# 18 The Molecular Biology of Cancer

*The term **cancer** applies to a group of diseases in which **cells grow abnormally** and form a **malignant tumor**. Malignant cells can invade nearby tissues and **metastasize** (i.e., travel to other sites in the body, where they establish secondary areas of growth). This aberrant growth pattern results from **mutations in genes that regulate proliferation, differentiation, and survival of cells** in a multicellular organism. Because of these genetic changes, cancer cells no longer respond to the signals that govern growth of normal cells (Fig. 18.1.)*

***Oncogenes and Tumor Suppressor Genes.** The genes involved in the development of cancer are classified as **oncogenes** or **tumor suppressor genes. Oncogenes** are mutated derivatives of normal genes (**proto-oncogenes**) whose function is to promote proliferation or cell survival. These genes can code for **growth factors, growth factor receptors, signal transduction proteins, intracellular kinases, and transcription factors.** The process of transformation into a malignant cell may begin with a **"gain of function"** mutation in only one copy of a proto-oncogene. As the mutated cell proliferates, additional mutations can occur. **Tumor suppressor genes** (normal growth suppressor genes) encode proteins that **inhibit proliferation, promote cell death,** or **repair DNA;** both alleles need to be inactivated for transformation (**a loss of function**). Growth suppressor genes have been called the guardians of the cell.*

***Cell Cycle Suppression and Apoptosis.** Normal cell growth depends on a balanced regulation of **cell cycle** progression and **apoptosis** (programmed cell death) by proto-oncogenes and growth suppressor genes. At **checkpoints** in the **cell cycle** products of **tumor suppressor genes** slow growth in response to signals from the cell's environment, including external growth inhibitory factors, or to allow time for repair of damaged DNA, or in response to other adverse circumstances in cells. Alternately, cells with damaged DNA are targeted for apoptosis so that they will not proliferate. Many growth-stimulatory pathways involving proto-oncogenes, and growth-inhibitory controls involving a variety of tumor suppressor genes, converge to regulate the activity of some key protein kinases, the **cyclin-dependent kinases.** These kinases act to control progression at specific points in the cell growth cycle. Apoptosis is initiated by either **death receptor activation** or intracellular signals leading to release of the **mitochondrial protein, cytochrome c.***

***Mutations.** Mutations in DNA that give rise to cancer may be inherited or caused by **chemical carcinogens, radiation, viruses,** and by **replication errors** that are not repaired. A cell population must accumulate **multiple mutations** for transformation to malignant cells.*

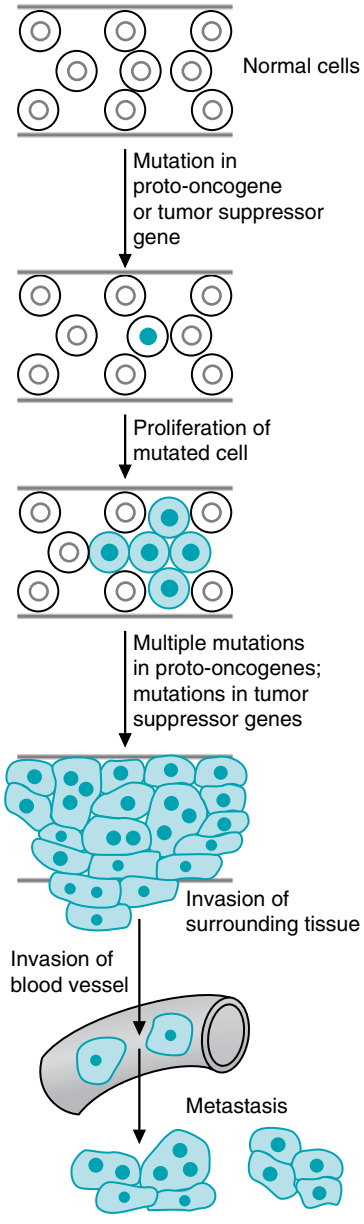



**Fig. 18.1.** Development of cancer. Accumulation of mutations in a number of genes results in transformation. Cancer cells change morphologically, proliferate, invade other tissues, and metastasize.

## THE WAITING ROOM

**Mannie Weitzels** has chronic myelogenous leukemia, a disease in which a single line of myeloid cells in the bone marrow proliferate abnormally, causing a large increase in the number of nonlymphoid white blood cells (see Chapter 16). His myeloid cells contain the abnormal Philadelphia chromosome, which increases their proliferation. He has recently complained of pain and tenderness in various areas of his skeleton, possibly stemming from the expanding mass of myeloid cells within his bone marrow. He also reports a variety of hemorrhagic signs, including bruises (ecchymoses), bleeding gums, and the appearance of small red spots (petechiae caused by release of red cells into the skin).

**Nick O'Tyne** was diagnosed with a well-differentiated adenocarcinoma of the lungs (see Chapter 13). He underwent anatomic staging procedures to determine the location and severity of the tumor. As a result of these tests, he was considered a candidate for surgical resection of the primary tumor aimed at cure. He survived the surgery and was recovering uneventfully until 6 months later when he complained of an increasingly severe right temporal headache. A CT scan of his brain was performed. Results indicated that the cancer, which had originated in his lungs, had metastasized to his brain.

**Colin Tuma** has had an intestinal adenocarcinoma resected, but there were several metastatic nodules in his liver (see Chapters 12 and 13). He completed his second course of chemotherapy with 5-fluorouracil (5-FU) and had no serious side effects. He assured his physician at his most recent checkup that, this time, he intended to comply with any instructions his physicians gave him. He ruefully commented that he wished he had returned for regular examinations after his first round of surgery for benign intestinal polyps.

**Mel Anoma** returned to his physician after observing a brownish-black irregular mole on his forearm (see Chapter 13). His physician thought the mole looked suspiciously like a malignant melanoma, and performed an excision biopsy (surgical removal for the purposes of biopsy).

Patients with leukemia experience a variety of hemorrhagic (bleeding) manifestations caused by a decreased number of platelets. Platelets are small cells that initiate clot formation at the site of endothelial injury. Because of the uncontrolled proliferation of white cells within the limited space of the marrow, the normal platelet precursor cells (the megakaryocytes) in the marrow are "squeezed" or crowded and fail to develop into mature platelets. Consequently, the number of mature platelets (thrombocytes) in the circulation falls, and a thrombocytopenia develops. Because there are fewer platelets to contribute to clot formation, bleeding problems are common.

Malignant neoplasms (new growth, a tumor) of epithelial cell origin (including the intestinal lining, cells of the skin, and cells lining the airways of the lungs) are called carcinomas. If the cancer grows in a gland-like pattern, it is an adenocarcinoma. Thus, **Nick O'Tyne** and **Colin Tuma** have adenocarcinomas. **Mel Anoma** had a carcinoma arising from melanocytes, which is technically a melanocarcinoma, but is usually referred to as a melanoma.

Moles (also called nevi) are tumors of the skin. They are formed by melanocytes that have been transformed from highly dendritic single cells interspersed among other skin cells to round oval cells that grow in aggregates or "nests." (Melanocytes produce the dark pigment melanin that protects against sunlight by absorbing UV light.) Additional mutations may transform the mole into a malignant melanoma.

## I. CAUSES OF CANCER

*Cancer* is the term applied to a group of diseases in which cells no longer respond to normal restraints on growth. Normal cells in the body respond to signals, such as contact inhibition, that direct them to stop proliferating. Cancer cells do not require growth stimulatory signals and they are resistant to growth inhibitory signals. They are also resistant to apoptosis, the programmed death process whereby unwanted or

The study of cells in culture was a great impetus to the study of cancer, because the development of a tumor in animals could take months. Once cells could be removed from an animal and propagated in a tissue culture dish, the onset of transformation (the normal cell becoming a cancer cell) could be seen in days.

What are the criteria that distinguish transformed cells from normal cells in culture? The first is the requirement for serum in the cell culture medium to stimulate growth. Transformed cells have a reduced requirement for serum, approximately 10% that required for normal cells to grow. The second is the ability to grow without attachment to a supporting matrix (anchorage dependence). Normal cells (such as fibroblasts, smooth muscle cells) require adherence to a substratum (in this case, the bottom of the plastic dish) and will not grow if suspended in a soft agar mixture (the consistency of loose jello). Transformed cells, however, have lost this anchorage dependence for growth. An additional criterion used to demonstrate that cells are truly transformed is that they form tumors when injected into mice lacking an immune system.

irreparably damaged cells self-destruct. They have an infinite proliferative capacity and do not become senescent (i.e., they are immortalized). Furthermore, they can grow independently of structural support, such as the extracellular matrix (loss of anchorage dependence).

A single cell that divides abnormally eventually forms a mass called a tumor. A tumor can be benign and harmless; the common wart is a benign tumor formed from a slowly expanding mass of cells. In contrast, a malignant neoplasm (malignant tumor) is a proliferation of rapidly growing cells that progressively infiltrate, invade, and destroy surrounding tissue. Tumors develop angiogenic potential, which is the capacity to form new blood vessels and capillaries. Thus, tumors can generate their own blood supply to bring in oxygen and nutrients. Cancer cells also can metastasize, separating from the growing mass of the tumor and traveling through the blood or lymph to unrelated organs, where they establish new growths of cancer cells.

The transformation of a normal cell to a cancer cell begins with damage to DNA (base changes or strand breaks) caused by chemical carcinogens, UV light, viruses, or replication errors (see Chapter 13). Mutations result from the damaged DNA if it is not repaired properly or if it is not repaired before replication occurs. A mutation that can lead to transformation also may be inherited. When a cell with one mutation proliferates, this clonal expansion (proliferation of cells arising from a single cell) results in a substantial population of cells containing this one mutation, from which one cell may acquire a second mutation relevant to control of cell growth or death. With each clonal expansion, the probability of another transforming mutation increases. As mutations accumulate in genes controlling proliferation, subsequent mutations occur even more rapidly until the cells acquire the multiple mutations (in the range of 4–7) necessary for full transformation.

The transforming mutations occur in genes that regulate cellular proliferation and differentiation (proto-oncogenes), suppress growth (tumor suppressor genes), target irreparably damaged cells for apoptosis, or repair damaged DNA. The genes regulating cellular growth are called proto-oncogenes, and their mutated forms are called oncogenes. The term *oncogene* is derived from the Greek word "onkos" meaning bulk or tumor. A transforming mutation in a proto-oncogene increases the activity or amount of the gene product (a gain-of-function mutation). Tumor suppressor genes (normal growth suppressor genes) and repair enzymes protect against uncontrolled cell proliferation. A transforming mutation in these protective genes results in a loss of activity or a decreased amount of the gene product. In summary, cancer is caused by the accumulation of mutations in the genes involved in normal cellular growth and differentiation. These mutations give rise to cancer cells capable of unregulated, autonomous, and infinite proliferation.

