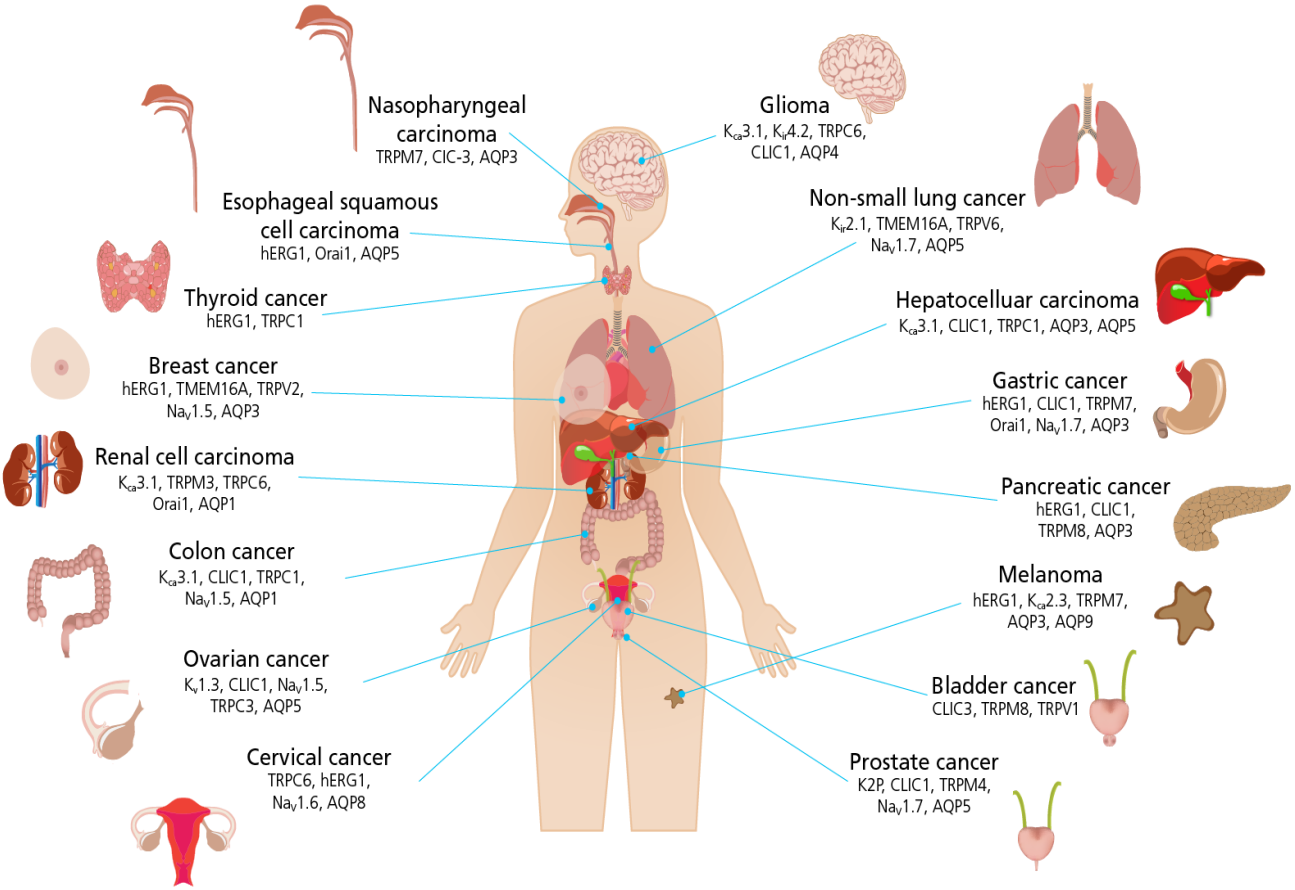
Course Materials Title: Cancer

Class: Zoology (Hons) Semester II



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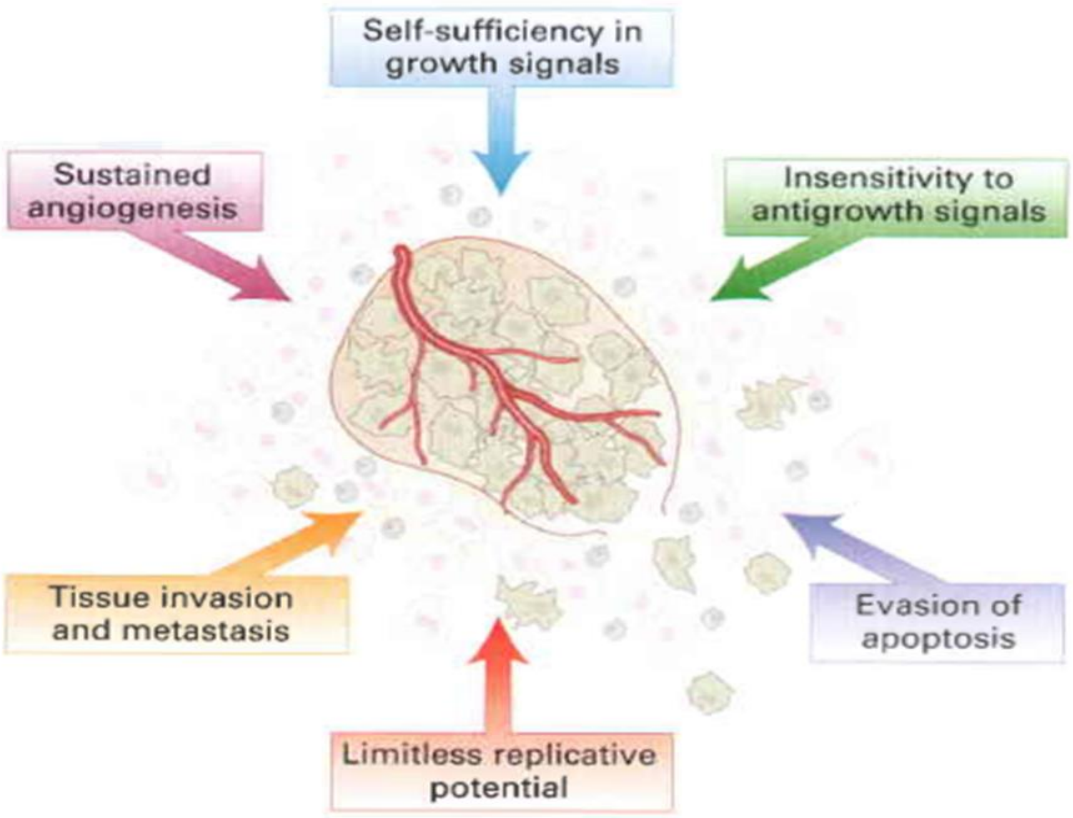
What Is Cancer?

Clinically, cancer is defined as a large number of complex diseases, up to a hundred, that behave differently depending on the cell types from which they originate. Cancers vary in their ages of onset, growth rates, invasiveness, prognoses, and responsiveness to treatments. However, at the molecular level, all cancers exhibit common characteristics that unite them as a family.

All cancer cells share two fundamental properties: (1) abnormal cell growth and division (proliferation), and (2) defects in the normal restraints that keep cells from spreading and colonizing other parts of the body (metastasis). In normal cells, these functions are tightly controlled by genes that are expressed appropriately in time and place. In cancer cells, these genes are either mutated or are expressed inappropriately.

