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

Tumors destroy man in a unique and appalling way, as flesh of his own flesh which has somehow been rendered proliferative, rampant, predatory and ungovernable. They are the most concrete and formidable of human maladies, yet despite more than 70 years of experimental study they remain the least understood.

Francis Peyton Rous, tumor virologist, Nobel lecture, 1966

The 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 pose a grave danger, in that individual cells within the organism may gain access to information in their genomes that is normally denied to them and assume roles that are inappropriate for normal tissue

Movies in this chapter
2.1 Embryonic Origins of Tissues
2.2 Mammary Cancer Cells
2.3 Visualization of Cancer I: Lymphoma
maintenance and function. 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 proliferation 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 collaborate with one another 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 preexisting 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 an afflicted person. Now, tumors, like normal tissues, could be examined under the microscope by researchers in the then-new science of histology. These examinations of tissue sections (thin slices) revealed that tumors, like normal tissues, were composed of masses of cells (Figure 2.1). Contemporary cancer research makes frequent use of a variety of histological techniques; the most frequently used of these are illustrated in Supplementary Sidebar 2.1.
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 disease first began, a consequence of the tendency of cancers to spread throughout the body and to establish 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 than the architecture of nearby normal tissues (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.

While the microarchitecture of tumors differed from that of normal tissue, tumors nevertheless bore certain histological features that resembled those of normal tissue.

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) Melanoma metastases can be quickly identified in mice because of their distinctive dark pigmentation. Seen here are the lungs of two mice, in one of which the formation of metastases was almost entirely blocked (left) and one in which hundreds of metastases (black spots) were allowed to form (right), as observed two weeks after B16 mouse melanoma cells were injected into the tail veins of these mice. This injection route causes many of the cells to become mechanically trapped in the lungs, where they seed numerous colonies. (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 (see Figure 14.45), 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; shown intact at right) of the brain has been removed. (A, from F. Nimmerjahn et al., Immunity 23:41–51, 2005. B, courtesy of Peter Isaacson. C, from H. Okazaki and B.W. Scheithauer, Atlas of Neuropathology. Gower Medical Publishing, 1988.)
This suggested that all tumors should, in principle, be traceable back to the specific tissue or organ site in which they first arose, using the histopathological analyses of tumor sections to provide critical clues. This simple idea led 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 (that is, 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. Another type of basement membrane separates endothelial cells, which form the inner linings of capillaries and larger vessels, 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, uterus, prostate, gallbladder, and urinary bladder. Examples of normal epithelial tissues are presented in Figure 2.4.

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

**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.

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**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 are tethered to one side of the basement membrane, sometimes termed “basal lamina.” Seen here as a continuous sheet, it is formed as meshwork of extracellular matrix proteins. A network of collagen fibers anchors the underside of the basement membrane to the extracellular matrix (ECM) of the stroma. (B) The epithelium of the mouse trachea is viewed here at far higher magnification through a transmission electron microscope. Several epithelial cells are seen above the basement membrane, while below are collagen fibrils, a fibroblast, and elastin fibers. Note that the basement membrane is not interrupted at the intercellular space between the epithelial cells. (C) While basement membranes cannot be detected using conventional staining techniques, use of immunofluorescence with an antibody against a basement membrane protein—in this case laminin 5 (red)—allows its visualization. The epithelial cells coating the villi of the mouse small intestine have been stained with an antibody against E-cadherin (green), while all cell nuclei are stained blue. Here the convoluted basement membrane separates the outer villus layer of epithelial cells, termed enterocytes, from the mesenchymal cells forming the core of each villus (not stained). [A, courtesy of Robert Trelstad. B, from B. Young et al., Wheater’s Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003. C, from Z.X. Mahoney et al., *J. Cell Sci.* 121:2493–2502, 2008 (cover image).]
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 lumen (cavity) that they are lining. Panel C illustrates another property that is characteristic of the epithelial cells forming an epithelium: the state of apico-basal polarity, in which individual epithelial cells are organized to present their apical surface toward the lumen (right) and their basal surface toward the underlying basement membrane. This polarization involves the asymmetric localization of the nuclei, which are more basally located, along with hundreds of cell-surface (and associated cytoskeletal) proteins (not shown) that are specifically localized either to the apical or basal surfaces of these cells. In addition, the lateral surfaces of the epithelial cells establish several distinct types of junctions with their adjacent epithelial neighbors. (From B. Young et al., Wheater’s Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003.)
Most 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 termed squamous cell carcinomas. For example, the epithelial cells lining the skin (keratinocytes) and most of the oral cavity 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 (lumina) 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 adenocarcinomas or pure squamous cell carcinomas; quite frequently, however, tumors in these organs are found in which both types of carcinoma cells coexist.