## II. DAMAGE TO DNA LEADING TO MUTATIONS

### A. Chemical and Physical Alterations in DNA

An alteration in the chemical structure of DNA, or of the sequence of bases in a gene, is an absolute requirement for the development of cancer. The function of DNA depends on the presence of various polar chemical groups in DNA bases, capable of forming hydrogen bonds between DNA strands or other chemical reactions. The oxygen and nitrogen atoms in DNA bases are targets for a variety of electrophiles (electron-seeking chemical groups). A typical sequence of events leading to a mutation is shown for dimethylnitrosamine in Figure 18.2. Chemical carcinogens (compounds that can cause transforming mutations) found in the environment and ingested in foods are generally stable lipophilic compounds that, like dimethylnitrosamine, must be activated by metabolism in the body to react with DNA (see also benz[o]pyrene, Action of Mutagens, Chapter 13, section III.A.). Many

Drs. Michael Bishop and Harold Varmus demonstrated that cancer is not caused by unusual and novel genes, but rather by mutation within existing cellular genes and that for every gene that causes cancer (an oncogene) there was a corresponding cellular gene, called the proto-oncogene. Although this concept seems straightforward today, it was a significant finding when it was first announced and, in 1989, Drs. Bishop and Varmus were awarded the Nobel Prize in Medicine.

The first experiments to show that oncogenes were mutant forms of proto-oncogenes in human tumors involved cells cultured from a human bladder carcinoma. The DNA sequence of the *ras* oncogene cloned from these cells differed from the normal *c-ras* proto-oncogene. Similar mutations were subsequently found in the *ras* gene of lung and colon tumors. **Colin Tuma's** malignant polyp had a mutation in the *ras* proto-oncogene.

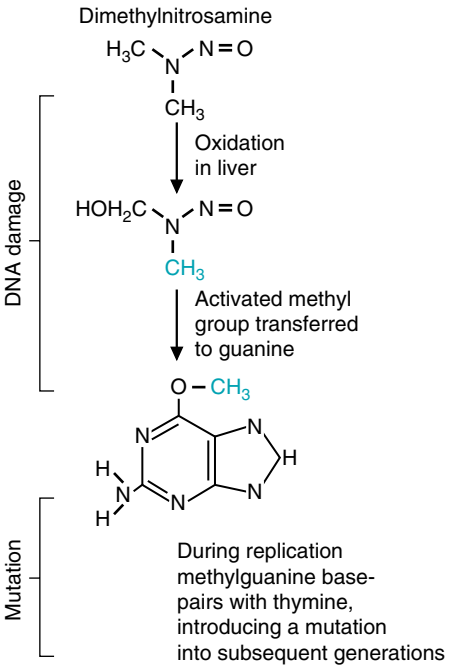



**Fig. 18.2.** Mutations in DNA caused by nitrosamines. Nitrosamines are consumed in many natural products and are produced in the stomach from nitrites used as preservatives and secondary amines found in foods such as fish. They are believed to be responsible for the high incidence of gastric cancer found in Japan and Iceland, where salt-preserved fish was a major dietary item. Nitrosamine metabolites methylate guanine (the transferred methyl group is shown in blue).

UV rays derived from the skin induce an increased incidence of all skin cancers, including squamous cell carcinoma, basal cell carcinoma, and malignant melanoma of the skin. The wavelength of UV light most associated with skin cancer is UVB (280–320 nm), which forms pyrimidine dimers in DNA. This type of DNA damage is repaired by nucleotide excision repair pathways that require products of at least 20 genes. With excessive exposure to the sun, the nucleotide excision repair pathway is overwhelmed, and some damage remains unrepaired.

Burkitt's lymphoma, a general name for a number of types of B-cell malignancies, results from a translocation between chromosomes 8 and 14. The translocation of genetic material moves the proto-oncogene transcription factor *c-myc* (normally found on chromosome 8) to chromosome 14. The translocated gene is now under the control of the promoter region for the immunoglobulin heavy chain gene, which leads to inappropriate and overexpression of *c-myc*. The result may be uncontrolled cell proliferation and tumor development. All subtypes of Burkitt's lymphoma contain this translocation. Epstein-Barr virus infection of B cells is also associated with certain types of Burkitt's lymphoma.

**Mannie Weitzels'** bone marrow cells contain the Philadelphia chromosome, typical of chronic myelogenous leukemia (CML). The Philadelphia chromosome results from a reciprocal translocation between the long arms of chromosome 9 and 22. As a consequence, a fusion protein is produced containing the N-terminal region of the Bcr protein from chromosome 22 and the C-terminal region of the Abl protein from chromosome 9. *Abl* is a proto-oncogene, and the resulting fusion protein (Bcr-Abl) has lost its regulatory region and is constitutively active. When active, Abl stimulates the Ras pathway of signal transduction, leading to cell proliferation.

The oncogene N-myc (a cell proliferation transcription factor, related to c-myc) is amplified in some neuroblastomas, and amplification of the erb-B2 oncogene (a growth factor receptor) is associated with several breast carcinomas.

chemotherapeutic agents, which are designed to kill proliferating cells by interacting with DNA, may also may act as carcinogens and cause new mutations and tumors while eradicating the old. Structural alterations in DNA also occur through radiation and through UV light, which causes the formation of pyrimidine dimers. More than 90% of skin cancers occur in sunlight-exposed areas. Thus, each chemical carcinogen or reactant creates a characteristic modification in a DNA base. The DNA damage, if not repaired, introduces a mutation into the next generation when the cell proliferates.

## B. Gain-of-Function Mutations in Proto-oncogenes

Proto-oncogenes are converted to oncogenes by mutations in the DNA that cause a "gain- in-function," that is, the protein can now function better in the absence of the normal activating events. Several mechanisms that lead to the conversion of proto-oncogenes to oncogenes are known:

- Radiation and chemical carcinogens act (a) by causing a mutation in the regulatory region of a gene, increasing the rate of production of the proto-oncogene protein, or (b) by producing a mutation in the coding portion of the oncogene that results in the synthesis of a protein of slightly different amino acid composition capable of transforming the cell (Fig. 18.3A).
- The entire proto-oncogene or a portion of it may be transposed or translocated, that is, moved from one position in the genome to another (Fig. 18.3B). In its new location, the proto-oncogene may be controlled by a more active promoter and, therefore, overexpressed (increased amounts of the protein product may be produced). If only a portion of the proto-oncogene is translocated, it may be expressed as a truncated protein with altered properties, or it may fuse with another gene and produce a fusion protein containing portions of what normally were two separate proteins. The truncated or fusion protein would be hyperactive and cause inappropriate cell growth.
- The proto-oncogene may be amplified (Fig. 18.3C), so that multiple copies of the gene are produced in a single cell. If more genes are active, more proto-oncogene protein will be produced, increasing the growth rate of the cells.
- If an oncogenic virus infects a cell, its oncogene may integrate into the host cell genome, permitting production of the abnormal oncogene protein. The cell may be transformed and exhibit an abnormal pattern of growth. Rather than inserting an oncogene, a virus may simply insert a strong promoter into the host cell genome. This promoter may cause an increased or untimely expression of a normal proto-oncogene.

The important point to remember is that transformation results from abnormalities in the normal growth regulatory program caused by gain-of-function mutations in proto-oncogenes. However, loss-of-function mutations also must occur in the tumor suppressor genes, repair enzymes, or activators of apoptosis for full transformation to a cancer cell.

## C. Mutations in Repair Enzymes

Repair enzymes are the first line of defense preventing conversion of chemical damage in DNA to a mutation (see Chapter 13, section III.B). DNA repair enzymes are tumor suppressor genes in the sense that errors repaired before replication do not become mutagenic. DNA damage is constantly occurring from exposure to sunlight, background radiation, toxins, and replication error. If DNA repair enzymes are absent, mutations accumulate much more rapidly, and once a mutation develops in a growth regulatory gene, a cancer may arise. As an example, inherited mutations in the tumor suppressor genes *brca1* and *brca2* predispose women to the development of breast cancer.

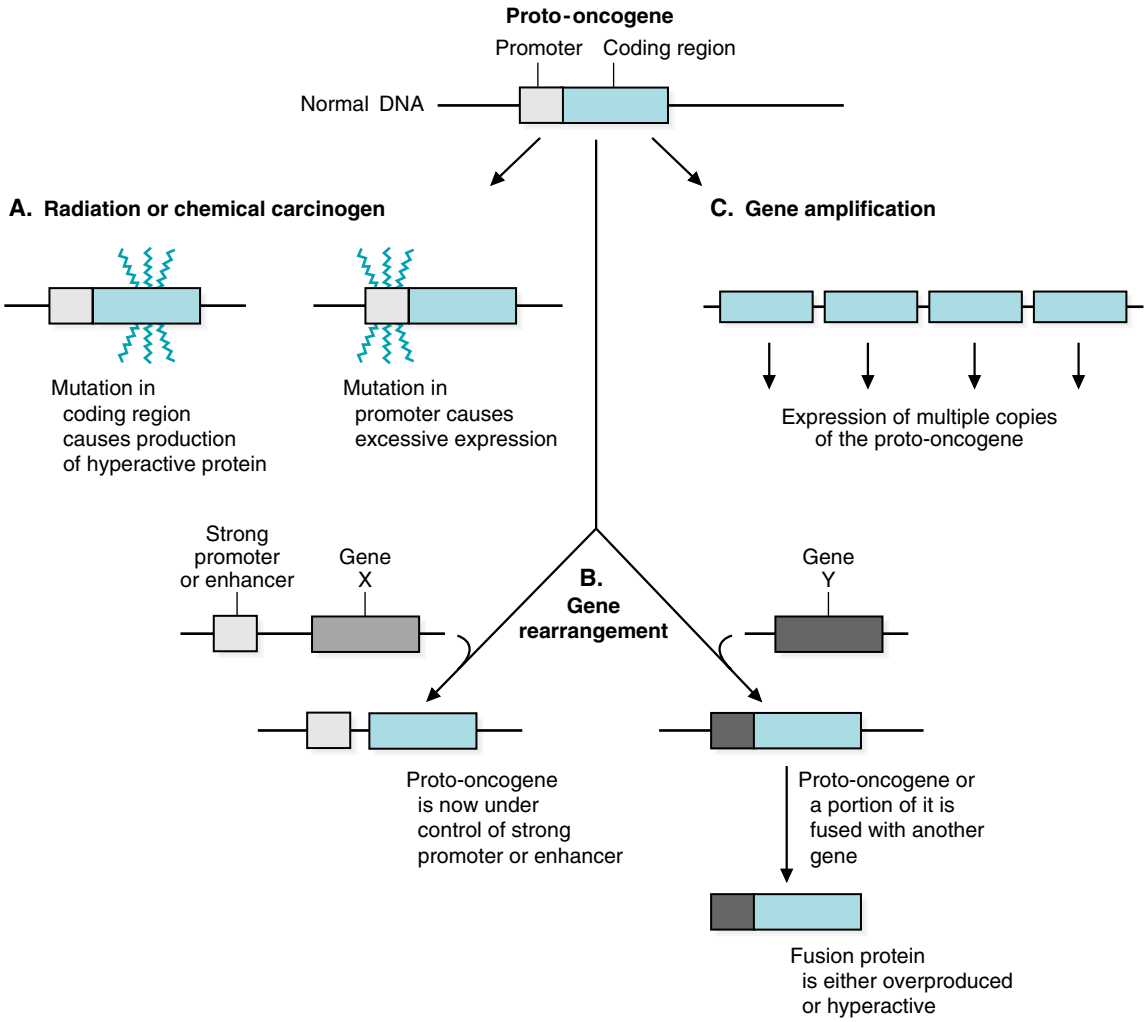



**Fig. 18.3.** Transforming mutations in proto-oncogenes. (A) Effect of radiation or chemical carcinogens on proto-oncogenes or their promoters. The mutations may be point mutations, deletions, or insertions. (B) Gene rearrangements as caused by transposition or translocation of a proto-oncogene or proto-oncogene fragment. (C) Amplification of a proto-oncogene allows more protein to be produced. The proto-oncogene and the resulting oncogene are shown in blue.

