanoma)	Australia (Queensland)	Japan	155
Lip	Canada (Newfoundland)	Japan	151
Nasopharynx	Hong Kong	United Kingdom	100
Prostate	U.S. (African American)	China	70
Liver	China (Shanghai)	Canada (Nova Scotia)	49
Penis	Brazil	Israel (Ashkenazic)	42
Cervix (uterus)	Brazil	Israel (non-Jews)	28
Stomach	Japan	Kuwait	22
Lung	U.S. (Louisiana, African American)	India (Madras)	19
Pancreas	U.S. (Los Angeles, Korean American)	India	11
Ovary	New Zealand (Polynesian)	Kuwait	8

Geographic areas showing highest and lowest death rates from specific types of cancer <sup>c</sup>			
Cancer site	Area of highest risk	Area of lowest risk	Relative risk H/L <sup>b</sup>
Lung, male	Eastern Europe	West Africa	33
Esophagus	Southern Africa	West Africa	16
Colon, male	Australia, New Zealand	Middle Africa	15
Breast, female	Northern Europe	China	6

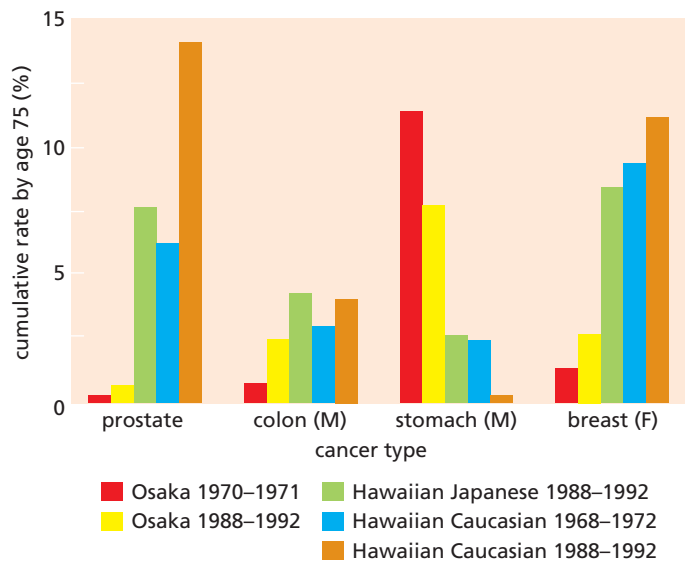
<sup>a</sup>See C. Muir, J. Waterhouse, T. Mack et al., eds., *Cancer Incidence in Five Continents*, vol. 5, Lyon: International Agency for Research on Cancer, 1987; excerpted by V.T. DeVita, S. Hellman, and S.A. Rosenberg, *Cancer: Principles and Practice of Oncology*, Philadelphia: Lippincott, 1993.

<sup>b</sup>Relative risk: age-adjusted incidence or death rate in highest country or area (H) divided by age-adjusted incidence or death rate in lowest country or area (L). These numbers refer to age-adjusted rates, e.g., the relative risk of a 60-year-old dying from a specific type of tumor in one country compared with a 60-year-old in another country.

<sup>c</sup>See P. Pisani, D.M. Parkin, F. Bray and J. Ferlay, *Int. J. Cancer* 83:18–29, 1999. This survey divided the human population into 23 geographic areas and surveyed the relative mortality rates of various cancer types in each area.

Which of these two alternatives—heredity or environment—is the dominant determinant of the country-to-country variability of cancer incidence? While many types of disease-causing alleles are distributed unequally in the gene pools of different human populations, these alleles do not seem to explain the dramatically different incidence rates of various cancers throughout the world. This point is demonstrated most dramatically by measuring cancer rates in migrant populations. For example, Japanese experience rates of stomach cancer that are 6 to 8 times higher than those of Americans (Figure 2.20). However, when Japanese settle in the United States, within a generation their offspring exhibit a stomach cancer rate that is comparable to that of the surrounding population. For the great majority of cancers, disease risk therefore seems to be “environmental,” where this term is understood to include both physical environment and lifestyle.

As indicated in Table 2.5, the incidence of some types of cancer may vary enormously from one population to the next. Thus, breast cancer in China is about one-sixth as common as in the United States or Northern Europe. Having excluded genetic contributions to this difference, we might then conclude that as much as 85% of the breast cancers in the United States might in theory be avoidable, if only American women were to experience an environment and lifestyle comparable to those of their Chinese counterparts. Even within the American population, there are vast differences in cancer mortality: the



**Figure 2.20 Country-to-country comparisons of cancer incidence** Public health records reveal dramatic differences in the incidence of certain cancers in different countries. Here, the relative incidence of a group of cancers in Japan and in the American island of Hawaii are presented. Invariably, after Japanese have immigrated to Hawaii, within a generation their cancer rates approach those of the population that settled there before them. This indicates that the differing cancer rates are not due to genetic differences between the Japanese and the American populations. (From J. Peto, *Nature* 411:390–395, 2001.)

Seventh-Day Adventists, whose religion discourages smoking, heavy drinking, and the consumption of meat, die from cancer at a rate that is only about half that of the general population.

For those who wish to understand the **etiologic** (causative) mechanisms of cancer, these findings lead to an inescapable conclusion: the great majority of the commonly occurring cancers are caused by factors or agents that are external to the body, enter into the body, and somehow attack and corrupt its tissues. In a minority of cancers, substantial variations in cancer risk may be attributable to differences in reproductive behavior and the resulting dramatic effects on the hormonal environment within the human female body.

Let us imagine, for the sake of argument, that avoidance of certain obvious cancer-causing factors in diet and lifestyle resulted in a 50% reduction in the risk of dying from cancer in the West, leaving the disease of cancer as the cause of about 10% of overall mortality in this population. Under these conditions, given the approximately  $10^{16}$  mitoses occurring in each human body during a normal life span, we calculate that only 1 in  $10^{17}$  cell divisions would lead directly or indirectly to a clinically detectable cancer. Now, we become persuaded that in spite of the enormous intrinsic risk of developing cancer, the body must be able to mount highly effective defenses that usually succeed in holding off the disease for the 70 or 80 years that most of us spend on this planet. These defenses are the subject of many discussions throughout this book.