Table 2.1 Carcinomas

<table>
<thead>
<tr>
<th>Tissue sites of more common types of adenocarcinoma</th>
<th>Tissue sites of more common types of squamous cell carcinoma</th>
<th>Other types of carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>lung, colon, breast, pancreas, stomach, esophagus, prostate, endometrium, ovary</td>
<td>skin, nasal cavity, oropharynx, larynx, lung, esophagus, cervix</td>
<td>small-cell lung carcinoma, large-cell lung carcinoma, hepatocellular carcinoma, renal cell carcinoma, transitional-cell carcinoma (of urinary bladder)</td>
</tr>
</tbody>
</table>
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, all of which 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

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 (left) and the skin (right) are organized quite similarly, with mature flattened cells at the surface being continually shed (for example, the dead keratinocytes of the skin) and replaced by less differentiated cells that move upward and proceed to differentiate. (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.)
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). Hemangiomas, which are relatively common in children, arise from precursors of the endothelial cells. The stromal layers of epithelial tissues include some 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); these cells also derive from the embryonic mesoderm. Among them 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. This class of tumors is responsible for ~7% of cancer-associated mortality in the United States.

The third and last major grouping of nonepithelial tumors arises from cells that form various components of the central and peripheral nervous systems (Table 2.4). These are often termed neuroectodermal tumors to reflect their origins in the outer cell

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### Table 2.2 Various types of more common sarcomas

<table>
<thead>
<tr>
<th>Type of tumor</th>
<th>Presumed cell lineage of founding cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteosarcoma</td>
<td>osteoblast (bone-forming cell)</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>adipocyte (fat cell)</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>smooth muscle cell (e.g., in gut)</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>striated/skeletal muscle cell</td>
</tr>
<tr>
<td>Malignant fibrous histiocytoma</td>
<td>adipocyte/muscle cell</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>fibroblast (connective tissue cell)</td>
</tr>
<tr>
<td>Angiosarcoma</td>
<td>endothelial cells (lining of blood vessels)</td>
</tr>
<tr>
<td>Chondrosarcoma</td>
<td>chondrocyte (cartilage-forming cell)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type of leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphocytic leukemia (ALL)</td>
</tr>
<tr>
<td>Acute myelogenous leukemia (AML)</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia (CML)</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia (CLL)</td>
</tr>
<tr>
<td>Multiple myeloma (MM)</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma a (NHL)</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma (HL)</td>
</tr>
</tbody>
</table>

aThe 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.
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.

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

Not all tumors fall neatly into one of these four major 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,
Some types of tumors do not fit into the major classifications

Figure 2.8 Hematopoietic malignancies (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 (red-purple) exhibited the antigenic markers indicating origin from pre-B cells. (B) As in many hematopoietic malignancies, these acute myelogenous leukemia (AML) cells (blue) 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, in turn, into macrophages, dendritic cells, osteoclasts, and other tissue-specific phagocytic cells. (C) The large erythroblasts in this erythroleukemia (red-purple) closely resemble the precursors of differentiated red blood cells—erythrocytes. (D) In chronic myelogenous leukemia (CML), a variety of leukemic cells of the myeloid (marrow) lineage are apparent (red nuclei), 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 secrete antibody molecules, explaining their relatively large cytoplasms in which proteins destined for secretion are processed and matured. Seen here are plasma cells of MM at various stages of differentiation (purple nuclei). In some of these micrographs, numerous lightly staining erythrocytes are seen in the background. (From A.T. Skarin, Atlas of Diagnostic Oncology, 4th ed. Philadelphia: Elsevier Science Ltd., 2010.)
the melanocytes end up during development as wanderers that settle in the skin and the eye, provide pigment to these tissues, but 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 a 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.

Of the atypical tumor types, teratomas are arguably the most bizarre of all, in part because they defy all attempts at classification. While only ~10,000 cases are diagnosed worldwide annually, teratomas deserve mention because they are unique and shed light on the biology of embryonic stem (ES) cells, which have become so important to biologists; ES cells enable genetic manipulation of the mouse germ line and are central to certain types of stem cell therapies currently under development. Teratomas

<table>
<thead>
<tr>
<th>Name of tumor</th>
<th>Lineage of founding cell</th>
</tr>
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<tbody>
<tr>
<td>Glioblastoma multiforme</td>
<td>highly progressed astrocytoma</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>astrocyte (type of glial cell)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>arachnoidal cells of meninges</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>Schwann cell around axons</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>cone cell in retina</td>
</tr>
<tr>
<td>Neuroblastoma(^{e})</td>
<td>cells of peripheral nervous system</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>cells lining ventricles of brain</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>oligodendrocyte covering axons</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>granular cells of cerebellum</td>
</tr>
</tbody>
</table>

\(^{a}\)Nonneuronal cell of central nervous system that supports neurons.

\(^{b}\)Membranous covering of brain.

\(^{c}\)Constructs insulating myelin sheath around axons in peripheral nervous system.

\(^{d}\)Photosensor for color vision during daylight.

\(^{e}\)These tumors arise from cells of the sympathetic nervous system.

\(^{f}\)Fluid-filled cavities in brain.

\(^{g}\)Similar to Schwann cells but in brain.