The protein products of these genes play roles in DNA repair, recombination, and regulation of transcription. A second example, HNPCC (hereditary non-polyposis colorectal cancer), was previously introduced in Chapter 13. It results from inherited mutations in enzymes involved in the DNA mismatch repair system.

## III. ONCOGENES

Proto-oncogenes control normal cell growth and division. These genes encode proteins that are growth factors, growth factor receptors, signal transduction proteins, transcription factors, cell cycle regulators, and regulators of apoptosis (Table 18.1). (The name representing the gene of an oncogene is referred to in lowercase letters and italics [e.g., *myc*], but the name of the protein product is capitalized and italics are not used [e.g., Myc]). The mutations in oncogenes giving rise to transformation are usually gain-of-function mutations; either a more active protein is produced or an increased amount of the normal protein is synthesized.

Although mutations in both the brca1 and brca2 genes are linked to breast cancer development in women, there are some fundamental differences in how these genes interact with other proteins at the molecular level. As a result, there are some differences in the diseases expressed by mutations within these genes. For example, *brca1* mutations are also linked to ovarian cancer and *brca2* mutations are not. *Brca2* mutations have been linked to pancreatic cancer, whereas *brca1* mutations have not. Men with inherited *brca2* mutations develop breast cancer; but men who carry *brca1* mutations do not.

**Table 18.1 Classes of Oncogenes, Mechanism of Activation, and Associated Human Tumors**

| Class | Proto-oncogene | Activation Mechanism | Location | Disease |
|---|---|---|---|---|
| **Growth Factors** | | | | |
| Platelet-Derived Growth Factor-β Chain | *sis* | Overexpression | Secreted | Astrocytoma<br>Osteosarcoma |
| Fibroblast growth factors | *int-2* | Amplification | Secreted | Breast cancer<br>Bladder cancer<br>Melanoma |
| **Growth Factor Receptors** | | | | |
| Epidermal Growth Factor Receptor Family | *erb-B1*<br>*erb-B2* | Overexpression<br>Amplification | Cell membrane<br>Cell membrane | Squamous cell carcionoma of the lung<br>Breast, ovarian, lung, stomach cancer |
| Platelet- Derived Growth Factor-Receptor | *PDGFR* | Translocation | Cell membrane | Chronic myelomonocytic leukemia |
| Hedgehog Receptor | *SMO* | Point mutation | Cell membrane | Basal cell carcinoma |
| **Signal Transduction Proteins** | | | | |
| G proteins | *ras* | Point mutation | Cytoplasm | Multiple cancers including lung, colon, thyroid, pancreas, many leukemias |
| Serine/threonine kinase | *akt2* | Amplification | Cytoplasm | Ovarian carcinoma |
| | *raf* | Overexpression | Cytoplasm | Myeloid leukemia |
| Tyrosine kinase | *abl* | Translocation | Cytoplasm | Chronic myeloid leukemia<br>Acute lymphoblastic leukemia |
| | *src* | Overexpression | Cytoplasm | Breast |
| **Hormone Receptors** | | | | |
| Retinoid receptor | *RARα* | Translocation | Nucleus | Acute promyelocytic leukemia |
| **Transcription Factors** | | | | |
| | *Hox11* | Translocation | Nucleus | Acute T cell leukemia |
| | *Myc* | Translocation<br>Amplification | Nucleus<br>nucleus | Burkitt's lymphoma<br>Neuroblastoma, small cell carcinoma of the lung |
| | *fos, jun* | Phosphorylation | Nucleus | Breast cancer |
| **Apoptosis Regulators** | | | | |
| | *Bcl-2* | Translocation | Mitochondria | Follicular B-cell lymphoma |
| **Cell Cycle Regulators** | | | | |
| Cyclins | Cyclin D | Translocation<br>Amplification | Nucleus<br>Nucleus | Lymphoma<br>Breast, liver, esophageal cancer |
| Cyclin-dependent kinase | CDK4 | Amplification<br>Point mutation | Nucleus<br>Nucleus | Gglioblastoma, sarcoma<br>Melanoma |

The gene for the human epidermal growth factor receptor (*HER2, c-erbB-2*) is over-expressed in 10 to 20% of breast cancer cases. When this gene is over-expressed, the prognosis for recovery is poor, as the patients display shorter disease-free intervals, increased risks for metastasis, and a resistance to therapy. Clinical trials are underway using a monoclonal antibody directed against this receptor (herceptin) to inactivate it. Preliminary results are encouraging in that use of this drug, either alone or in combination with others, appears to control the growth of some tumors overexpressing the *HER2* gene. However, not all tumors over-expressing *HER2* are responsive to herceptin. Thus, it appears that a complete genotyping of breast cancer cells may be necessary (using the microarray techniques described in Chapter 17) to specifically develop an effective therapy for each patient with the disease.

## A. Oncogenes and Signal Transduction Cascades

All of the proteins in growth factor signal transduction cascades are proto-oncogenes (Fig18.4).

### 1. GROWTH FACTORS AND GROWTH FACTOR RECEPTORS

The genes for both growth factors and growth factor receptors are oncogenes.

Growth factors generally regulate growth by serving as ligands that bind to cellular receptors located on the plasma membrane (cell-surface receptors) (see Chapter 11). Binding of ligands to these receptors stimulates a signal transduction pathway in the cell activating the transcription of certain genes. If too much of a growth factor or a growth factor receptor is produced, the target cells may respond by proliferating inappropriately. Growth factors receptors may also become oncogenic through translocation or point mutations in domains affecting binding of the growth factor, dimerization, kinase activity, or some other aspect of their signal transmission. In such cases, the receptor transmits a proliferative signal even though the growth factor normally required to activate the receptor is absent. In other words, the receptor is stuck in the "on" position.

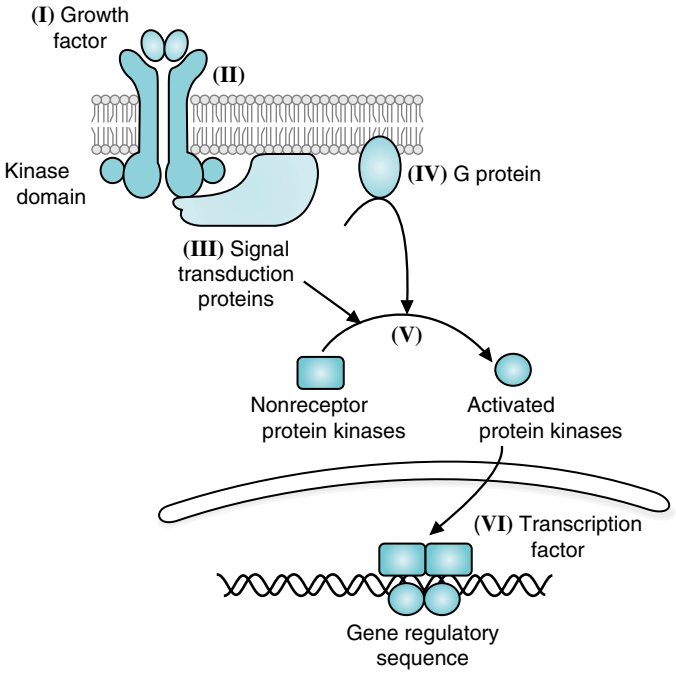



**Fig. 18.4.** Proto-oncogene sites for transforming mutations in growth factor signaling pathways. I. The amount of growth factor. II. The receptor, which normally must bind the growth factor to dimerize and activate a kinase domain. III. Signal transduction proteins. Some, such as PI 3-kinase, form second messengers. IV. G proteins, and their regulators, which are also signal transduction proteins. V. Nonreceptor protein kinase cascades, which lead to phosphorylation of transcription factors. VI. Nuclear transcription factors normally activated through phosphorylation or binding of a ligand.

## 2. SIGNAL TRANSDUCTION PROTEINS

The genes encoding proteins involved in growth factor signal transduction cascades may also be proto-oncogenes. Consider, for example, the monomeric G protein Ras. Binding of growth factor leads to the activation of Ras (see Fig 11.11). When Ras binds GTP, it is active, but Ras slowly inactivates itself by hydrolyzing its bound GTP to GDP and Pi. This controls the length of time that Ras is active. Ras is converted to an oncogenic form by point mutations that decrease the activity of the GTPase domain of Ras, thereby increasing the length of time it remains in the active form.

Ras, when active, activates the serine/threonine kinase Raf (a MAP kinase kinase kinase), which activates MEK (a MAP kinase kinase), which activates MAP kinase (Fig. 18.5). Activation of MAP kinase results in the phosphorylation of cytoplasmic and nuclear proteins, followed by increased transcription of the transcription factor proto-oncogenes *myc* and *fos* (see below). Note that mutations in the genes for any of the proteins that regulate MAP kinase activity, as well as those proteins induced by MAP kinase activation, can lead to uncontrolled cell proliferation.

## 3. TRANSCRIPTION FACTORS

Many transcription factors, such as Myc and Fos, are proto-oncoproteins (the products of proto-oncogenes). MAP kinase, in addition to inducing *myc* and *fos,* also directly activates the AP-1 transcription factor through phosphorylation (see Fig.18.5). AP-1 is a heterodimer formed by the protein products of the *fos* and *jun* families of proto-oncogenes. The targets of AP-1 activation are genes involved in

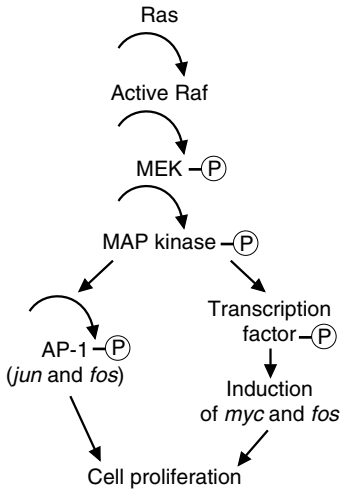



**Fig. 18.5.** Phosphorylation cascade leading to activation of proto-oncogene transcription factors *myc, fos,* and *jun.*

The synthesis of the transcription factor C-myc is tightly regulated in normal cells, and it is expressed only during the S phase of the cell cycle. In a large number of tumor types, this regulated expression is lost, and c-myc becomes inappropriately expressed or overexpressed throughout the cell cycle, driving cells continuously to proliferation.