## 2.7 The risks of cancers often seem to be increased by assignable influences including lifestyle

Evidence that certain kinds of cancers are associated with specific exposures or lifestyles is actually quite old, predating modern epidemiology by more than a century. The first known report comes from the observations of the English physician John Hill, who in 1761 noted the connection between the development of nasal cancer and the excessive use of tobacco snuff. Fourteen years later, Percivall Pott, a surgeon in London, reported that he had encountered a substantial number of skin cancers of the scrotum in adolescent men who, in their youth, had worked as chimney sweeps. Within three years, the Danish sweepers guild urged its members to take daily baths to remove the apparently cancer-causing material from their skin. This practice was likely to be the cause of the markedly lower rate of scrotal cancer in continental Europe when compared with Britain even a century later.

Beginning in the mid-sixteenth century, silver was extracted in large quantities from the mines in St. Joachimsthal in Bohemia, today Jáchymov in the Czech Republic. (The silver Joachimsthaler coins that were soon in wide circulation came to be called “thaler,” which eventually yielded the word “dollar”!) By the first half of the nineteenth century, lung cancer was documented at high rates in the miners, a disease that was otherwise almost unheard of at the time. Once again, an occupational exposure had been correlated with a specific type of cancer.

In 1839, an Italian physician reported that breast cancer was a scourge in the nunneries, being present at rates that were six times higher than among women in the general population who had given birth multiple times. By the end of the nineteenth century, it was clear that occupational exposure and lifestyle were closely connected to and apparently causes of a number of types of cancer.

The range of agents that might trigger cancer was expanded with the discovery in the first decade of the twentieth century that physicians and others who experimented with the then-recently invented X-ray tubes experienced increased rates of cancer, often developing tumors that arose at the site of irradiation. These observations led, many years later, to an understanding of the lung cancer in the St. Joachimsthaler miners: their greatly increased lung cancer incidence could be attributed to the high levels of radioactivity in the ores coming from these mines.

Perhaps the most compelling association between environmental exposure and cancer incidence was forged in 1949 and 1950 when two groups of epidemiologists reported that individuals who were heavy cigarette smokers ran a lifetime risk of lung cancer that was more than twentyfold higher than that of nonsmokers. The initial results of one of these landmark studies are given in Table 2.6. These various epidemiologic correlations proved to be critical for subsequent cancer research, since they suggested that cancers often had specific, assignable causes, and that a chain of causality might one day be traced between these ultimate causes and the cancerous changes observed in certain human tissues. Indeed, in the half century that followed the 1949–1950 reports, epidemiologists identified a variety of environmental and lifestyle factors that were strongly correlated with the incidence of certain cancers (Table 2.7); in some of these cases, researchers have been able to discover the specific biological mechanisms through which these factors act to cause increased incidence of some of these cancers.

## 2.8 Specific chemical agents can induce cancer

Coal tar condensates, much like those implicated in cancer causation by Percivall Pott’s work, were used in Japan at the beginning of the twentieth century to induce skin cancers in rabbits. Repeated painting of localized areas of the skin of their ears resulted, after many months, in the outgrowth of carcinomas.

**Table 2.6** Relative risk of lung cancer as a function of the number of cigarettes smoked per day<sup>a</sup>

Most recent number of cigarettes smoked (by subjects) per day before onset of disease	Lifelong nonsmoker		Smokers		
	—	≥1, <5	≥5, <15	≥15, <25	≥25
Relative risk	1	8	12	14	27

<sup>a</sup>The relative risk indicates the risk of contracting lung cancer compared with that of a nonsmoker, which is set at 1. (From R. Doll and A.B. Hill, *BMJ* 2:739–748, 1950.)

**Table 2.7** Known or suspected causes of human cancers

Environmental and lifestyle factors known or suspected to be etiologic for human cancers in the United States <sup>a</sup>	
Type	% of total cases <sup>b</sup>
Cancers due to occupational exposures	1–2
Lifestyle cancers	
Tobacco-related (sites: e.g., lung, bladder, kidney)	34
Diet (low in vegetables, high in nitrates, salt) (sites: e.g., stomach, esophagus)	5
Diet (high fat, lower fiber, broiled/fried foods) (sites: e.g., bowel, pancreas, prostate, breast)	37
Tobacco and alcohol (sites: mouth, throat)	2

Specific carcinogenic agents implicated in the causation of certain cancers <sup>c</sup>	
Cancer	Exposure
Scrotal carcinomas	chimney smoke condensates
Liver angiosarcoma	vinyl chloride
Acute leukemias	benzene
Nasal adenocarcinoma	hardwood dust
Osteosarcoma	radium
Skin carcinoma	arsenic
Mesothelioma	asbestos
Vaginal carcinoma	diethylstilbestrol
Oral carcinoma	snuff

<sup>a</sup>Adapted from Cancer Facts and Figures, American Cancer Society, 1990.

<sup>b</sup>A large number of cancers are thought to be provoked by a diet high in calories acting in combination with many of these lifestyle factors.

<sup>c</sup>Adapted from S. Wilson, L. Jones, C. Coussens and K. Hanna, eds., Cancer and the Environment: Gene-Environment Interaction, Washington, DC: National Academy Press, 2002.

This work, first reported by Katsusaburo Yamagiwa in 1915, was little noticed in the international scientific community of the time (Figure 2.21). In retrospect, it represented a stunning advance, because it directly implicated chemicals (those in coal tar) in cancer causation. Equally important, Yamagiwa's work, together with that of Peyton Rous (to be described in Chapter 3), demonstrated that cancer could be induced at will in laboratory animals. Before these breakthroughs, researchers had been forced to wait for tumors to appear spon-



**Figure 2.21** The first induction of tumors by chemical carcinogens

(A) In 1915, Katsusaburo Yamagiwa reported the first successful induction of cancer by repeated treatment of rabbit ears with a chemical carcinogen, in this case coal tars. (B) The skin carcinomas (arrows) that he induced on the ears of these rabbits are preserved to this day in the medical museum of the University of Tokyo. This particular carcinoma was harvested and fixed following 660 days of painting with coal tar. (Courtesy of T. Taniguchi.)

taneously in wild or domesticated animals. Now, cancers could be produced according to a predictable schedule, often involving many months of experimental treatment of animals.