\(^{h}\)Cells of the lower level of cerebellar cortex (for example, see Figure 2.9B).
Some types of tumors do not fit into the major classifications

**Figure 2.9 Neuroectodermal tumors**

(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 of the cerebellum (left panel) reside below Purkinje cells and cells of the molecular layer 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 cytoplasms). About one-third of these tumors show the rosettes of cells seen here. (C) Shown is an oligodendroglioma (right), which derives from oligodendrocytes, nonneuronal cells of ectodermal origin that support and insulate axons in the central nervous system. Each of the neoplastic cell nuclei here has a halo around it, which is characteristic of this tumor. The cultured normal oligodendrocyte shown here (left) exhibits a number of branching (dendritic) arms—each of which associates with one or several axons and proceeds to form an insulating myelin sheath around a segment of each of these axons. The cell body has been immunostained (yellow/orange) for the O4 oligodendrocyte marker, while the tips of the dendritic arms (green) have been stained for CNPase, an enzyme associated with myelination of axons. (D) Rods, cones, and other neuronal cell types (left panel) constitute important components of the normal retina. Retinoblastomas (right panel) arise from cells with attributes of the cone precursors present in the normal developing retina. Retinoblastomas often show the characteristic rosettes, indicated here with arrows. (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, D, and E, left panels, from B. Young et al., Wheater’s Functional Histology, 4th ed. Edinburgh: Churchill Livingstone, 2003. A–C, right panels, from H. Okazaki, B.W. Scheithauer, Atlas of Neuropathology. Gower Medical Publishing, 1988. B, left panel, Thomas Deerinck, NCMIR/Science Source. C, left panel, courtesy of R. Hardy and R. Reynolds. D, E, right panels, from A.T. Skarin, Atlas of Diagnostic Oncology, 3rd ed. Philadelphia: Elsevier Science Ltd., 2003.)
seem to arise from germ cell (egg and sperm) precursors (see Section 1.3) that fail to migrate to their proper destinations during embryonic development and persist at ectopic (inappropriate) sites in the developing fetus. They retain the pluripotency of early embryonic cells—the ability to generate most and possibly all of the tissues present in the fully developed fetus. The cells in different sectors of common “mature” teratomas—which are largely benign, localized growths—differentiate to create tissues that are very similar to those found in a variety of adult tissues (Figure 2.11). Typically, representatives of the three cell layers of the embryo—endoderm, mesoderm, and ectoderm (see Figure 2.5)—coexist within a single tumor and often develop into recognizable structures, such as teeth, hair, and bones. Occasionally these tumors progress to become highly malignant and thus life-threatening.

Of special interest is the fact that careful karyotypic and molecular analyses of benign, mature teratomas have indicated that the associated tumor cells are genetically wild type. This suggests that such teratoma cells are unique, being the only type of tumorigenic cell whose genomes are truly wild type, in contrast to the cells of all other tumor types described in this book, which carry multiple genetic aberrations.
The occasional rule-breaking exceptions, such as those represented by teratomas and the products of the EMT, do not detract from one major biological principle that 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 sites from which these biopsies were prepared.

In a small minority of cases (2–4%), 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 2.12). A tumor of this type is often classified as a cancer of unknown primary (CUP), reflecting the difficulty of identifying the original site of tumor formation in the patient.

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 progressively toward greater degrees of aggressive and invasive behavior. Thus, each type of abnormal growth within a tissue 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 2.13). 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. 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 2.14). Even though these gastric cells have a quite normal appearance, this metaplasia is considered an early step in the development of esophageal adenocarcinomas. 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 2.15). 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 2.16). These are often large growths that can be readily detected with the naked eye. They contain all the cell types found in the normal epithelial tissue, but this assemblage 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. These tumors 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 the underlying stroma. 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. In the case of carcinoma cells, this incursion is signaled the moment carcinoma cells break through a basement membrane and invade into the adjacent stroma (Figure 2.17). Here, for the first time, we encounter malignant cells 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 neoplasms, that is, new types of tissue.
Figure 2.16 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 in a photograph (left) and a micrograph (right). These growths, also termed “adenomas,” have not penetrated the basement membrane and invaded the underlying stroma.

(B) The lobules of the normal human breast (purple islands, left half of figure), each containing numerous small alveoli in which milk is produced, are surrounded by extensive fibrous stroma (pink). The cells of an intraductal carcinoma, often called a ductal carcinoma in situ (DCIS; purple, to right of dashed line), fill and distend ducts but have not invaded through the basement membrane surrounding the ducts into the stroma. In the middle of one of these ducts is an island of necrotic carcinoma cells (dark red) that have died, ostensibly because of inadequate access to the circulation. (A, left, courtesy of John Northover and Cancer Research, UK; right, courtesy of Anne Campbell. B, courtesy of Tan A. Ince.)