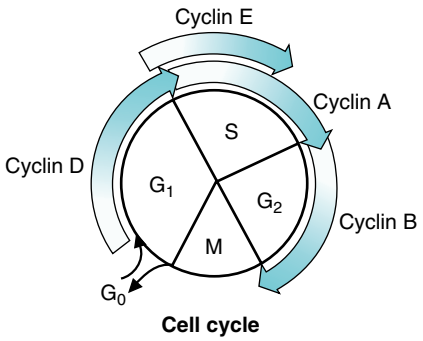



**Fig. 18.6.** Cyclin synthesis during different phases of the cell cycle.

The proteins induced by E2F include cyclin E, cyclin A, cdc25A (an activating protein phosphatase), and proteins required to bind at origins of replication to initiate DNA synthesis. The synthesis of cyclin E allows it to complex with cdk2, forming another active cyclin complex that retains activity into S phase (see Fig. 18.6). Cyclin A activates Cdk2. Thus, each phase of the cell cycle activates the next through cyclin synthesis. The cyclins are removed by regulated proteolysis.

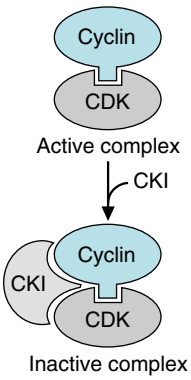



**Fig. 18.7.** CKI inhibition of cyclin/CDK activity.

cellular proliferation and progression through the cell cycle, as are the targets of the *myc* transcription factor. The net result is increased production of the proteins that carry out the processes required for proliferation.

## B. Oncogenes and the Cell Cycle

The growth of human cells, involving DNA replication and cell division in the cell cycle, is activated by growth factors, hormones, and other messengers. These activators work through cyclins and cyclin- dependent kinases (CDKs) that control progression from one phase of the cycle to another (Fig 18.6). For quiescent cells to proliferate, they must leave $G_0$ and enter the G1 phase of the cell cycle (see Chapter 13, Fig. 13.7). If the proper sequence of events occurs during G1, the cells enter the S phase and are committed to DNA replication and cell division. Similarly, during G2 cells make a commitment to mitotic division. CDKs are made constantly throughout the cell cycle but require binding of a specific cyclin to be active. Different cyclins made at different times in the cell cycle control each of the transitions (G1/S, S/G2, G2/M).

The activity of the cyclin–CDK complex is further regulated through phosphorylation and through inhibitory proteins called cyclin-dependent kinase inhibitors (CKIs) (Fig. 18.7). CKIs slow cell cycle progression by binding and inhibiting the CDK–cyclin complexes. CDKs are also controlled through activating phosphorylation by CAK (cyclin-activating kinases) and inhibitory hyperphosphorylation kinases.

To illustrate the role of these proteins, consider some of the events occurring at the G1/S checkpoint (Fig. 18.8). Because the cell is committed to DNA replication and division once it enters the S phase, multiple regulatory proteins are involved in determining whether the cell is ready to pass this checkpoint. These regulatory proteins include cdk4 and cdk6 (which are constitutively produced throughout the cell cycle), cyclin D (whose synthesis is only induced after growth factor stimulation of a quiescent cell), the retinoblastoma gene product (Rb), and a class of transcription factors known collectively as E2F. In quiescent cells, Rb is complexed with E2F, resulting in inhibition of these transcription factors. On growth factor stimulation, the cyclin Ds are induced (there are three types of cyclin D; D1, D2, and D3). They bind to cdk4 and cdk6, converting them to active protein kinases. One of the targets of cyclin/cdk phosphorylation is the Rb protein. Phosphorylation of Rb releases it from E2F, and E2F is then free to activate the transcription of genes required for entry into S. The Rb protein is a tumor suppressor gene (more below).

Progression through the cell cycle is opposed by the CKIs (see Fig. 18.8). The CKIs regulating cyclin/cdk expression in the G1 phase of the cell cycle fall into two categories: the Cip/Kip family and the INK4 family. The Cip/Kip family members (p21, p27, and p57) have a broad specificity and inhibit all cyclin–CDK complexes. The INK4 family, which consists of p15, p16, p18, and p19, are specific for the cyclin D–cdk4/6 family of complexes (**in**hibitors of cyclin-dependent **k**inase-**4**). The regulation of synthesis of different CKIs is complex, but some are induced by DNA damage to the cell and halt cell cycle progression until the damage can be repaired. For example, the CKI p21 (a protein of 21,000 Daltons) is a key member of this group that responds to specific signals to block cell proliferation. If the damage cannot be repaired, an apoptotic pathway is selected, and the cell dies.

In addition to sunlight and a preexisting nevus, hereditary factors also play a role in the development of malignant melanoma. Ten percent of melanomas tend to run in families. Some of the suspected melanoma-associated genes include the tumor suppressor gene p16 (an inhibitor of cdk 4) and CDK4. **Mel Anoma** was the single child of parents who had died of a car accident in their 50s, and thus, a familial tendency could not be assessed.

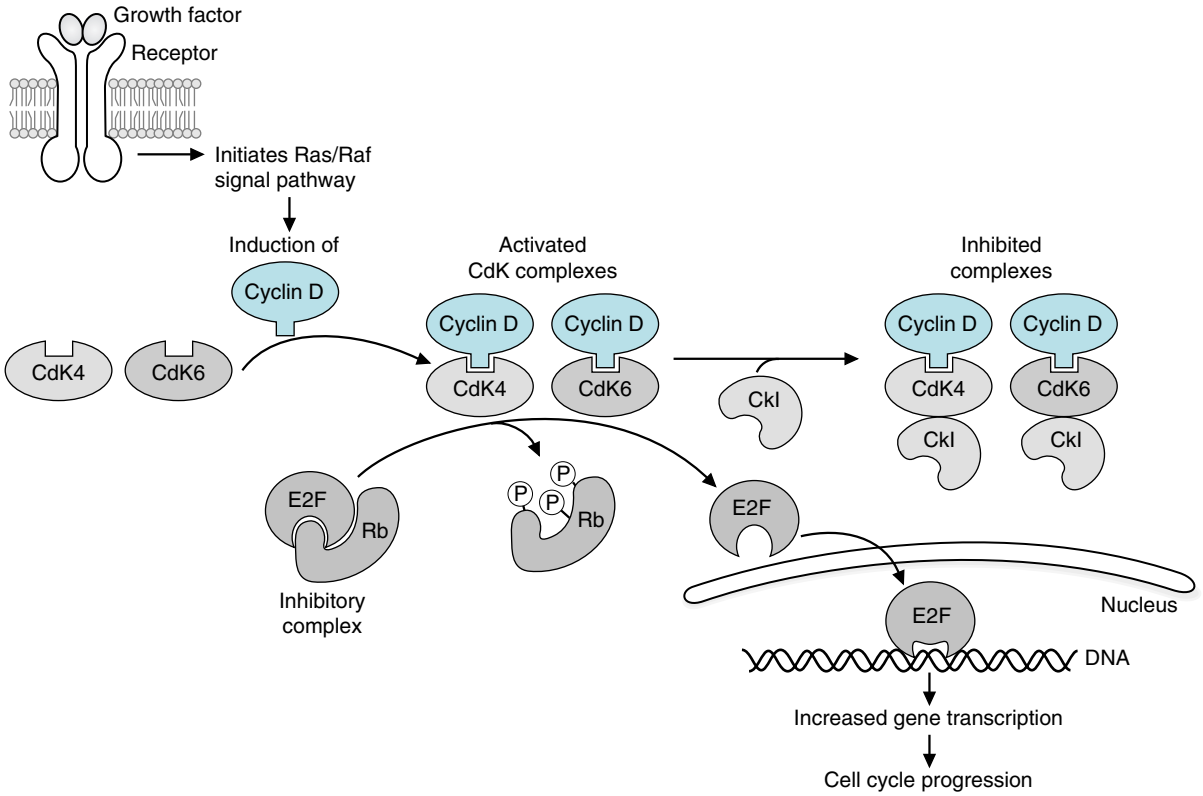



**Fig. 18.8.** Control of the G1/S transition in the cell cycle. The genes encoding cyclins and CDKs are oncogenes, and the gene encoding the Retinoblastoma protein (Rb) is a tumor suppressor gene. Abbreviations: CDK, cyclin- dependent kinase; CDKI, cyclin-dependent kinase inhibitor.

## IV. TUMOR SUPPRESSOR GENES

Like the oncogenes, the tumor suppressor genes encode molecules involved in the regulation of cell proliferation. Table 18.2 provides several examples. The normal function of tumor suppressor proteins is generally to inhibit proliferation in response to certain signals such as DNA damage. The signal is removed when the cell is fully equipped to proliferate; the effect of their elimination of tumor suppressor genes is to remove the brakes on cell growth. They affect cell cycle regulation, signal transduction, transcription, and cell adhesion. The products of tumor suppressor genes frequently modulate pathways that are activated by the products of proto-oncogenes.

**Table 18.2 Tumor Suppressors**

| Class | Protein | Location | Associated Diseases |
|---|---|---|---|
| Adhesion protein | E-cadherin | Cell membrane | Stomach cancer |
| Receptor | PATCHED | Cell membrane | Basal cell carcinoma |
| | TGF–β receptor | Cell membrane | Colon cancer |
| Signal transduction | NF-1 | Under cell membrane | Neurofibrosarcoma |
| | SMAD4/DPC | Cytoplasm/nucleus | Pancreatic and colorectal cancer |
| Transcription factor | WT-1 | Nucleus | Wilms tumor |
| Cell cycle regulator | p16(INK4) | Nucleus | Melanoma, lung, pancreatic cancer |
| | Retinoblastoma | Nucleus | Retinblastoma, sarcomas |
| Cell cycle/apoptosis | p53 | Nucleus | Most cancers |
| DNA repair | BRACA 1 | Nucleus | Breast cancer |

Tumor suppressor genes contribute to the development of cancer when both copies of the gene are inactivated. This is different from the case of proto-oncogene mutations because only one allele of a proto-oncogene needs to be converted to an oncogene to initiate transformation.

## A. Tumor Suppressor Genes That Directly Regulate the Cell Cycle

The two best understood cell cycle regulators that are also tumor suppressors are the retinoblastoma (*rb*) and *p53* genes.

### 1. THE RETINOBLASTOMA (*rb*) GENE

As previously discussed, the retinoblastoma gene product, Rb, functions in the transition from G1 to S phase and regulates the activation of members of the E2F family of transcription factors (see Fig. 18.8). If an individual inherits a mutated copy of the *rb* allele, there is a 100% chance of that individual developing retinoblastoma, because of high probability that the second allele of *rb* will gain a mutation (Fig.18.9). This is considered familial retinoblastoma. Individuals who do not inherit mutations in *rb*, but who develop retinoblastoma, are said to have sporadic retinoblastoma, and acquire two specific mutations, one in each *rb* allele of the retinoblast, during their lifetime.

### 2. p53, THE GUARDIAN OF THE GENOME

The p53 protein is a transcription factor regulating the cell cycle and apoptosis, programmed cell death. Loss of both *p53* alleles is found in over more than 50% of human tumors. p53 acts as the "guardian of the genome" by halting replication in cells that have suffered DNA damage and targeting unrepaired cells to apoptosis.

Inheritance of a mutation in *p53* leads to Li-Fraumeni syndrome, which is characterized by multiple types of tumors. Mutations in *p53* are present in more than 50% of human tumors. These are secondary mutations within the cell, and if *p53* is mutated the overall rate of cellular mutation will increase because there is no p53 to check for DNA damage, to initiate the repair of the damaged DNA, or to initiate apoptosis if the damage is not repaired. Thus, damaged DNA is replicated, and the frequency of additional mutations within the same cell increases remarkably.