By 1940, British chemists had purified several of the components of coal tar that were particularly **carcinogenic** (i.e., cancer-causing), as demonstrated by the ability of these compounds to induce cancers on the skin of laboratory mice. Compounds such as 3-methylcholanthrene, benzo[*a*]pyrene, and 1,2,4,5-dibenz[*a,h*]anthracene were common products of combustion, and some of these hydrocarbons, notably benzo[*a*]pyrene, were subsequently found in the condensates of cigarette smoke as well (Figure 2.22). These findings suggested that certain chemical species that entered into the human body could perturb tissues and cells and ultimately provoke the emergence of a tumor. The same could be said of X-rays, which were also able to produce cancers, ostensibly through a quite different mechanism of action.

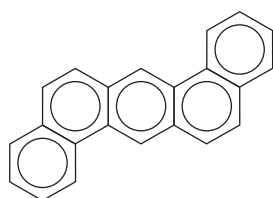
While these discoveries were being reported, an independent line of research developed that portrayed cancer as an infectious disease. As described in detail in Chapter 3, researchers in the first decade of the twentieth century found that viruses could cause leukemias and sarcomas in infected chickens. By mid-century, a wide variety of viruses had been found able to induce cancer in rabbits, chickens, mice, and rats. As a consequence, those intent on uncovering the origins of human cancer were pulled in three different directions, since the evidence of cancer causation by chemical, viral, and physical (i.e., radiation) agents had become compelling.

### Figure 2.22 Structures of carcinogenic hydrocarbons

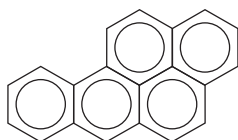
These chemical species arise from the incomplete combustion of organic (i.e., carbon-containing) compounds. Each of the chemical structures shown here, which were already determined before 1940, represents a chemical species that was found, following purification, to be potentially carcinogenic. The four compounds shown in the top row are all polycyclic aromatic hydrocarbons (PAHs). (From E.C. Miller, *Cancer Res.* 38:1479–1496, 1978.)

## 2.9 Both physical and chemical carcinogens act as mutagens

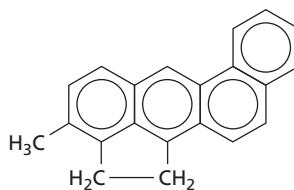
The confusion caused by the three competing theories of carcinogenesis was reduced significantly by discoveries made in the field of fruit fly genetics. In 1927, Hermann Muller discovered that he could induce mutations in the genome of *Drosophila melanogaster* by exposing these flies to X-rays. Most important, this discovery revealed that the genome of an animal was mutable, that is, that its information content could be changed through specific treatments, notably irradiation. At the same time, it suggested at least one mechanism by which X-rays could induce cancer: perhaps radiation was able to mutate the genes of normal cells, thereby creating mutant cells that grew in a malignant fashion.