Figure 2.17 Invasive carcinomas

Tumors are considered malignant only after they have breached the basement membrane and invaded the surrounding stroma. (A) These breast cancer cells (dark red), which previously constituted a ductal carcinoma in situ (DCIS; see Figure 2.16B), have now broken through on a broad front (dashed line) the layer of myoepithelial cells (dark brown) and underlying attached basement membrane (not visible) into the stroma; this indicates that they have acquired a new trait: invasiveness. (B) After breaching the basement membrane, invasive cancer cells can appear in various configurations amid the stroma. In this invasive ductal carcinoma of the breast, islands of epithelial cancer cells (dark purple) are interspersed amid the stroma (dark pink). The ductal nature of this carcinoma is revealed by the numerous rudimentary ducts formed by the breast cancer cells. (C) In this invasive lobular carcinoma of the breast, individual carcinoma cells (dark purple nuclei) have ventured into the stroma (red-orange), often doing so in single-file formation. (A, from F. Koerner, Diagnostic Problems in Breast Pathology. Philadelphia: Saunders/Elsevier, 2008. B and C, courtesy of Tan A. Ince.)
A summary of the overall pathological classification scheme of tumors is provided in Figure 2.18. A short discussion of the organizing principles underlying these classifications can be found in Supplementary Sidebar 2.2.
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 (that is, normal → hyperplastic → dysplastic → neoplastic → metastatic) are 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 2.19)? Do the tumor cells descend from a single ancestral cell that crossed over the boundary 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 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 is present in all cells within a tumor, this would suggest that they all descend from an initially mutated cell. 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 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 in Chapter 1, 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 almost 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 (see Supplementary Sidebar 1.1). Once an X chromosome (of maternal or paternal origin) has been inactivated in a cell, all descendant cells in adult tissues appear to respect this decision and thus continue to inactivate the same X chromosome.

Thus, the lineage of a cell can be followed in vivo from its embryonic ancestor, a term called lineage tracing. 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 susceptibility to heat inactivation. Because of X-chromosome silencing, each of the cells in these heterozygous females 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 2.20). 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 cancer cells all descended from a single founding 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 in the tumor mass constitute a monoclonal growth. (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 one from the father (P) soon undergoes inactivation at random. Such inactivation silences expression of almost all genes 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 had inactivated 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 from a G6PD heterozygous patient express the same version of this enzyme. This indicated their likely descent from a common ancestral cell that already had this particular pattern of X-inactivation, suggesting 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.)

![Figure 2.20 X-chromosome inactivation patterns and the monoclonality of tumors](image-url)
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 (antibody) 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 2.21A).

Perhaps the most vivid demonstrations of tumor monoclonality have come from cancer cells sporting a variety of chromosomal aberrations that can be visualized microscopically when chromosomes condense during 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 (see Figure 2.21B). This observation makes it obvious that all 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 10 normal cells in a tissue simultaneously crossed over the border from being normal to being malignant (or at least premalignant) and that each of these cells, and its descendants in turn, proliferated uncontrollably (see Figure 2.19). Each of these founding cells would spawn a large monoclonal population, and the tumor mass, as a whole, consisting of a mixture of these 10 cell populations, would be polyclonal.

It is highly likely that each of these 10 clonal populations varies subtly from the other 9 in a number of characteristics, among them the time required for their cells 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 may become quite heterogeneous because of the continual acquisition of new mutant alleles by some of its cells, a term called genetic instability. The resulting genetic 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 advanced human tumors are monoclonal growths descended from single normal progenitor cells that took the first small steps to becoming cancerous. Such progenitors are often termed cells-of-origin, and it is increasingly appreciated that the differentiation programs of these cells continue to influence the behavior of derived tumor cell populations decades later. Indeed, in the great majority of human tumor types, one can identify the tissues in which these cells-of-origin resided, but the precise identities of these normal cells, including their state of differentiation, often remain obscure.

2.6 Cancer cells exhibit an altered energy metabolism

The monoclonality of tumor cell populations was first demonstrated in 1965. Another equally interesting peculiarity of tumors was already appreciated more than four decades earlier: the energy metabolism of most cancer cells differs markedly from that of normal cells, a trait first reported in 1924 by Otto Warburg, the Nobelist later honored for discovering the respiratory enzyme now known as cytochrome c oxidase. As was documented in the decades that followed, normal cells that experience aerobic conditions break down glucose into pyruvate in the cytosol through the process of glycolysis and then dispatch the pyruvate into mitochondria, where it is broken down further into carbon dioxide in the citric acid cycle (known also as the Krebs cycle; Figure 2.22A). Under anaerobic or hypoxic (low oxygen tension) conditions, however, normal cells are limited to using only glycolysis, generating pyruvate that is reduced to lactate, which is then secreted from cells. Warburg discovered that even when exposed to ample oxygen, many types of cancer cells rely largely on glycolysis, generating lactate as the breakdown product of glucose (see Figure 2.22B).

The use by cancer cells of “aerobic glycolysis,” as Warburg called it, would seem to make little sense energetically, since the breakdown of one molecule of glucose yields only two molecules of ATP through glycolysis. In contrast, when under aerobic conditions glycolysis is followed by oxidation of pyruvate in the citric acid cycle, as many as 36 ATPs per glucose molecule are generated. In fact, most types of normal cells in the body have continuous access to O2 conveyed by the blood and therefore metabolize glucose through this energetically far more efficient route. The tendency of cancer cells to limit themselves to glycolysis, even when provided with adequate oxygen, stands out as exceedingly unusual behavior.