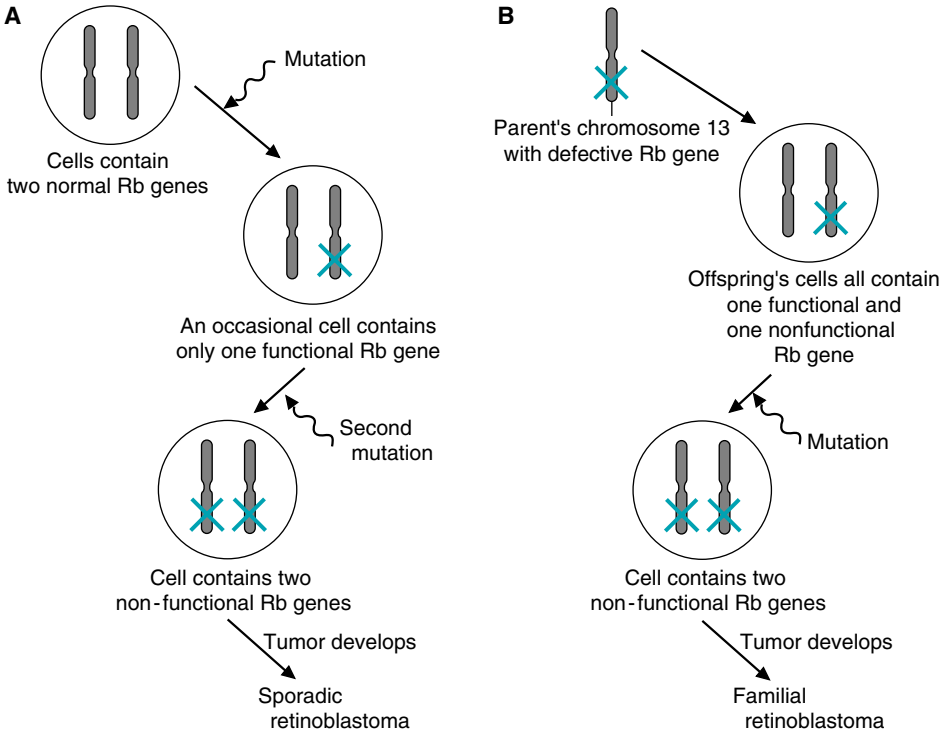



**Fig. 18.9.** Mutations in the retinoblastoma (Rb) gene. A. Sporadic retinoblastoma. B. Familial retinoblastoma.

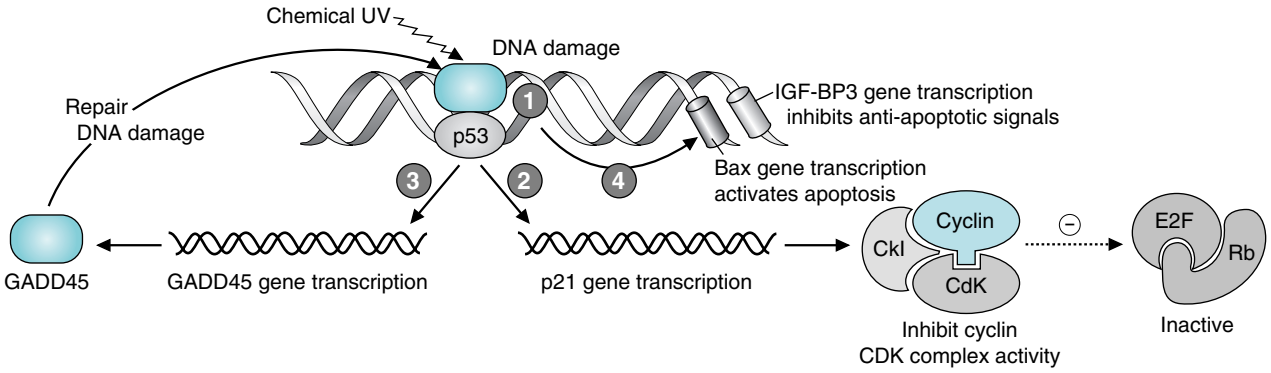



**Fig. 18.10.** p53 and cell cycle arrest. Mechanisms that recognize DNA damage stop p53 degradation and modify the p53 protein (circle 1). p53 stimulates the transcription of p21 (circle 2) and GADD45 (circle 3). p21 blocks the cyclin/CDK phosphorylation of Rb, which continues to inhibit the E2F family of transcription factors, thereby blocking cell progression through the cell cycle. GADD45 allows the DNA damage to be repaired. If the damage is not repaired, apoptotic genes are activated (circle 4).

In response to DNA-damaging mutagens, ionizing radiation, or ultraviolet light, the level of p53 rises (Fig. 18.10, circle 1). p53, acting as a transcription factor, stimulates transcription of *p21* (a member of the Cip/Kip family of CKIs), as shown in Figure 18.10, circle 2. The p21 gene product inhibits the cyclin/CDK complexes, which prevents the phosphorylation of Rb and release of E2F proteins. The cell is thus prevented from entering S phase. P53 also stimulates the transcription of GADD45 (Growth Arrest and DNA Damage), a DNA repair enzyme (Fig. 18.10, circle 3). If the DNA is successfully repaired, p53 induces its own downregulation. If the DNA repair was not successful, p53 activates two apoptosis genes, *bax* (discussed below) and *IGF-BP3* (Fig. 18.10, circle 4). The IGF-BP3 protein product binds the receptor for insulin-like growth factor, which presumably induces apoptosis by blocking the anti-apoptotic signaling by growth factors, and the cell enters a growth factor deprivation mode.

## B. Tumor Suppressor Genes That Affect Receptors and Signal Transduction

Tumor suppressor genes may encode receptors, components of the signaling transduction pathway, or transcription factors.

### 1. REGULATORS OF RAS

The Ras family of proteins is involved in signal transduction for many hormones and growth factors (see above), and is, therefore, oncogenic. The activity of these pathways is interrupted by GAPs (GTPase-activating proteins; see Chapter 9), which vary among cell types. Neurofibromin, the product of the tumor suppressor gene *NF-1*, is a nervous system-specific GAP that regulates the activity of Ras in neuronal tissues (Fig. 18.11). The growth signal is transmitted so long as the Ras protein binds GTP. Binding of NF-1 to Ras activates the GTPase domain of Ras, which hydrolyzes GTP to GDP, thereby inactivating Ras. Without a functional neurofibromin molecule, Ras is perpetually active.

### 2. PATCHED AND SMOOTHENED

A good example of tumor suppressors and oncogenes working together is provided by the co-receptor genes *patched* and *smoothened,* which encode the receptor for the hedgehog class of signaling peptides. These co-receptors normally function to control growth during embryogenesis and illustrate the importance of maintaining

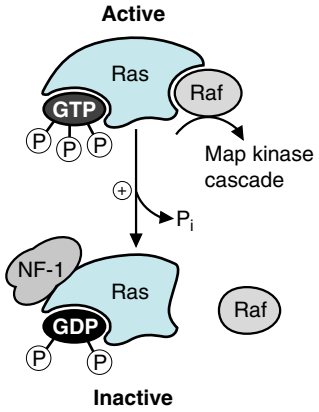
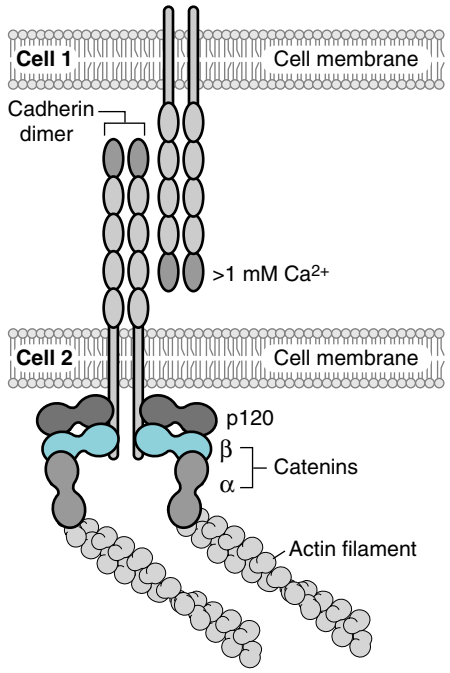



**Fig.18.11.** NF-1 (neurofibromin) binds to Ras and activates its GTPase activity, thereby decreasing cell proliferation. A mutation in neurofibromin leads to prolonged GTP binding and activation of Ras.

An inherited mutation in *NF-1* can lead to neurofibromatosis, a disease primarily of numerous benign, but painful, tumors of the nervous system. The movie *Elephant Man* was based on an individual who was believed to have this disease. Recent analysis of the patient's remains, however, indicates that he may have suffered from the rare Proteus syndrome, and not neurofibromatosis.

The strange names of some of the tumor suppressor genes arose because they were first discovered in Drosophila (fruit fly), and the names of Drosophila mutations are often based on the appearance of the fly expressing the mutation. Once the human homolog is found, it is given the same name as the Drosophila gene.

**A. Catenins and cadherins in cell attachment**




**B. β-catenin and APC in gene transcription**

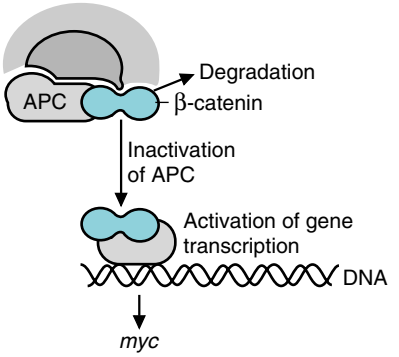



**Fig. 18.12.** A. Catenins and cadherins. E-cadherin molecules form intercellular, calcium-dependent homodimers with cadherins from another cell, resulting in cell–cell adhesion. The cytoplasmic portion of E-cadherin is complexed to a variety of catenins, which anchor the cadherin to the actin cytoskeleton. B. β-Catenin and APC in transcription. The APC complex activates β-catenin for proteolytic degradation. If APC is inactivated, β-catenin levels increase. It acts as a transcription factor that increases synthesis of *myc* and other genes regulating cell cycle progression.

a balance between oncogenes and tumor suppressor genes. The Patched receptor protein inhibits Smoothened, its co-receptor protein. Binding of a hedgehog ligand to Patched releases the inhibition of Smoothened, which then transmits an activating signal to the nucleus, stimulating new gene transcription. *Smoothened* is a proto-oncogene, and *patched* is a tumor suppressor gene. If *patched* loses its function (definition of a tumor suppressor), then Smoothened can signal the cell to proliferate, even in the absence of a hedgehog signal. Conversely, if *smoothened* undergoes a gain of function mutation (definition of an oncogene), it can signal in the absence of the hedgehog signal, even in the presence of Patched. Inherited mutations in either *smoothened* or *patched* will lead to an increased incidence of basal cell carcinoma.