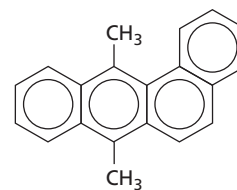
dibenz[*a,h*]anthracene



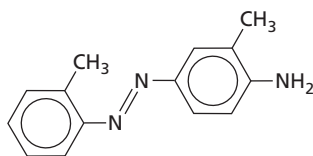
benzo[*a*]pyrene



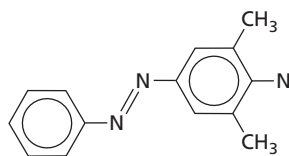
3-methylcholanthrene



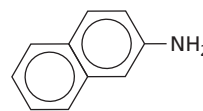
7,12-dimethylbenz[*a*]anthracene



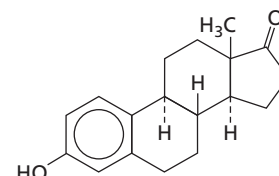
2',3-dimethyl-4-aminoazobenzene



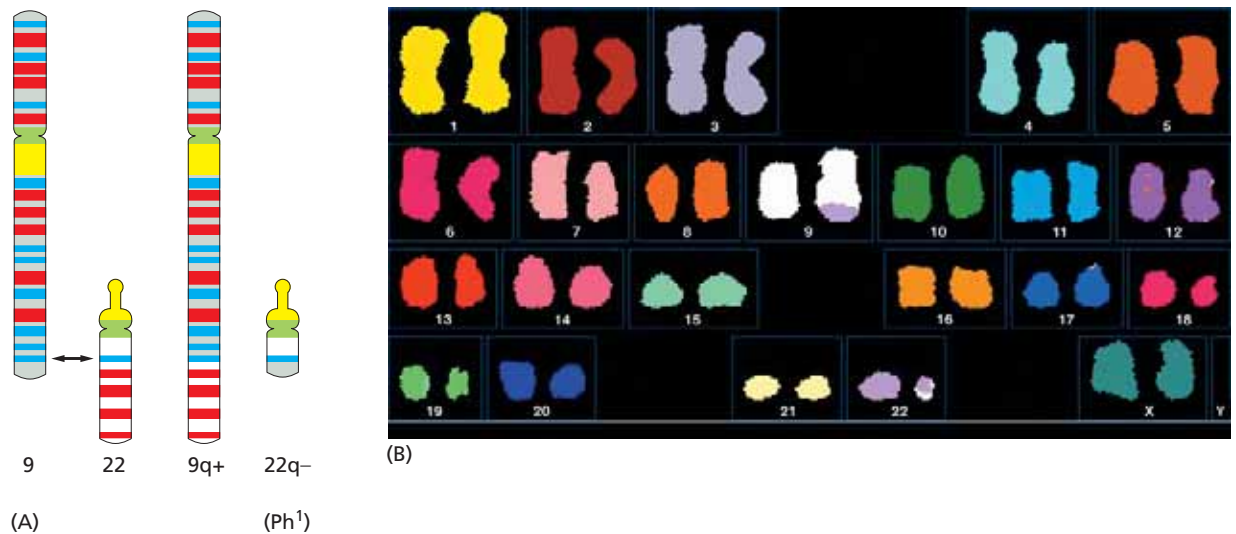
*N,N*-dimethyl-4-aminoazobenzene



2-naphthylamine



estrone



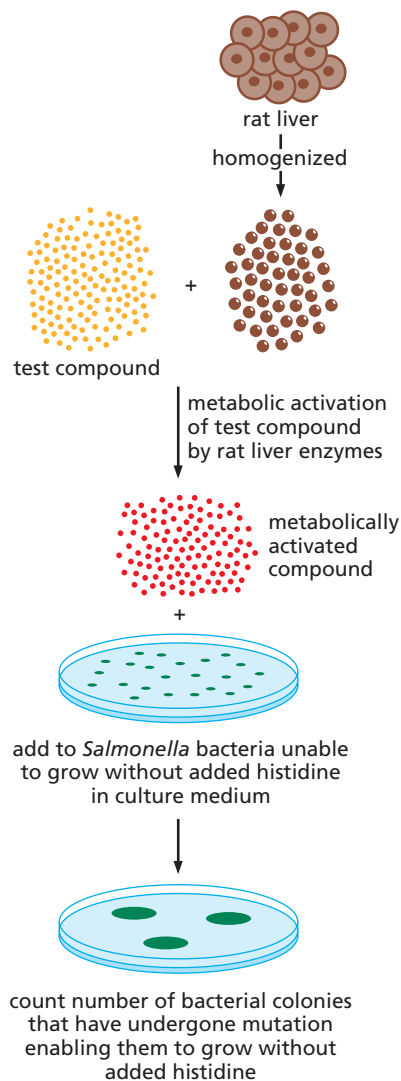
**Figure 2.23 Structure of the Philadelphia chromosome** Analyses of the banding patterns of metaphase chromosomes of chronic myelogenous leukemia (CML) cells first revealed the characteristic tiny chromosome (called the “Philadelphia chromosome” or Ph<sup>1</sup>) that is present in the great majority of CML cells. (A) This banding pattern, determined through light-microscopic surveys of stained metaphase chromosomes, is illustrated here schematically. While the chromosomal translocation generating the two altered chromosomes (9q+,22q-) is *reciprocal* (i.e., involving a loss and a gain by each of the two chromosomes), the sizes of the exchanged chromosomal arms are unequal, leading to the greatly truncated Chromosome 22 (i.e., 22q-). The arrow indicates the point of crossing over, known as the translocation *breakpoint*. (B) The relatively minor change to the tumor cell karyotype that is created by the CML translocation is apparent in this SKY analysis, in which chromosome-specific probes are used, together with fluorescent dyes and computer-generated coloring, to visualize the entire chromosomal complement of CML cells. As is apparent, one of the two Chromosomes 9 has acquired a *light purple* segment (a color assigned to Chromosome 22) at the end of its long arm. Reciprocally, one of the two Chromosomes 22 has acquired a white region (characteristic of Chromosome 9) at the end of its long arm. (A, from B. Alberts et al., *Molecular Biology of the Cell*, 4th ed. New York: Garland Science, 2002; B, courtesy of Thomas Ried and Nicole McNeil.)

By the late 1940s, a series of chemicals, many of them alkylating agents of the type that had been used in World War I mustard gas warfare, were also found to be **mutagenic** for fruit flies. Soon thereafter, some of these same compounds were shown to be carcinogenic in laboratory animals. These findings caused several geneticists to speculate that cancer was a disease of mutant genes, and that carcinogenic agents, such as X-rays and certain chemicals, succeeded in inducing cancer through their ability to mutate genes.

These speculations were hardly the first ones of this sort. As early as 1914, the German biologist Theodor Boveri, drawing on yet older observations of others, suggested that chromosomes, which by then had been implicated as carriers of genetic information, were aberrant within cancer cells, and that cancer cells might therefore be mutants. Boveri’s notion, along with many other speculations on the origin of cancer, gained few adherents, however, until the discovery in 1960 of an abnormally configured chromosome in a large proportion of cases of chronic myelogenous leukemia (CML). This chromosome, soon called the Philadelphia chromosome after the place of its discovery, was clearly a distinctive characteristic of this type of cancer (Figure 2.23). Its reproducible association with this class of tumor cells suggested, but hardly proved, that it played a causal role in tumorigenesis.

In 1975 Bruce Ames, a bacterial geneticist working at the University of California in Berkeley, reported experimental results that lent great weight to the theory that carcinogens can function as mutagens. Decades of experiments with laboratory mice and rats had demonstrated that chemical carcinogens acted with vastly different potencies, differing by as much as 1 million-fold in their ability to induce cancers. Such experiments showed, for example, that one microgram of aflatoxin, a compound produced by molds growing on peanuts and wheat, was as potentially carcinogenic as was a 10,000 times greater weight of the synthetic compound benzidine. Ames posed the question whether these various compounds were also mutagenic, more specifically, whether compounds that were potent carcinogens also happened to be potent mutagens.

The difficulty that Ames faced in his initial attempts to address this question was a simple one: there were no good ways of measuring the relative mutagenic potencies of various chemical species. So Ames set out to devise his own method for quantifying mutagenic potency. He developed an experimental protocol that consisted of applying various carcinogenic chemicals to a population of *Salmonella* bacteria growing in Petri dishes and then scoring for the abilities of these carcinogens to mutate the bacteria. The readout here was the number of colonies of *Salmonella* that grew out following exposure to one or another chemical.