The fact that cancer cells metabolize glucose so inefficiently requires them to compensate by importing enormous amounts of glucose. This behavior is seen in many types of cancer cells, including both carcinomas and hematopoietic tumors; they express greatly elevated levels of glucose transporters, particularly GLUT1, which span the plasma membrane and drive the high rates of glucose uptake by these cells. Radiologists take advantage of this elevated glucose uptake by injecting into the circulation radiolabeled glucose [2-deoxy-2-(18F)fluoro-d-glucose, FDG] and observing its rapid concentration in tumors (see Figure 2.22C).

In the 1950s, Warburg proposed that this altered energy metabolism was the driving force in the formation of cancer cells, a notion that was discredited in the decades that followed. However, the process of aerobic glycolysis that he discovered was ultimately found to operate in a wide variety of human cancer cells and is now thought to represent one of the many consequences of cell transformation.

Aerobic glycolysis, sometimes called the Warburg effect, remains a subject of much contention, as its rationale in cancer cell biology has never been fully resolved: why do as many as 80% of cancer cells metabolize most of their glucose via glycolysis when completion of glucose degradation in mitochondria by the citric acid cycle would afford them vastly more ATP to fuel their own growth and proliferation? Is aerobic glycolysis required for maintenance of the cancer cell phenotype, or does it represent nothing more than a side effect of cell transformation that plays no causal role in cell transformation and tumor growth?
Figure 2.22 Changes in glucose metabolism in cancer cells

(A) In most normal nonproliferating cells having access to adequate oxygen, glucose is imported into the cells by glucose transporters (GLUTs) and then broken down by glycolysis and the citric acid cycle. During the last step of glycolysis, pyruvate kinase form M1 (PK-M1) ensures that its product, pyruvate, is imported into the mitochondria, where it is oxidized by pyruvate dehydrogenase (PDH) into acetyl CoA for processing in the citric acid cycle. Altogether, the mitochondria can generate as much as 36 ATP molecules per glucose molecule. (B) In cancer cells, including those with access to ample oxygen, the GLUT1 glucose transporter imports large amounts of glucose into the cytosol, where it is processed by glycolysis. However, as the last step of glycolysis, pyruvate kinase M2 (PK-M2) causes its pyruvate product to be diverted to lactate dehydrogenase (LDH), yielding the lactate that is secreted in abundance by cancer cells. Because relatively little of the initially imported glucose is metabolized by the mitochondria, as few as 2 ATPs are generated per glucose molecule. Moreover, many of the intermediates generated during glycolysis are diverted toward biosynthetic uses. This mode of metabolic regulation resembles the metabolic state of normal, rapidly dividing cells, which also divert a significant portion of their glycolytic intermediates to biosynthetic pathways. Enzymes are in rectangles, glucose metabolites are in ovals, low–molecular-weight compounds are in hexagons, regulatory proteins are in pentagons. (C) 2-Deoxy-2-(18F)fluoro-d-glucose positron-emission tomography (FDG-PET) makes it possible to visualize tumors in the body that have concentrated large amounts of glucose because of the hyperactivity of the GLUT1 transporter in the associated cancer cells. In the case shown here, FDG-PET revealed a small tumor (bright orange; arrow) in the region near an ovary of a woman who was under treatment for breast cancer but was otherwise without symptoms. X-ray-computed tomography (CT) was used at the same time to image the outlines of the tissues of this patient. This highly sensitive technology provided the first indication of an incipient ovarian cancer in this patient. (C, from R.A. Milam, M.R. Milam and R.B. Iyer, J. Clin. Oncol. 25:5657–5658, 2007.)
One explanation of aerobic glycolysis comes from the observation that the cancer cells within a tumor often have inadequate access to oxygen, as we will discuss in detail in Chapter 13. The resulting hypoxic state limits cancer cells to glycolysis and thus to inefficient ATP production—just as normal cells would be limited under these conditions. Because of the Warburg effect, cancer cells would seem to be well adapted to this oxygen starvation, since glycolysis operates normally under hypoxic conditions. Still, this fails to explain why cancer cells, even when provided with abundant oxygen, do not take advantage of this oxygen to generate ATP in far larger quantities.

Another rationale for aerobic glycolysis derives from the fact that glycolysis actually serves a second role independent of ATP generation: the intermediates in the glycolytic pathway function as precursors of many molecules involved in cell growth, including the biosynthesis of nucleotides and lipids. By blocking the last step of glycolysis (see below), cancer cells ensure the accumulation of earlier intermediates via feedback reactions in this pathway. These glycolytic intermediates can then be diverted into critically important biosynthetic reactions. This behavior contrasts with that of normal cells, which are generally not actively proliferating, do not require large-scale biosynthetic reactions, and depend largely on ATP to sustain their metabolic activity. (By some estimates, normal cells use more than 30% of their imported glucose to make ATP, while cancer cells use only ~1% of their glucose for this purpose—a striking contrast in metabolic organization.)