It is this combination of uncontrolled cell proliferation and metastatic spread that makes cancer cells dangerous. When a cell simply loses genetic control over cell growth, it may grow into a multicellular mass, a benign tumor. Such a tumor can often be removed by surgery and may cause no serious harm. However, if cells in the tumor also have the ability to break loose, enter the bloodstream, invade other tissues, and form secondary tumors (metastases), they become malignant. Malignant tumors are often difficult to treat and may become life threatening. As we will see later in the chapter, there are multiple steps and genetic mutations that convert a benign tumor into a dangerous malignant tumor.

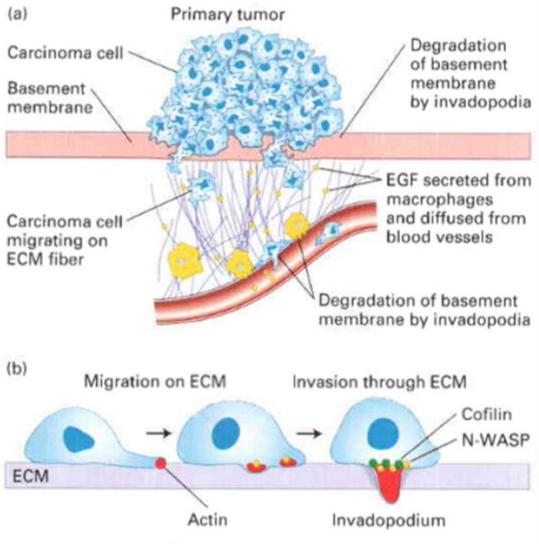


▲ FIGURE 25-1 Overview of changes in cells that cause cancer.