**Figure 2.24 The Ames test for gauging mutagenicity** The Ames test makes it possible to quantitatively assess the mutagenic potency of a test compound. To begin, the liver of a rat (or other species) is homogenized, releasing the enzymes that can metabolically activate a chemical to its mutagenic form. The rat liver homogenate (*brown dots*) is then mixed with the test compound (*orange*), which often results in the conversion of the test compound to a chemically activated state (*red*). This mixture (still containing the liver homogenate, *not shown*) is applied to a dish of mutant *Salmonella* bacteria (*small green dots*) that require the amino acid histidine in their culture medium in order to grow. Since histidine is left out of the medium, only those bacteria that are mutated to a histidine-independent genotype (and phenotype) will be able to grow, and each of these will yield a large colony (*green*) that can be counted with the naked eye, indicating how many mutant bacteria (and thus mutant alleles) were generated by the brief exposure to the activated compound.

In detail, Ames used a strain of *Salmonella* that was already mutant and therefore unable to grow in medium lacking the amino acid histidine. The mutant allele that caused this phenotype was susceptible to back-mutation to a wild-type allele. Once the wild-type allele was formed in response to exposure to a mutagen, a bacterium carrying this allele became capable of growing in Ames's selective medium, multiplying until it formed a colony that could be scored by eye (Figure 2.24).

In principle, Ames needed only to introduce a test compound into a Petri dish containing his special *Salmonella* strain. By counting the number of bacterial colonies that appeared later on, he could gauge the mutagenic potency of this compound. But there remained one substantial obstacle to the success of this mutagenesis assay. Detailed studies of a number of chemical carcinogens had shown that after carcinogenic molecules entered into the tissues of laboratory animals, they were metabolized into yet other chemical species. In many cases, the resulting products of metabolism, rather than the initially introduced chemicals, seemed to be the agents that were directly responsible for the observed cancer induction. These metabolized compounds were found to be highly reactive chemically and able to form covalent bonds with the various macromolecules known to be present in cells—DNA, RNA, and protein.

The original, unmodified compounds that were introduced into laboratory animals came to be called **procarcinogens** to indicate their ability to become converted into actively carcinogenic compounds, which were labeled **ultimate carcinogens**. This chemical conversion complicated the design of Ames's mutagenesis assay. If many compounds required metabolic activation before their carcinogenicity was apparent, it seemed plausible that their mutagenic powers would also be evident only after such metabolic conversion. Given the radically different metabolisms of bacteria and mammalian cells, it was highly unlikely that Ames's *Salmonella* bacteria would be able to accomplish the metabolic activation of procarcinogens that occurred in the tissues of laboratory animals.

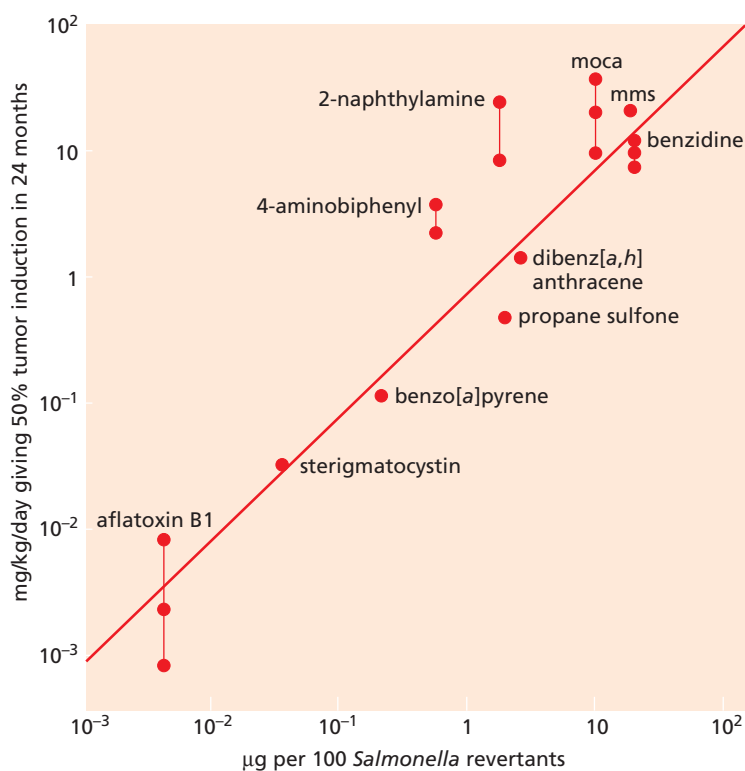
This information forced Ames to add an extra step to his mutagenesis assay, a step suggested by earlier work of others. It was known that a great many chemicals introduced into the body undergo metabolic conversion in the liver. Many of these conversions could be achieved in the test tube simply by mixing such chemicals with homogenized liver. So Ames mixed rat liver homogenates with his test compounds and then introduced this mixture into the Petri dishes carrying *Salmonella*. (We now know that the metabolic activation of procarcinogens in the liver is often mediated by enzymes that are normally involved, paradoxically, in the **detoxification** of compounds introduced into the body; see Section 12.6).

When Ames added liver homogenate to his *Salmonella* cultures, his assay of mutagenic potency worked beautifully. It revealed that a number of known car-

cinogens were also actively mutagenic. Even more important were the correlations that Ames found. Chemicals that were potently mutagenic were also powerful carcinogens. Those that were weakly mutagenic induced cancer poorly. These correlations, as plotted by others, extended over five orders of magnitude of potency (Figure 2.25).

As we have read, the notion that carcinogens are mutagens predated Ames's work by a quarter of a century. Nonetheless, the results of his analyses galvanized researchers interested in the origins of cancer, since they addressed the carcinogen–mutagen relationship so directly. Their reasoning went like this: Ames had demonstrated the mutagenic powers of certain chemical compounds in bacteria. Since the genomes of bacterial and animal cells are both made of the same chemical substance—double-stranded DNA—it was likely that the compounds that induced mutations in the *Salmonella* genome were similarly capable of inducing mutations in the genomes of animal cells. Hence, the “Ames test,” as it came to be known, should be able to predict the mutagenicity of these compounds after they had been introduced into the body of a mammal. And in light of the correlation between mutagenic and carcinogenic potency, the Ames test could be employed to screen various substances for their carcinogenic powers, and thus for their threat to human health. By 1976, Ames and his group reported on the mutagenic potencies of 300 distinct organic compounds. Yet other tests for mutagenic potency were developed in the years that followed (Sidebar 2.1).