A complete rationale for why cancer cells use aerobic glycolysis is still not in hand. However, independent of how this question is resolved, there is yet another: how do cancer cells actually manage to avoid mitochondrial processing of glucose metabolites? Pyruvate kinase (PK) catalyzes the last step of glycolysis—the conversion of phosphoenolpyruvate (PEP) to pyruvate. As noted earlier, this end product of glycolysis is normally destined for import into the mitochondria, where it is broken down in the citric acid cycle (see Figure 2.22). The M1 isoform of PK typically is expressed in most adult tissues, while the M2 isoform is expressed by early embryonic cells, rapidly growing normal cells, and cancer cells. For reasons that are still poorly understood, the commonly expressed M1 isoform of PK ensures that its product, pyruvate, is dispatched from the cytosol into the mitochondria, while the M2 isoform that is expressed instead in cancer cells causes its pyruvate product to be reduced to lactate in the cytosol. Relative to the M1 form of PK, the M2 enzyme has a very slow turnover number, which results in a backup of glycolytic intermediates and their diversion into biosynthetic pathways. Importantly, the relative inactivity of the citric acid cycle in cancer cells is not due to defects in the mitochondria: they are normal and fully capable of receiving pyruvate and processing it in the citric acid cycle.

Experimental evidence indicates that the growth of tumors actually depends on the expression of the M2 form of PK and on the elevated expression of the glucose importer GLUT1 and lactate dehydrogenase-A (LDH-A), the latter being involved in reducing pyruvate to lactate, which is then secreted (see Figure 2.22B). When any one of these is inhibited, tumor growth slows down, sometimes dramatically. Observations like these provide the first indications that the bizarre glucose metabolism of cancer cells creates a physiologic state on which cancer cell growth and proliferation depend.

2.7 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.

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

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Countries showing highest and lowest incidence of specific types of cancer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relative risk H/L&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Skin (melanoma)</td>
<td>Australia (Queensland) Japan</td>
<td>155</td>
</tr>
<tr>
<td>Lip</td>
<td>Canada (Newfoundland) Japan</td>
<td>151</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>Hong Kong United Kingdom China</td>
<td>100</td>
</tr>
<tr>
<td>Prostate</td>
<td>U.S. (African American) China</td>
<td>70</td>
</tr>
<tr>
<td>Liver</td>
<td>China (Shanghai) Canada (Nova Scotia)</td>
<td>49</td>
</tr>
<tr>
<td>Penis</td>
<td>Brazil Israel (Ashkenazic)</td>
<td>42</td>
</tr>
<tr>
<td>Cervix (uterus)</td>
<td>Brazil Israel (non-Jews)</td>
<td>28</td>
</tr>
<tr>
<td>Stomach</td>
<td>Japan Kuwait</td>
<td>22</td>
</tr>
<tr>
<td>Lung</td>
<td>U.S. (Louisiana, African American) India (Madras)</td>
<td>19</td>
</tr>
<tr>
<td>Pancreas</td>
<td>U.S. (Los Angeles, Korean American) India</td>
<td>11</td>
</tr>
<tr>
<td>Ovary</td>
<td>New Zealand (Polynesian) Kuwait</td>
<td>8</td>
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<table>
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<tr>
<th>Geographic areas showing highest and lowest death rates from specific types of cancer&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Relative risk H/L&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>Lung, male</td>
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<td>Esophagus</td>
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<td>Colon, male</td>
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</tr>
<tr>
<td>Breast, female</td>
<td>6</td>
</tr>
</tbody>
</table>


<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, for example, 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.
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.

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 2.23). 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 many 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 Seventh-Day Adventists, whose religion discourages smoking, heavy drinking, and the consumption of pork, die from cancer at a rate that is only about three-quarters 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.

![Figure 2.23 Country-to-country comparisons of cancer incidence](image)

Public health records reveal dramatic differences in the incidence of certain cancers in different countries. Here, the relative incidences 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.)
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—the total number of cell divisions occurring in the bodies of 10 individuals during their lifetimes—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.

2.8 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 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. 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 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.
2.9 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. This work, first reported by Katsusaburo Yamagiwa in 1915, was little noticed in the international scientific community of the time (Figure 2.24). 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 spontaneously 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 (that is, 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.

Table 2.6 Relative risk of lung cancer as a function of the number of cigarettes smoked per day

| Most recent number of cigarettes smoked (by subjects) per day before onset of disease | Lifelong nonsmoker | Smokers |
|---|---|---|---|---|---|---|
| — | 1 | 8 | 12 | 14 | 27 |

The relative risk indicates the risk of contracting lung cancer compared with that of a nonsmoker, which is set at 1.


Figure 2.24 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 tar. (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.)
Chapter 2: The Nature of Cancer

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 radioactive agents had become compelling.

2.10 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.