### C. Tumor Suppressor Genes That Affect Cell Adhesion

The cadherin family of glycoproteins mediates calcium- dependent cell–cell adhesion. Cadherins from intercellular complexes binding cells together (Fig. 18.12A). They are anchored intracellularly by catenins, which bind to actin filaments. Loss of E-cadherin expression may contribute to the ability of cancer cells to detach and migrate in metastasis. Individuals who inherit a mutation in E cadherin (this mutation is designated as CDH1) are sharply predisposed to developing diffuse type gastric cancer.

The catenin proteins have two functions; in addition to anchoring cadherins to the cytoskeleton, they act as transcription factors (Fig. 18.12B). β-Catenin also binds to a complex containing the regulatory protein APC (adenomatous polyposis coli), which activates it for degradation. When the appropriate signal activates APC, β-catenin levels increase, and it travels to the nucleus, where it activates *myc* and *cyclin D1* transcription, leading to cell proliferation. APC is a tumor suppressor gene. If it is inactivated, it cannot bind β-catenin and inhibit cell proliferation. Mutations in APC or proteins interacting with it are found in the vast majority of sporadic human colon cancer. Inherited mutations in APC lead to the most common form of hereditary colon cancer, familial adenomatous polyposis.

## V. CANCER AND APOPTOSIS

In the body, superfluous or unwanted cells are destroyed by a pathway called apoptosis, or programmed cell death. Apoptosis is a regulated energy-dependent sequence of events by which a cell self-destructs. In this suicidal process, the cell shrinks, the chromatin condenses, and the nucleus fragments. The cell membrane forms blebs (outpouches), and the cell breaks up into membrane-enclosed apoptotic vesicles (apoptotic bodies) containing varying amounts of cytoplasm, organelles, and DNA fragments. Phosphatidylserine, a lipid on the inner leaflet of the cell membrane, is exposed on the external surface of these apoptotic vesicles. It is one of the phagocytic markers recognized by macrophages and other nearby phagocytic cells that engulf the apoptotic bodies.

Apoptosis is a normal part of multiple processes in complex organisms: embryogenesis, the maintenance of proper cell number in tissues, the removal of infected or otherwise injured cells, the maintenance of the immune system, and aging. It can be initiated by injury, radiation, free radicals or other toxins, withdrawal of growth

A form of apoptosis is a normal part of embryonic development. For example, the development of the nervous system uses apoptosis to destroy neurons that have not made the proper connections with target cells. Neurons are produced in excess, and more than 50% of developing neurons are eliminated by programmed cell death. Those neurons that have made the correct connections survive by secreting growth factors that block apoptosis.

factors or hormones, binding of pro-apoptotic cytokines, or interactions with cytotoxic T cells in the immune system. Apoptosis can protect organisms from the negative effect of mutations by destroying cells with irreparably damaged DNA before they proliferate. Just as an excess of a growth signal can produce an excess of unwanted cells, the failure of apoptosis to remove excess or damaged cells can contribute to the development of cancer.

## A. Normal Pathways to Apoptosis

Apoptosis can be divided into three general phases: an initiation phase, a signal integration phase, and an execution phase. Apoptosis can be initiated by external signals that work through death receptors, such as tumor necrosis factor (TNF), or deprivation of growth hormones (Fig. 18.13). It can also be initiated by intracellular events that affect mitochondrial integrity (e.g., oxygen deprivation, radiation), and irreparably damaged DNA. In the signal integration phase, these pro-apoptotic signals are balanced against anti-apoptotic cell survival signals by several pathways, including members of the Bcl-2 family of proteins. The execution phase is carried out by proteolytic enzymes called caspases.

### 1. CASPASES

Caspases are cysteine proteases that cleave peptide bonds next to an aspartate residue. They are present in the cell as procaspases, zymogen-type enzyme precursors activated by proteolytic cleavage of the inhibitory portion of their polypeptide chain. The different caspases are generally divided into two groups according to their function: initiator caspases, which specifically cleave other procaspases, and execution caspases, which cleave other cellular proteins involved in maintaining cellular integrity (see Fig. 18.13). The initiator caspases are activated through two major signaling pathways; the death receptor pathway and the mitochondrial integrity pathway. They activate the execution caspases, which cleave protein kinases involved in cell adhesion, lamins that form the inner lining of the nuclear envelope, actin and other proteins required for cell structure, and DNA repair enzymes. They also cleave an inhibitor protein of the endonuclease CAD (**c**aspase-**a**ctivated **D**Nase). With destruction of the nuclear envelope, additional endonucleases ($Ca^{2+}$- and $Mg^{2+}$ -dependent) also become activated.

### 2. THE DEATH RECEPTOR PATHWAY TO APOPTOSIS

The death receptors are a subset of TNF-1 receptors, which includes Fas/CD95, TNF-Receptor 1 (TNF-R1) and Death Receptor 3 (DR3). These receptors form a trimer that binds TNF-1 or another death ligand on its external domain and adaptor proteins to its intracellular domain (Fig.18.14). The activated TNF–receptor complex forms the scaffold for binding two molecules of procaspase 8, which autocatalytically cleave each other to form active caspase 8. Caspase 8 is an initiator caspase that activates execution caspases 3, 6, and 7. Caspase 8 also cleaves a Bcl-2 protein, Bid, to a form that activates the mitochondrial integrity pathway to apoptosis.

### 3. THE MITOCHONDRIAL INTEGRITY PATHWAY TO APOPTOSIS

Apoptosis is also induced by intracellular signals indicating that cell death should occur. Examples of these signals include growth factor withdrawal, cell injury, the release of certain steroids, and an inability to maintain low levels of intracellular calcium. All of these treatments, or changes, lead to release of cytochrome c from the mitochondria (Fig. 18.15). Cytochrome c is a necessary protein component of the mitochondrial electron transport chain that is a loosely bound to the outside of the inner mitochondrial membrane. Its release initiates apoptosis.

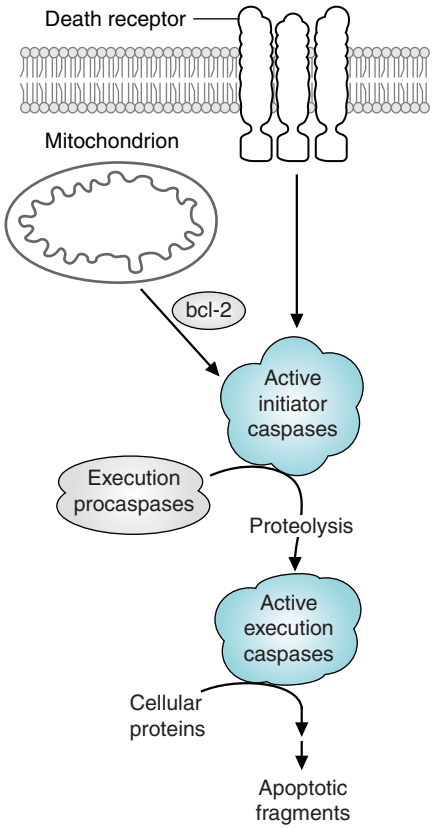



**Fig. 18.13.** Major components in apoptosis.

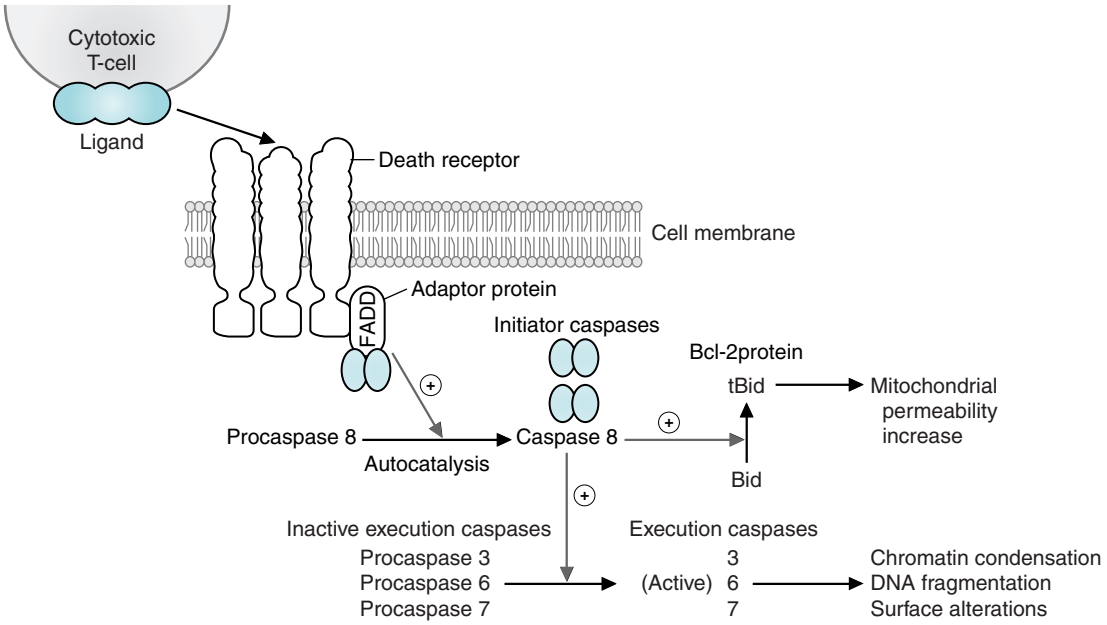



**Fig. 18.14.** The death receptor pathway to apoptosis. The ligand (usually a cell surface protein on another cell) binds to the death receptor, which makes a scaffold for autocatalytic activation of caspase 8. Active caspase 8 directly cleaves apoptotic execution caspases. However, the pathway also activates Bid, which acts on mitochondrial membrane integrity.

In the cytosol, cytochrome c binds Apaf (pro-**a**poptotic **p**rotease **a**ctivating **f**actor). The Apaf/cytochrome c complex binds caspase 9, an initiator caspase, to form an active complex called the apoptosome. The apoptosome in turn activates execution caspases by zymogen cleavage.

## 4. INTEGRATION OF PRO- AND ANTI-APOPTOTIC SIGNALS BY THE BCL-2 FAMILY OF PROTEINS

**Table 18.3 Bcl-2 Family Members**

| |
|---|
| *Anti-apoptotic* |
| Bcl-2 |
| Bcl-x |
| Bcl-w |
| *Proapoptotic* |
| *Channel Forming* |
| Bax |
| Bak |
| Bok |
| *Pro-apoptotic* |
| *BH3-Only* |
| Bad |
| Bid |
| Bod/Bim |

Roughly 30 Bcl-2 family members are currently known. These proteins play tissue-specific as well as signal pathway–specific roles in regulating apoptosis. The tissue-specificity is overlapping. For example, Bcl-2 is expressed in hair follicles, kidney, small intestines, neurons, and the lymphoid system, whereas Bcl-x is expressed in the nervous system and hematopoietic cells.

The Bcl-2 family members are decision-makers that integrate prodeath and antideath signals to determine whether the cell should commit suicide. Both pro-apoptotic and anti-apoptotic members of the *Bcl-2* family exist (Table 18.3).

The antiapoptotic *Bcl-2* –type proteins (including Bcl-2, Bcl-xL, Bcl-wL) have at least two ways of antagonizing death signals. They insert into the outer mitochondrial membrane to antagonize channel-forming pro-apoptotic factors, therby decreasing cytochrome c release. They may also bind cytoplasmic Apaf so that it cannot form the apoptosome complex (Fig. 18.16).