Ames's results led to the next deduction, really more of a speculation: if, as Ames argued, carcinogens are mutagens, then it followed that the carcinogenic powers of various agents derived directly from their ability to induce mutations in the cells of target tissues. As a further deduction, it seemed inescapable that the cancer cells created by chemical carcinogens carry mutated genes. These mutated genes, whatever their identity, must in some way be responsible for the aberrant growth phenotypes of such cancer cells.



**Figure 2.25 Mutagenic versus carcinogenic potency** On this log–log plot, the relative carcinogenic potencies of a group of chemicals (*ordinate*) that have been used to treat laboratory animals (rats and mice) are plotted as a function of their mutagenic potencies (*abscissa*) as gauged by the Ames test (see Figure 2.24). Since both the ordinate and abscissa are plotted as the amount of compound required to elicit an observable effect (yielding tumors in 50% of treated animals or 100 colonies of mutant *Salmonella* bacteria, termed here “revertants”), the compounds that are the most potent mutagens and most potent carcinogens appear in the lower left of this graph. Note that both parameters vary by five orders of magnitude. (Adapted from M. Meselson et al., in H.H. Hiatt et al., eds., *Origins of Human Cancer*, Book C: Human Risk Assessment. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1977.)

**Sidebar 2.1 Other tests for mutagenicity help assess possible carcinogenicity** The Ames test is only one of a number of biological assay systems that can be used to assess the mutagenic potency of suspected carcinogenic chemicals. Many of these other assays depend upon exposing mammalian cells directly to the chemical compounds being tested and the subsequent use of a diverse array of biological readouts. For example, a test for sister chromatid exchange (SCE) depends on the ability to measure cross-over between the two paired chromatids that together constitute a chromosome in the late (i.e., G<sub>2</sub>) phase of a cell's growth-and-division cycle. Many mutagenic agents have been shown to provoke this SCE. Mutagenic agents may also register as being capable of inducing the formation of fragmented cell nuclei, that is, **micronuclei**. Use of genetics has made it possible to test mammalian cells for their mutation-induced loss of thymidine kinase enzyme or the HGPRT enzyme (hypoxanthine guanine phosphoribo-

syl transferase). The ability to examine under the light microscope the chromosomal array (i.e., the karyotype) of cells in the metaphase of mitosis makes it possible to screen for chromosomal aberrations inflicted by test compounds. Yet another assay gauges the degree of DNA labeling in those cells that are in the G<sub>1</sub> or G<sub>2</sub> phase of the cell cycle (described in Chapter 8); such non-S-phase labeling, sometimes referred to as “unscheduled DNA synthesis,” has also been shown to be a good indicator of the genomic damage that has been inflicted on a cultured cell, since this type of DNA synthesis represents one key step in the process used by cells to repair damaged DNA.

None of these tests has proven to be ideal as a predictor of the carcinogenicity of a test substance. The Ames test, as an example, has been found by some to have a sensitivity (% of established carcinogens identified as mutagens) of about 54% and a specificity (% of noncarcinogens identified as nonmutagens) of 70%.

This logic was transferable to X-ray carcinogenesis as well. Since X-rays were mutagens and carcinogens, it followed that they also induced cancer through their ability to mutate genes. This convergence of cancer research with genetics had a profound effect on researchers intent on puzzling out the origins of cancer. Though still unproven, it appeared likely that the disease of cancer could be understood in terms of the mutant genes carried by cancer cells.

### 2.10 Mutagens may be responsible for some human cancers

The connection between carcinogenesis and mutagenesis seemed to shed light on how human tumors arise. Perhaps many of these neoplasms were the direct consequence of the mutagenic actions of chemical and physical carcinogens. The mutagenic chemicals, specifically, procarcinogens, need not derive exclusively from the combustion of carbon compounds and the resulting formation of coal tars. It seemed plausible that chemical species present naturally in foodstuffs or generated during cooking could also induce cancer. Even if many foods did not contain ultimate carcinogens, chemical conversions carried out by liver cells or by the abundant bacteria in the colon might well succeed in creating actively mutagenic and thus carcinogenic chemical species.

As this research on the causes of human cancer proceeded, it became apparent that virtually all compounds that are mutagenic in human cells are likely to be carcinogenic as well. However, the converse does not seem to hold: chemical compounds that are carcinogenic are not necessarily mutagenic (see Sidebar 2.2).

By 1991, Ames and others had used his test to catalog the mutagenic powers of a diverse group of chemicals and natural foodstuffs, including many of the plants that are common and abundant in the Western diet. As Ames argued, the presence of such compounds in foodstuffs derived from plants was hardly surprising, since plants have evolved thousands, possibly millions of distinct toxic chemical compounds in order to defend themselves from predation by insects and larger animals. Some of these naturally toxic compounds, initially developed as anti-predator defenses, might also, as an unintended side-effect, be mutagenic (Table 2.8).

**Sidebar 2.2 Not all carcinogens are mutagenic** By the early 1990s, it became apparent that the carcinogen–mutagen equivalence no longer held. By then, a more extensive use of the Ames test showed that as many as 40% of the compounds that were known to be carcinogenic in rodents showed no obvious mutagenicity in the *Salmonella* mutation assay. So the conclusions drawn earlier from the initial applications of Ames's test required major revision: some carcinogens act through their ability to mutate DNA, while others promote the appearance of tumors through nongenetic mechanisms. We will encounter these nonmutagenic carcinogens, often called tumor **promoters**, again in Chapter 11.

A diverse set of discoveries led to the model, which remains unproven in many of its aspects to this day, that a significant proportion of human cancer is attributable directly to the consumption of foodstuffs that are mutagenic and hence carcinogenic. Included among these foodstuffs is, for example, red meat, which upon cooking at high temperatures generates compounds such as heterocyclic amines, which are potently mutagenic (see Section 12.6).