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 2.26). 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 potently carcinogenic as 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 was that there were no good ways of measuring the relative mutagenic potencies of various chemical species. So Ames devised his own method. It 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.

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**Figure 2.25 Structures of carcinogenic hydrocarbons** These chemical species arise from the incomplete combustion of organic (for example, 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 potently 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.)
In detail, Ames used a mutant strain of *Salmonella* that was 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 2.27).

In principle, Ames needed only to introduce a test compound into a Petri dish containing his special *Salmonella* strain and count the bacterial colonies that later appeared. There remained, however, one substantial obstacle to the success of this mutagenesis assay. Detailed studies had shown that after carcinogenic molecules entered 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 conversion. Given the radically different metabolisms of bacteria and mammalian cells, it was highly unlikely that Ames’s *Salmonella* would be able to accomplish the metabolic activation of procarcinogens that occurred in the tissues of laboratory animals.

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**Figure 2.26 Structure of the Philadelphia chromosome**

Analyses of the banding patterns of stained metaphase chromosomes of chronic myelogenous leukemia (CML) cells first revealed the characteristic tiny chromosome (called the “Philadelphia chromosome” or Ph¹) that is present in the leukemia cells of most CML patients. (A) This banding pattern, determined through light-microscopic surveys, is illustrated here schematically. While the chromosomal translocation generating the two altered chromosomes (9q+,22q−) is reciprocal (for example, involving a loss and a gain by both), the sizes of the exchanged chromosomal arms are unequal, leading to the greatly truncated Chromosome 22 (for example, 22q−). The small 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* region (characteristic of Chromosome 9) 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 (arrows). (A, from B. Alberts et al., Molecular Biology of the Cell, 5th ed. New York: Garland Science, 2008. B, courtesy of Thomas Ried and Nicole McNeil.)
Earlier work of others had shown that a great many chemicals introduced into the body undergo metabolic conversion, specifically in the liver. Moreover, 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.)

With the addition of this extra step, Ames’s assay revealed that a number of known carcinogens 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 2.28).

As we have read, the notion that carcinogens are mutagens predated Ames’s work by a quarter of a century. Nonetheless, his analyses galvanized researchers interested in the origins of cancer, since the results 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 in mammals. 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.

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.
2.11 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.

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) measures crossing over between the two paired chromatids that are formed by DNA replication during the S phase and persist in paired form during the late (that is, G₂) 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 select mammalian cells that have lost by mutation their thymidine kinase or HGPRT (hypoxanthine guanine phosphoribosyl transferase) enzymes. The ability to examine under the light microscope the chromosomal array (that is, the karyotype; see Figure 1.11) of cells in 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 G₁ or G₂ phase of the cell cycle (described in Chapter 8); since cellular DNA synthesis normally occurs in the S phase, such non-S-phase labeling, which is 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%.

Figure 2.28 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 2.27). 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. moca—4,4’-methylenebis(2-chloroaniline), used in manufacture of polyurethane; mms—methyl methanesulfonate, an alkylating mutagen. (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.)
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. Thus, by the 1990s, 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 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.

Ames and others eventually 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).

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.

Table 2.8 A sampling of Bruce Ames’s roster of carcinogens identified in the normal diet

<table>
<thead>
<tr>
<th>Foodstuff</th>
<th>Compound</th>
<th>Concentration in foodstuff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td>piperine</td>
<td>100 mg/g</td>
</tr>
<tr>
<td>Common mushroom</td>
<td>agaritine</td>
<td>3 mg/g</td>
</tr>
<tr>
<td>Celery</td>
<td>furocoumarins, psoralensb</td>
<td>1 μg/g, 0.8 μg/g</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>anthraquinones</td>
<td>varies</td>
</tr>
<tr>
<td>Cocoa powder</td>
<td>theobromine</td>
<td>20 mg/g</td>
</tr>
<tr>
<td>Mustard, horseradish</td>
<td>allyl isothiocyanate</td>
<td>varies</td>
</tr>
<tr>
<td>Alfalfa sprouts</td>
<td>canavanine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15 mg/g</td>
</tr>
<tr>
<td>Burnt materials&lt;sup&gt;d&lt;/sup&gt;</td>
<td>large number</td>
<td>varies</td>
</tr>
<tr>
<td>Coffee</td>
<td>caffeic acid</td>
<td>11.6 mg/g</td>
</tr>
</tbody>
</table>

<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, pepper (black), 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.

Sidebar 2.2 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 (see Section 2.10), and in vivo tests—exposure of laboratory animals to agents suspected of causing cancer (see Section 2.9). 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 phenobarbital and isoniazid, register positively in the Ames test, and saccharin registers as a carcinogen in male laboratory rats, 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.

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 ingested, 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 may then be concentrated in some cells or quickly metabolized and excreted, creating 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 (Sidebar 2.2).

2.12 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 model drew attention to the nature of the cells that founded tumors and to the mechanisms that led to their transformation into cancer cells. If one could understand why a 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, suggesting 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 were unable, at least initially, 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 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 is the subject of Chapter 3.