The Clonal Origin of Cancer Cells:

Although malignant tumors may contain billions of cells, and may invade and grow in numerous parts of the body, all cancer cells in the primary and secondary tumors are clonal, meaning that they originated from a common ancestral cell that accumulated specific mutations. This is an important concept in understanding the molecular causes of cancer and has implications for its diagnosis.

As for example, Cancer cells from patients with Burkitt's lymphoma show reciprocal translocations between chromosome 8 (with translocation breakpoints at or near the c-myc gene) and chromosomes 2, 14, or 22 (with translocation breakpoints at or near one of the immunoglobulin genes). Each Burkitt's lymphoma patient exhibits unique breakpoints in his or her c-myc and immunoglobulin gene DNA sequences; however, all lymphoma cells within that patient contain identical translocation breakpoints. This demonstrates that all cancer cells in each case of Burkitt's lymphoma arise from a single cell, and this cell passes on its genetic aberrations to its progeny.



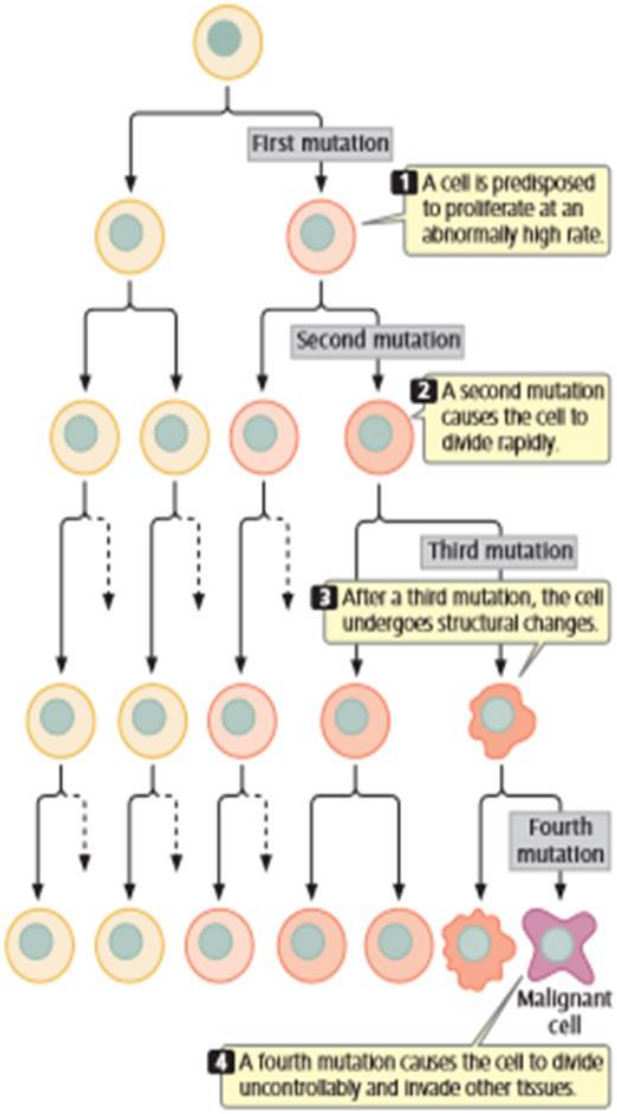
▲ **FIGURE 25-3 Metastasis.** (a) First steps in metastasis, using breast carcinoma cells as an example. Cancer cells leave the main tumor and attack the basement membrane, using extracellular matrix (ECM) fibers to reach the blood vessels. The cancer cells can be attracted by signals such as epidermal growth factor (EGF), which can be secreted by macrophages (yellow). At the blood vessels they penetrate the layer of endothelial cells that forms the vessel walls, and enter the bloodstream. (b) Carcinoma cells penetrate the extracellular matrix and blood vessel wall by extending "invadopodia," which produce matrix metalloproteases and other proteases to open up a path. (c) Proteins used by invadopodia. N-WASP (Chapter 17) controls assembly of actin into filaments that form the internal skeleton of the invadopodia. One hope for fighting cancer is to develop drugs that target components of the invasion and metastasis process without blocking normal essential functions. [Adapted from Yamaguchi et al., 2005, *Curr. Opin. Cell Biol.* **17**:559.]

The Cancer Stem Cell Hypothesis:

A concept that is related to the clonal origin of cancer cells is that of the cancer stem cell. Many scientists now believe that tumors are comprised of a mixture of cells, many of which do not proliferate. Those that do proliferate and give rise to all the cells within the tumor are known as cancer stem cells. Stem cells are cells that have the capacity for self-renewal—a process in which the stem cell divides unevenly, creating one daughter cell that goes on to differentiate into a mature cell type and one that remains a stem cell. For example, human acute myeloid leukemias contain less than 1 cancer stem cell in 10,000. In contrast, some solid tumors may contain as many as 40 percent cancer stem cells.