The difficulties in proving this model derive from several sources. Each of the plant and animal foodstuffs in our diet is composed of thousands of diverse chemical species present in vastly differing concentrations. Almost all of these compounds undergo metabolic conversions once they are inside our bodies, first in the gastrointestinal tract and often thereafter in the liver. Accordingly, the number of distinct chemical species that are introduced into our bodies is incalculable. Each of these introduced compounds, once it is present in the body, may then be concentrated in some cells or quickly metabolized and excreted. This creates a further dimension of complexity.

Moreover, the actual mutagenicity of various compounds in different cell types may vary enormously because of metabolic differences in these cells. For example, some cells, such as **hepatocytes** in the liver, express high levels of biochemical species designed to scavenge and inactivate mutagenic compounds, while others, such as fibroblasts, express far lower levels. In sum, the ability to relate the mutagenicity of foodstuffs to actual rates of mutagenesis and carcinogenesis in the human body is far beyond our reach at present—a problem of intractable complexity (see also Sidebar 2.3).

**Table 2.8** A sampling of Bruce Ames's roster of carcinogens identified in the normal diet<sup>a</sup>

Foodstuff	Compound	Concentration in foodstuff
Black pepper	piperine	100 mg/g
Common mushroom	agaritine	3 mg/g
Celery <sup>b</sup>	furocoumarins, psoralens	1 µg/g, 0.8 µg/g
Rhubarb	anthraquinones	varies
Cocoa powder	theobromine	20 mg/g
Mustard, horseradish	allyl isothiocyanate	varies
Alfalfa sprouts	canavanine <sup>c</sup>	15 mg/g
Burnt materials <sup>d</sup>	large number	varies
Coffee	caffeic acid	11.6 mg/g

<sup>a</sup>Ames has cited 37 naturally occurring compounds that have registered as carcinogens in laboratory animals; one or more have been found in each of the following foodstuffs: absinthe, allspice, anise, apple, apricot, banana, basil, beet, broccoli, Brussels sprouts, cabbage, cantaloupe, caraway, cardamom, carrot, cauliflower, celery, cherries, chili pepper, chocolate, cinnamon, cloves, coffee, collard greens, comfrey herb tea, coriander, corn, currants, dill, eggplant, endive, fennel, garlic, grapefruit, grapes, guava, honey, honeydew melon, horseradish, kale, lemon, lentils, lettuce, licorice, lime, mace, mango, marjoram, mint, mushrooms, mustard, nutmeg, onion, orange, paprika, parsley, parsnip, peach, pear, peas, black pepper, pineapple, plum, potato, radish, raspberries, rhubarb, rosemary, rutabaga, sage, savory, sesame seeds, soybean, star anise, tarragon, tea, thyme, tomato, turmeric, and turnip

<sup>b</sup>The levels of these can increase 100-fold in diseased plants.

<sup>c</sup>Canavanine is indirectly genotoxic because of oxygen radicals that are released, perhaps during the inflammatory reactions associated with elimination of canavanine-containing proteins.

<sup>d</sup>On average, several grams of burnt material are consumed daily in the form of bread crusts, burnt toast, and burnt surfaces of meats cooked at high temperature.

Adapted from B.N. Ames, Dietary carcinogens and anticarcinogens, *Science* 231:1256–1264, 1983; B.N. Ames and L.S. Gold, Dietary pesticides (99.99% all natural), *Proc. Natl. Acad. Sci. USA* 87:7777–7781, 1990; and S.W. Gold, B.N. Ames and T.H. Slone, Misconceptions about the causes of cancer, in D. Paustenbach, ed., *Human and Environmental Risk Assessment: Theory and Practice*, New York: John Wiley & Sons, 2002, pp. 1415–1460.

**Sidebar 2.3 The search for elusive human carcinogens** Ideally, the identification of important human carcinogens should have been aided by the use of *in vitro* assays, such as the Ames test (Section 2.9), and *in vivo* tests—exposure of laboratory animals to agents suspected of causing cancer (Section 2.8). In truth, however, these various types of laboratory tests have failed to register important human carcinogens. Instead, we have learned

about their carcinogenicity because of various epidemiologic studies. For example, the most important known human carcinogen—tobacco smoke—would likely have escaped detection because it is a relatively weak carcinogen in laboratory rodents; and another known human carcinogen—*asbestos*—would have eluded detection by both *in vitro* and *in vivo* laboratory tests. Conversely, some frequently used drugs, such as pheno-

barbital and isoniazid, register positively in the Ames test, and saccharin registers as a carcinogen in male laboratory rats (see Sidebar 11.19), but epidemiologic evidence indicates conclusively that none of these is actually associated with increased cancer risk in humans who have been exposed to these compounds over long periods of time. Hence, the development of truly useful, predictive tests of human carcinogens still lies in the future.

### 2.11 Synopsis and prospects

The descriptions of cancer and cancer cells developed during the second half of the nineteenth century and the first half of the twentieth indicated that tumors were nothing more than normal cell populations that had run amok. Moreover, many tumors seemed to be composed largely of the descendants of a single cell that had crossed over the border from normalcy to malignancy and proceeded to spawn the billions of descendant cells constituting these neoplastic masses. This scenario drew attention to the nature of the cells that founded tumors and to the mechanisms that led to their transformation into cancer cells. It seemed that each tumor mass was composed largely of replicas of a founding, transformed cell. If one could understand why this cell multiplied uncontrollably, somehow other pieces of the cancer puzzle were likely to fall into place.

Still, existing observations and experimental techniques offered little prospect of revealing precisely why a cell altered its behavior, transforming itself from a normal into a malignant cell. The carcinogen = mutagen theory seemed to offer some clarification, since it implicated mutant cellular genes as the agents responsible for disease development and, therefore, for the aberrant behavior of cancer cells. Perhaps there were mutant genes operating inside cancer cells that programmed the runaway proliferation of these cells, but the prospects for discovering such genes and understanding their actions seemed remote. No one knew how many genes were present in the human genome and how to analyze them. If mutant genes really did play a major part in cancer causation, they were likely to be small in number and dwarfed by the apparently vast number of genes present in the genome as a whole. They seemed to be the proverbial needles in the haystack, in this case a vast haystack of unknown size.