A major challenge for the future is to understand how various biological and environmental factors, the latter including lifestyle, contribute to the incidence of cancers, many of them quite common ones. For example, as indicated in part in Table 2.5, the incidence of cancers, such as those of the colon, breast, and prostate, shows enormous geographic variation—dramatic differences that cannot be ascribed to differing genetic susceptibilities. In fact, epidemiologists have uncovered many correlations between the frequencies of these and other cancer types and various lifestyle factors (for example, those listed in Table 2.9). However, with rare exception, our understanding of the biological and biochemical mechanisms by which these factors increase (or reduce) disease incidence is either incomplete or nonexistent. Indeed, these correlations represent one of the major unsolved mysteries confronting contemporary cancer researchers.

Until we understand how various biological and lifestyle factors succeed in triggering or preventing tumor development, our ability to prevent new cancers (which is usually far more effective than trying to cure them after they have been diagnosed) will be limited. Many of the chapters that follow provide critical information that may ultimately help to unravel these mysteries of cancer etiology.

Table 2.9 Examples of etiologic mysteries: epidemiologic correlations between environmental/lifestyle factors and cancer incidence that lack a clear explanation of causal mechanism

<table>
<thead>
<tr>
<th>Lifestyle, dietary factor, or medical condition</th>
<th>Altered cancer risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>High birth weight</td>
<td>premenopausal breast cancer ↑</td>
</tr>
<tr>
<td></td>
<td>infant acute leukemia ↑</td>
</tr>
<tr>
<td>Processed red meatb</td>
<td>ER+ breast cancer ↑</td>
</tr>
<tr>
<td></td>
<td>squamous cell and adenocarcinoma of lung ↑</td>
</tr>
<tr>
<td>Childhood soy consumption</td>
<td>breast cancer ↓</td>
</tr>
<tr>
<td>Well-done red meat</td>
<td>prostate cancer ↑</td>
</tr>
<tr>
<td>Western diet—high in fat, high in red meat</td>
<td>colorectal, esophageal, liver, and lung cancer ↑</td>
</tr>
<tr>
<td>Exercise</td>
<td>hormone-responsive breast cancer ↓</td>
</tr>
<tr>
<td>Diet with cruciferous vegetables</td>
<td>prostate cancer ↓</td>
</tr>
<tr>
<td>High body-mass index (BMI)</td>
<td>multiple cancer types ↑</td>
</tr>
<tr>
<td>Higher ratio of number of daughters to number of sons born to a woman</td>
<td>ovarian carcinoma ↑</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>melanoma ↑</td>
</tr>
<tr>
<td>Low circulating vitamin D</td>
<td>breast cancer incidence, CRC mortality ↑</td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>esophageal carcinoma ↑</td>
</tr>
<tr>
<td>Coffee consumption</td>
<td>hepatocellular carcinoma ↓</td>
</tr>
</tbody>
</table>

*aRelative risk (RR) is not given, because not all studies used the same criteria to gauge RR.
↑ = increased risk; ↓ = decreased risk.
*bProcessed red meat generally refers to meat that has been preserved by smoking, curing, salting or adding chemical preservatives.
Abbreviations: ER+ = estrogen receptor–positive; CRC = colorectal cancer.
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 as 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.

- With some exceptions, most tumors are classified into four major groups according to their origin (epithelial, mesenchymal, hematopoietic, and neuroectodermal).

- Virtually all cell types 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 the precursors of blood cells; and (3) neuroectodermal tumors, which originate from components of the nervous system.

- If a tumor’s cells have dedifferentiated (lost all tissue-specific traits), its origin cannot be readily identified; 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 normal cell 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 other sites in the body. Metastasis requires not only invasiveness but also such newly acquired traits as motility and adaptation to foreign tissue environments.

- Biochemical and genetic markers seem to indicate that human tumors are monoclonal (descended from one ancestral cell) rather than polyclonal (descended from multiple ancestral cells, each of which independently spawned a population of cancer cells).

- Most normal cells start metabolizing glucose through glycolysis, and then transfer pyruvate (the product of glycolysis) into the mitochondria, where it is further processed to yield 36 ATPs and CO₂. Most cancer cells rely largely on glycolysis alone, which yields lactate and only 2 ATPs.

- The incidence of many (but not all) cancers varies dramatically by country, an indication that they cannot be due simply to a normal biologic process gone awry by chance. While differences in either heredity or environment could explain these variations, epidemiologic studies show that environment (including lifestyle factors) 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 when mutations were induced in fruit flies by exposing them to either X-rays or chemicals, indicating that they were mutagenic. Since these agents were also known to be carcinogenic in laboratory 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. Additional research showed that although almost all compounds that are mutagenic are likely to be carcinogens, 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 are attributable to the consumption of foodstuffs that are directly or indirectly mutagenic and hence carcinogenic.

Thought questions

1. What types of observation allow a pathologist to identify the tissue of origin of a tumor? And why are certain tumors 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 are avoidable (through virtuous lifestyles) and what percentage occur independently of lifestyle?

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