Carcinogenesis:

Carcinogenesis is the process that leads to genetic mutations induced by physical or chemical agents. Conceptually, this process can be divided into three distinct stages: initiation, promotion, and progression. Initiation involves an irreversible genetic change, usually a mutation in a single gene. Promotion is generally associated with increased proliferation of initiated cells, which increases the population of initiated cells. Progression is the accumulation of more genetic mutations that lead to the acquisition of the malignant or invasive phenotype.



23.4 Through clonal evolution, tumor cells acquire multiple mutations that allow them to become increasingly more aggressive and proliferative. To conserve space, a dashed arrow is used to represent a second cell of the same type in each case.

CANCER-RELATED GENES:

Oncogenes: Oncogenes are derived from normal host genes, also called protooncogenes, that become dysregulated as a consequence of mutation. Oncogenes contribute to the transformation process by driving cell proliferation or reducing sensitivity to cell death.

Historically, oncogenes were identified in four major ways: chromosomal translocation, gene amplification, RNA tumor viruses, and gene transfer experiments. Gene transfer experiments consist of transfecting DNA isolated from tumor cells into normal rodent cells (usually NIH3T3 cells) and observing any morphological changes. These morphological changes became the hallmarks for cell transformation, the process of becoming tumorigenic.

The characteristics of transformed cells are as follows: (1) the ability to form foci instead of a monolayer in tissue culture;(2)the ability to grow without adherence to a matrix, or “anchorage-independent growth”; and (3) the ability to form tumors when injected into immunologically compromised animals.

There are seven classes of oncogenes, classified by their location in the cell and their biochemical activity (Table). All of these oncogenes have different properties that can lead

to cancer. The classes of oncogenes are growthfactors, growthfactor receptors, membrane-associated guanine nucleotide-binding proteins, serine-threonine protein kinases, cytoplasmic tyrosine kinases, nuclear proteins, and cytoplasmic proteins that affect cell survival.

Table 1.3 Oncogenes

Oncogenes	Protein Function	Neoplasm(s)
Growth Factors		
sis	Platelet-derived growth factor	fibrosarcoma
int-2	Fibroblast growth factor	breast
trk	Nerve growth factor	neuroblastoma
Growth Factor Receptors		
erb-B1	Epidermal growth factor receptor	squamous cell carcinoma
erb-B2/HER2/neu	Heregulin	breast carcinoma
fms	Hematopoietic colony stimulating factor	sarcoma
ros	Insulin receptor	astrocytoma
Tyrosine kinases		
bcr-abl	Tyrosine kinase	chronic myelogenous leukemia
src	Tyrosine kinase	colon
lck	Tyrosine kinase	colon
Serine-Threonine protein kinases		
raf	Serine-threonine kinase	sarcoma
mos	Serine-threonine kinase	sarcoma
Guanine nucleotide binding proteins		
H-ras	GTPase	melanoma; lung, pancreas
K-ras	GTPase	leukemias; colon, lung, pancreas
N-ras	GTPase	carcinoma of the genitourinary tract and thyroid; melanoma
Cytoplasmic proteins		
bcl-2	Anti-apoptotic protein	non-Hodgkin's B-cell lymphoma
Nuclear proteins		
myc	Transcription factor	Burkitt's lymphoma
jun	Transcription factor (AP-1)	osteosarcoma
fos	Transcription factor (AP-1)	sarcoma

Tumor Suppressor Genes:

Tumor Suppressor Genes In contrast to oncogenes, tumor suppressor genes can directly or indirectly inhibit cell growth. Those that directly inhibit cell growth or promote cell death are known as "gatekeepers" and their activity is rate limiting for tumor cell proliferation.

Hence, both copies of gatekeeper tumor suppressors must be functionally eliminated for tumors to develop. This characteristic requirement is a hallmark of tumor suppressor genes. Mutations that inactivate one allele of a gatekeeper gene can be inherited through the germline, which in conjunction with somatic mutation of the remaining allele, leads to cancer predisposition syndromes.