These anti-apoptotic Bcl-2 proteins are opposed by pro-apoptotic family members that fall into two categories: ion-channel forming members and the BH3-only members. The pro-death ion channel forming members, such as Bax, are very similar to the anti-apoptotic family members, except that they do not contain the binding domain for Apaf. They have the other structural domains, however, and when they dimerize with

When *Bcl-2* is mutated, and oncogenic, it is usually overexpressed, for example, in follicular lymphoma and CML (chronic myelogenous leukemia). Overexpression of *Bcl-2* disrupts the normal regulation of pro and anti-apoptotic factors and tips the balance to an anti-apoptotic stand. This leads to an inability to destroy cells with damaged DNA, such that mutations can accumulate within the cell. Bcl-2 is also a multi-drug resistance protein and if over-expressed will block the induction of apoptosis by antitumor agents by rapidly removing them from the cell. Thus, strategies are being developed to reduce Bcl-2 levels in tumors over-expressing it before initiating drug or radiation treatment.

pro-apoptotic BH3-only members in the outer mitochondrial membrane, they form an ion channel that promotes cytochrome c release rather than inhibiting it (see Fig. 18.16). The pro-death BH3-only proteins (e.g., Bim and Bid) contain only the structural domain that allows them to bind to other bcl-2 family members (the BH3 domain), and not the domains for binding to the membrane or forming ion channels. Their binding activates the pro-death family members and inactivates the anti-apoptotic members. When the cell receives a signal from a pro-death agonist, a BH3 protein like Bid is activated (see Fig. 18.16). The BH3 protein activates Bax (an ion-channel forming pro-apoptotic channel member), which stimulates release of cytochrome c. Normally Bcl-2 acts as a death antagonist by binding Apaf and keeping it in an inactive state. However, at the same time that Bid is activating Bax, Bid also binds to Bcl-2, thereby disrupting the Bcl-2/Apaf complex and freeing Apaf to bind to released cytochrome c to form the apoptosome.

## B. Cancer Cells Bypass Apoptosis

Apoptosis should be triggered by a number of stimuli, such as withdrawal of growth factors, elevation of p53 in response to DNA damage, monitoring of DNA damage by repair enzymes, or by release of TNF or other immune factors. However, mutations in oncogenes can create apoptosis-resistant cells.

One of the ways this occurs is through activation of growth factor–dependent signaling pathways that inhibit apoptosis, such as the PDGF/Akt/BAD pathway. Nonphosphorylated BAD acts like Bid in promoting apoptosis (see Fig. 18.16). Binding of the platelet-derived growth factor to its receptor activates PI-3 kinase, which phosphorylates and activates the serine/threonine kinase Akt (protein kinase B, see Chapter 11, section III.B.3). Activation of Akt results in the phosphorylation of the pro-apoptotic BH3-only protein BAD, which inactivates it. The PDGF/Akt/BAD pathway illustrates the requirement of normal cells for growth factor stimulation to prevent cell death. One of the features of neoplastic transformation is the loss of growth factor dependence for survival.

The MAP kinase pathway is also involved in regulating apoptosis and sends cell survival signals. MAP kinase kinase phosphorylates and activates another protein kinase known as RSK. Like Akt, RSK phosphorylates BAD and inhibits its activity. Thus, BAD acts as a site of convergence for the PI-3 kinase/Akt and MAP kinase pathways in signaling cell survival. Gain-of-function mutations in the genes controlling these pathways, such as *ras*, creates apoptosis- resistant cells.

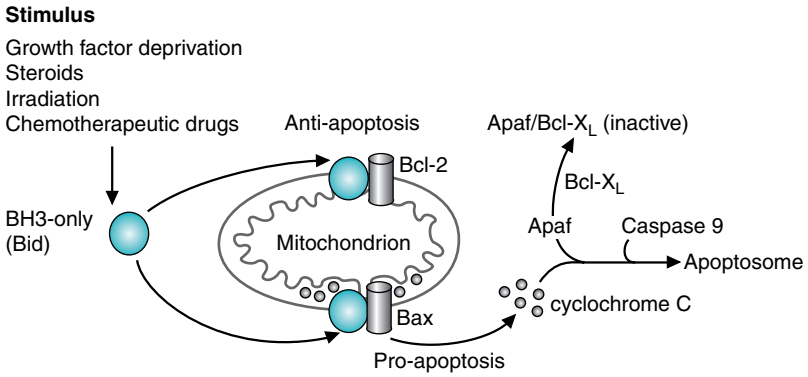



**Fig. 18.16.** Roles of the Bcl-2 family members in regulating apoptosis. Bcl-2, which is anti-apoptotic, binds Bid (or tBid) and blocks formation of channels that allow cytochrome c release from the mitochondria. Death signals result in activation of a BH3-only protein such as Bid, which can lead to mitochondrial pore formation, swelling, and release of cytochrome c. Bid binds to and activates the membrane ion-channel protein Bax, activating cytochrome c release, which binds to Apaf and leads to formation of the apoptosome.

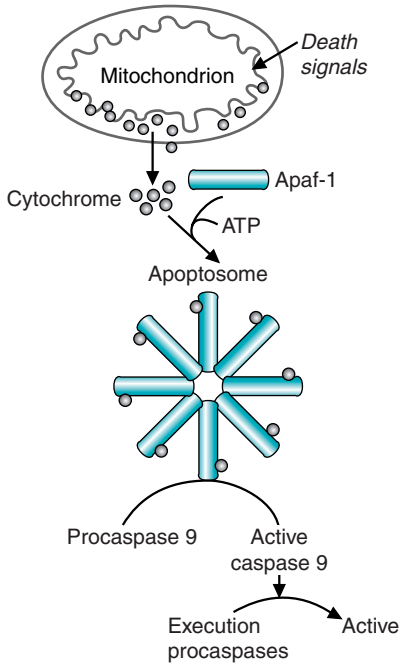



**Fig. 18.15.** The mitochondrial integrity pathway releases cytochrome c, which binds to Apaf and forms a multimeric complex called the apoptosome. The apoptosome converts procaspase 9 to active caspase, which it releases into the cytosol.

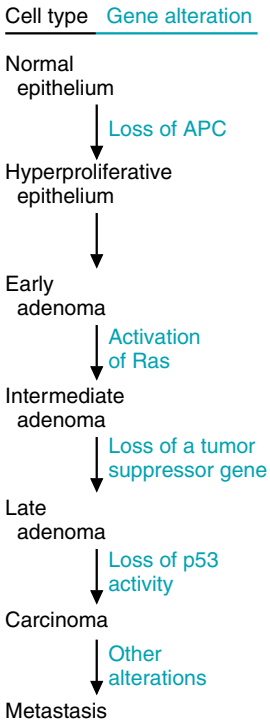



**Fig. 18.17.** Possible steps in the development of colon cancer. The changes do not always occur in this order, but the most benign tumors have the lowest frequency of mutations, and the most malignant have the highest frequency.

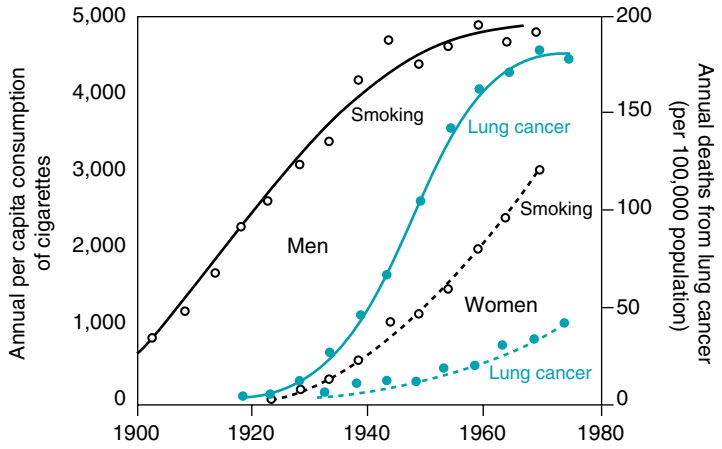
## VI. CANCER REQUIRES MULTIPLE MUTATIONS

Cancer takes a long time to develop in humans because multiple genetic alterations are required to transform normal cells into malignant cells (see Fig. 18.1). A single change in one oncogene or tumor suppressor gene in an individual cell is not adequate for transformation. For example, if cells derived from biopsy specimens of normal cells are not already "immortalized," that is, able to grow in culture indefinitely, addition of the *ras* oncogene to the cells is not sufficient for transformation. However, additional mutations in a combination of oncogenes, for example *ras* and *myc*, can result in transformation (Fig. 18.17). Epidemiologists have estimated that four to seven mutations are required for normal cells to be transformed.

Cells accumulate multiple mutations through clonal expansion. When DNA damage occurs in a normally proliferative cell, a population of cells with that mutation is produced. Expansion of the mutated population enormously increases the probability of a second mutation in a cell containing the first mutation. After one or more mutations in proto-oncogenes or tumor suppressor genes, a cell may proliferate more rapidly in the presence of growth stimuli and with further mutations grow autonomously, that is, independent of normal growth controls. Enhanced growth increases the probability of further mutations. Some families have a strong predisposition to cancer. Individuals in these families have inherited a mutation or deletion of one allele of a tumor suppressor gene, and as progeny of that cell proliferate, mutations can occur in the second allele, leading to a loss of control of cellular proliferation. These familial cancers include familial retinoblastoma, familial adenomatous polyps of the colon, and multiple endocrine neoplasia, one form of which involves tumors of the thyroid, parathyroid, and adrenal medulla (MEN type II).

Studies of benign and malignant polyps of the colon show that these tumors have a number of different genetic abnormalities. The incidence of these mutations increases with the level of malignancy. In the early stages, normal cells of the intestinal epithelium proliferate, develop mutations in the APC gene, and polyps develop. This change is associated with a mutation in the *ras* proto-oncogene that converts it to an active oncogene. Progression to the next stage is associated with a deletion or alteration of a tumor suppressor gene on chromosome 5. Subsequently, mutations occur in chromosome 18, inactivating a gene that may be involved in cell adhesion, and in chromosome 17, inactivating the *p53* tumor suppressor gene. The

**Nick O'Tyne** had been smoking for 40 years before he developed lung cancer. The fact that cancer takes so long to develop has made it difficult to prove that the carcinogens in cigarette smoke cause lung cancer. Studies in England and Wales show that cigarette consumption by men began to increase in the early 1900s. Followed by a 20-year lag, the incidence in lung cancer in men also began to rise. Women began smoking later, in the 1920s. Again the incidence of lung cancer began to increase after a 20-year lag.