This theorizing about cancer's origins was further complicated by two other important considerations. First, many apparent carcinogens failed the Ames test, providing strong suggestion that they were nonmutagenic. Second, certain viral infections seemed to be closely connected to the incidence of a small but significant subset of human cancer types. Somehow, their carcinogenic powers had to be reconciled with the actions of mutagenic carcinogens and mutant cellular genes.

By the mid-1970s, recombinant DNA technology, including gene cloning, began to influence a wide variety of biomedical research areas. While appreciating the powers of this new technology to isolate and characterize genes, cancer researchers remained frustrated as to how they should proceed to exploit it to track down the elusive mutant genes that were responsible for cancer. One thing was clear, however. Sooner or later, the process of cancer **pathogenesis** (disease development) needed to be explained and understood in molecular terms. Somehow, the paradigm of DNA, RNA, and proteins, so powerful in elucidating a vast range of biological processes, would need to be brought to bear on the cancer problem.

In the end, the breakthrough that opened up this logjam came from study of the tumor viruses, which by most accounts were minor players in human cancer development. Tumor viruses were genetically simple, and yet they possessed potent carcinogenic powers. To understand these viruses and their import, we need to move back, once again, to the beginning of the twentieth century and confront another of the ancient roots of modern cancer research. This we do in Chapter 3.

## Key concepts

- The nineteenth-century discovery that all cells of an organism descend from the fertilized egg led to the realization that tumors are not foreign bodies but growths derived from normal tissues. The comparatively disorganized tissue architecture of tumors pointed toward cancer being a disease of malfunctioning cells.
- Tumors can be either benign (localized, noninvasive) or malignant (invasive, metastatic). The metastases spawned by malignant tumors are responsible for almost all deaths from cancer.
- Tumors are classified into four major groups according to their origin (epithelial, mesenchymal, hematopoietic, and neuroectodermal).
- Virtually all cell types in the body can give rise to cancer, but the most common human cancers are of epithelial origin—the carcinomas. Most carcinomas fall into two categories: squamous cell carcinomas arise from epithelia that form protective cell layers, while adenocarcinomas arise from secretory epithelia.
- Nonepithelial malignant tumors include (1) sarcomas, which originate from mesenchymal cells; (2) hematopoietic cancers, which arise from cells of the circulatory and the immune systems; and (3) neuroectodermal tumors, which originate from components of the nervous system.
- Some tumors do not fit this classification scheme. Occasionally, the origin of a tumor cannot be identified because its cells have dedifferentiated (shed all tissue-specific traits); such tumors are said to be anaplastic.
- Cancers seem to develop progressively, with tumors demonstrating different gradations of abnormality along the way from benign to metastatic.
- Benign tumors may be hyperplastic or metaplastic. Hyperplastic tissues appear normal except for an excessive number of cells, whereas metaplastic tissues show displacement of normal cells by types not usually encountered at that site. Metaplasia is most frequent in epithelial transition zones.
- Dysplastic tumors contain cells that are cytologically abnormal. Dysplasia is a transitional state between completely benign and premalignant. Adenomatous growths (adenomas, polyps, papillomas, and warts) are dysplastic epithelial tumors that are considered to be benign because they respect the boundary created by the basement membrane.
- Tumors that breach the basement membrane and invade underlying tissue are malignant. An even further degree of abnormality is metastasis, the seeding of tumor colonies to different sites in the body. Metastasis requires not only invasiveness but also such newly acquired traits as motility and adaptation to foreign environments.
- Biochemical and genetic markers were used to determine that human tumors are monoclonal (descended from one ancestral cell) rather than polyclonal (descended from different subpopulations of cells), although confounding factors may mask the true nature of a tumor's origins.
- Although the incidence of some cancers (mostly pediatric ones) is comparable worldwide, many vary dramatically by country and therefore cannot be

due simply to a normal biologic process gone awry by chance. Differences in heredity or environment might well explain these differences; in fact, epidemiologic studies have shown that environment is the dominant determinant of the country-by-country variations in cancer incidence.

- Laboratory research supported the epidemiologic studies by directly implicating chemical and physical agents (tobacco, coal dust, X-rays) as causes of cancers. However, the possibility of cancer as an infectious disease arose when viruses were found to cause leukemias and sarcomas in chickens.
- A possible mechanism that supported carcinogenesis by physical and chemical agents surfaced in 1927 when mutations were induced in fruit flies by exposing them to X-rays. By 1950, a series of chemicals also were found to be mutagenic for fruit flies and carcinogenic in lab animals. This led to the speculation that cancer was a disease of mutant genes and that carcinogenic agents induced cancer through their ability to mutate genes.
- In 1975 the Ames test provided support for this idea by showing that many carcinogens can act as mutagens. However, additional research showed that almost all compounds that are mutagenic are likely to be carcinogens, but the converse does not hold true. So, some carcinogens act through their ability to mutate DNA, while others promote tumorigenesis through nongenetic mechanisms. Such nonmutagenic carcinogens are called tumor promoters.
- The Ames test combined with other discoveries led to the model, still unproven, that a significant portion of human cancers is attributable directly to the consumption of foodstuffs that are mutagenic and hence carcinogenic.

### Thought questions

1. What types of observation allow a trained pathologist to identify the tissue of origin of a tumor? And why are certain tumors (5–10%) extremely difficult to assign to a specific tissue of origin?
2. Under certain circumstances, all tumors of a class can be traced to a specific embryonic cell layer, while in other classes of tumors, no such association can be made. What tumors would fit into each of these two groupings?
3. What evidence persuades us that a cancer arises from the native tissues of an individual rather than invading the body from outside and thus being of foreign origin?
4. How compelling are the arguments for the monoclonality of tumor cell populations and what logic and observations undermine the conclusion of monoclonality?
5. How can we estimate what percentage of cancers in a population that are avoidable (through virtuous life styles) and what percentage occur because of an unavoidable background incidence that strikes a population independent of the specifics of its life style?
6. What limitations does the Ames test have in predicting the carcinogenicity of various agents?
7. In the absence of being able to directly detect mutant genes within cancer cells, what types of observation allow one to infer that cancer is a disease of mutant cells?

### Additional reading

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