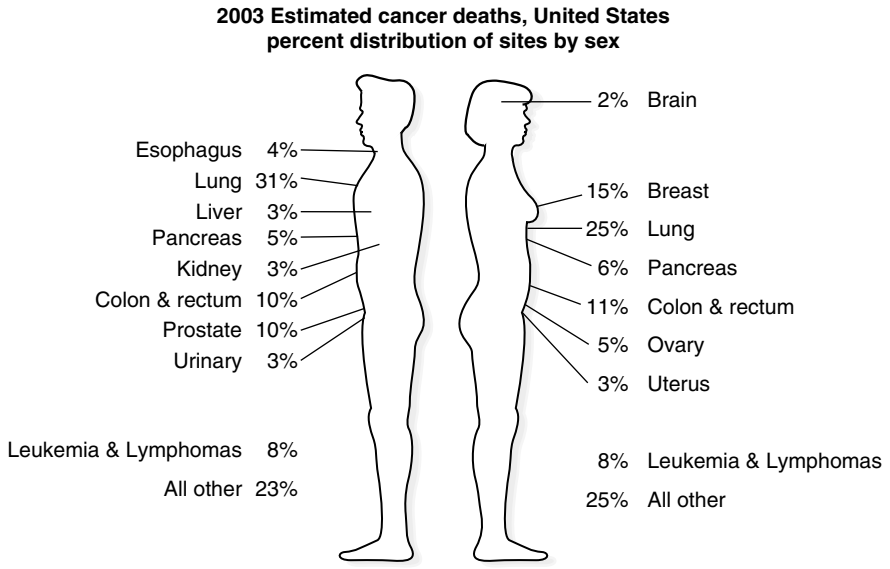



**Fig. 18.18.** Estimated cancer deaths by site and sex. Data from The American Cancer Society, Inc: Cancer Facts and Figures, 2003.

cells become malignant, and further mutations result in growth that is more aggressive and metastatic. This sequence of mutations is not always followed precisely, but an accumulation of mutations in these genes is found in a large percentage of colon carcinomas.

## VII. AT THE MOLECULAR LEVEL, CANCER IS MANY DIFFERENT DISEASES

More than 20% of the deaths in the United States each year are caused by cancer, with tumors of the lung, large intestine, and the breast being the most common (Fig. 18.18). Different cell types typically use different mechanisms through which they lose the ability to control their own growth. An examination of the genes involved in the development of cancer shows that a particular type of cancer can arise in multiple ways. For example, Patched and Smoothened are the receptor and co-receptor for the signaling peptide, sonic hedgehog. Either mutation of *smoothened,* an oncogene, or inactivation of *patched,* a tumor suppressor gene, can give rise to basal cell carcinoma. Similarly, transforming growth factor β and its signal transduction proteins SMAD4/DPC are part of the same growth-inhibiting pathway, and either may be absent in colon cancer. Thus, treatments which are successful for one patient with colon cancer may not be successful in a second patient with colon cancer because of the differences in the molecular basis of each individual's disease (this now also appears to be the case with breast cancer as well). Medical practice in the future will require identifying the molecular lesions involved in a particular disease and developing appropriate treatments accordingly. The use of gene chip technology (see Chapter 17) to genotype tumor tissues will aid greatly in allowing patient specific treatments to be developed.

A new treatment for CML based on rational drug design was recently introduced. The fusion protein Bcr-Abl is found only in the transformed cells expressing the Philadelphia chromosome and not in normal cells. Once the structure of Bcr-Abl was determined, the drug Gleevec was designed to specifically bind to and inhibit only the active site of the fusion protein and not the normal protein. Gleevec was successful in blocking Bcr-Abl function, thereby stopping cell proliferation, and in some cells would induce apoptosis, so the cells would die. Because normal cells do not express the hybrid protein, they were not affected by the drug. The problem with this treatment is that some patients suffered relapses, and when their Bcr-Abl proteins were studied it was found in some patients that the fusion protein had a single amino acid substitution near the active site that prevented Gleevec from binding to the protein. Other patients had an amplification of the Bcr-Abl gene product. Thus, Gleevec is a promising first step in designing drugs specifically targeted to tumor cells and is leading the way for rational drug design in the treatment of cancer.

## CLINICAL COMMENTS

**Mannie Weitzels.** The treatment of a symptomatic patient with CML whose white blood cell count is in excess of 50,000 cells/mL is usually initiated with busulfan. Alkylating agents such as cyclophos-

phamide have been used alone or in combination with busulfan. Purine and pyrimidine antagonists and hydroxyurea (an inhibitor of the enzyme ribonucleotide reductase, which converts ribonucleotides to deoxyribonucleotides for DNA synthesis) are sometimes effective in CML as well. In addition, trials with both γ- and β-interferon have shown promise in increasing survival in these patients. Interestingly, the latter agents have been associated with the disappearance of the Philadelphia chromosome in dividing marrow cells of some patients treated in this way.

The TNM system standardizes the classification of tumors. The T stands for the stage of tumor (the higher the number, the worse the prognosis), the N stands for the number of lymph nodes that are affected by the tumor (again, the higher the number, the worse the prognosis), and M stands for the presence of metastasis (0 for none, 1 for the presence of metastatic cells).

**Nick O' Tyne.** Surgical resection of the primary lung cancer with an attempt at cure was justified in Nick O'Tyne, who had a good prognosis with a $T_1,N_1,M_0$ staging classification preoperatively. Without some evidence of spread to the central nervous system at that time, a preoperative CT scan of the brain would not have been justified. This conservative approach would require scanning of all of the potential sites for metastatic disease from a non–small cell cancer of the lung in all patients who present in this way. In an era of runaway costs of health care delivery, such an approach could not be considered cost-effective.

Unfortunately, Mr. O'Tyne developed a metastatic lesion in the right temporal cortex of his brain. Because metastases were almost certainly present in other organs, Mr. O'Tyne's brain tumor was not treated surgically. In spite of palliative radiation therapy to the brain, Mr. O'Tyne succumbed to his disease just 9 months after its discovery, an unusually virulent course for this malignancy. On postmortem examination, it was found that his body was riddled with metastatic disease.

**Colin Tuma.** Colin Tuma requires yearly colonoscopies to check for new polyps in his intestinal tract. Because the development of a metastatic adenoma requires a number of years (because of the large numbers of mutations that must occur), yearly checks will enable new polyps to be identified and removed before malignant tumors develop.

Mutations associated with malignant melanomas include *ras* (gain-of-function in growth signal transduction oncogene), p53 (loss of function of tumor suppressor gene), p16 (loss of function in Cdk inhibitor tumor suppressor gene), Cdk4 (gain of function in a cell cycle progression oncogene) and cadherin/β-catenin regulation (loss of regulation that requires attachment).

**Mel Anoma.** The biopsy of Mel Anoma's excised mole showed that it was not malignant. The most important clinical sign of a malignant melanoma is a change in color in a pigmented lesion. Unlike benign (nondysplastic) nevi, melanomas exhibit striking variations in pigmentation, appearing in shades of black, brown, red, dark blue, and gray. Additional clinical warning signs of a melanoma are: enlargement of a preexisting mole, itching or pain in a preexisting mole, development of a new pigmented lesion during adult life, and irregularity of the borders of a pigmented lesion. **Mel Anoma** was advised to conduct a monthly self-examination, to have a clinical skin examination once or twice yearly, to avoid sunlight, and to use appropriate sunscreens.

## BIOCHEMICAL COMMENTS

**Viruses and human cancer.** Three RNA retroviruses are associated with the development of cancer in humans: HTLV-1, HIV, and hepatitis C. There are also DNA viruses associated with cancer.

**HTLV-1.** HTLV-1 causes adult T-cell leukemia. The HTLV-1 genome encodes a protein Tax, which is a transcriptional coactivator. The cellular proto-oncogenes c-*sis* and *c-fos* are activated by Tax, thereby altering the normal controls on cellular proliferation and leading to malignancy. Thus, *tax* is a viral oncogene without a counterpart in the host cell genome.

**HIV.** Infection with HIV, the virus causing acquired immunodeficiency disease (AIDS), leads to the development of neoplastic disease through several mechanisms. HIV infection leads to immunosuppression and, consequently, loss of immune-mediated tumor surveillance. HIV-infected individuals are predisposed to non-Hodgkins lymphoma, which results from an overproduction of T cell lymphocytes. The HIV genome encodes a protein, Tat, a transcription factor that activates transcription of the *interleukin-6* and *interleukin-10* genes in infected T cells. IL-6 and IL-10 are growth factors that promote proliferation of T cells and, thus, their increased production may contribute to the development of non-Hodgkins lymphoma. Tat can also be released from infected cells and act as an angiogenic (blood vessel forming) growth factor. This property is thought to contribute to the development of Kaposi's sarcoma.

**DNA viruses.** Some DNA viruses also cause human cancer, but by different mechanisms. Three DNA tumor virus families, SV40, papillomavirus, and adenovirus, encode proteins that inactivate pRb and p53. By interfering with the G1/S checkpoint, these oncoproteins increase the probability that mutations in oncogenes and tumor suppressor genes will be incorporated into the genome of infected cells, thereby increasing the probability of transformation. The Epstein-Barr virus encodes a Bcl-2 protein that restricts apoptosis of the infected cell.

## Suggested References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## REVIEW QUESTIONS—CHAPTER 18

1. The *ras* oncogene in Colin Tuma's malignant polyp differs from the *c-ras* proto-oncogene only in the region that encodes the N-terminus of the protein. This portion of the normal and mutant sequences is shown below:

```
                    10          20          30
Normal  ATGACGGAATATAAGCTGGTGGTGGTGGGCGCCGGCGGT
Mutant  ATGACGGAATATAAGCTGGTGGTGGTGGGCGCCGTCGGT
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This mutation is similar to the mutation found in the *ras* oncogene in various tumors. What type of mutation converts the *ras* proto-oncogene to an oncogene?

(A) An insertion that disrupts the reading frame of the protein
(B) A deletion that disrupts the reading frame of the protein
(C) A missense mutation that changes one amino acid within the protein
(D) A silent mutation that has no change in amino acid sequence of the protein
(E) An early termination that creates a stop codon in the reading frame of the protein

2. The mechanism through which Ras becomes an oncogenic protein is which of the following?

   (A) Ras remains bound to GAP.
   (B) Ras can no longer bind cAMP.
   (C) Ras has lost its GTPase activity.
   (D) Ras can no longer bind GTP.
   (E) Ras can no longer be phosphorylated by MAP kinase.

3. Which of the following statements best describes a characteristic of oncogenes?

   (A) All retroviruses contain at least one oncogene.
   (B) Retroviral oncogenes were originally obtained from a cellular host chromosome.
   (C) Proto-oncogenes are genes, found in retroviruses, that have the potential to transform normal cells when inappropriately expressed.
   (D) The oncogenes that lead to human disease are different from those that lead to tumors in animals.
   (E) Oncogenes are mutated versions of normal viral gene products.

4. When p53 increases in response to DNA damage, which of the following events occur?

   (A) p53 induces transcription of cdk4.
   (B) p53 induces transcription of cyclin D.
   (C) p53 binds E2F to activate transcription.
   (D) p53 induces transcription of p21.
   (E) p53 directly phosphorylates the transcription factor jun.

5. A tumor suppressor gene is best described by which of the following?

   (A) A gain-of-function mutation leads to uncontrolled proliferation.
   (B) A loss-of-function mutation leads to uncontrolled proliferation.
   (C) When expressed, the gene suppresses viral genes from being expressed.
   (D) When expressed, the gene specifically blocks the G1/S checkpoint.
   (E) When expressed, the gene induces tumor formation.