For example, mutations of the APC gene lead to colon tumors. Somatic mutations that inactivate both gatekeeper alleles occur in sporadic tumors. Those tumor suppressor genes

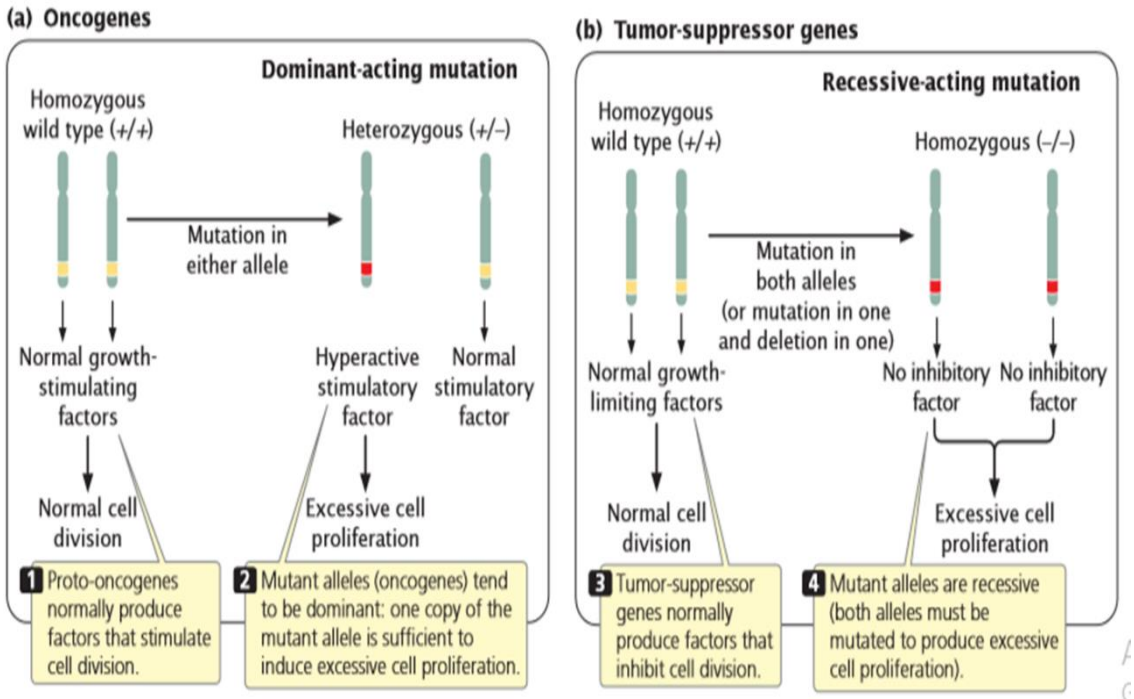
that do not directly suppress proliferation, but function to promote genetic stability are known as “caretakers.”

Caretakers function in DNA repair pathways and elimination of caretakers results in increased mutation rates. Because numerous mutations are required for the full development of a tumor, elimination of caretaker tumor suppressors can greatly accelerate tumor progression. As with gatekeepers, mutations can be inherited through the germline and can give rise to cancer predisposition syndromes. An example of a caretaker gene is MSH2, which functions in the mismatch DNA repair system, and inherited mutations in this gene gives rise to the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (Table).

Table 1.4 Tumor Suppressor Genes

TS Gene	Protein Function	Neoplasm(s)
APC	cell adhesion	colon
BRCA 1	transcription factor	breast and ovary
BRCA 2	DNA repair	breast and ovary
CDK4	cyclin D kinase	melanoma
hMLH1	DNA mismatch repair	HNPCC[a]
hMSH2	DNA mismatch repair	HNPCC
hPMS1	DNA mismatch repair	HNPCC
hPMS2	DNA mismatch repair	HNPCC
MEN1[b]	Ret receptor	thyroid
NF1	GTPase	neuroblastoma
p53	transcription factor	colon, lung, breast
Rb	cell cycle checkpoint	retinoblastoma
WT-1	transcription factor	childhood kidney

[a]Hereditary non-polyposis colon cancer.
[b]Multiple endocrine neoplasia.



23.5 Both oncogenes and tumor-suppressor genes contribute to cancer but differ in their modes

The ras Proto-oncogenes:

Some of the most frequently mutated genes in human tumors are those in the ras gene family. These genes are mutated in more than 30 percent of human tumors. The ras gene family encodes signal transduction molecules that are associated with the cell membrane and regulate cell growth and division. Ras proteins normally transmit signals from the cell membrane to the nucleus, stimulating the cell to divide in response to external growth factors (Figure 19–9). Ras proteins alternate between an inactive (switched off) and an active (switched on) state by binding either guanosine diphosphate (GDP) or guanosine triphosphate (GTP). When a cell encounters a growth factor (such as platelet-derived growth factor or epidermal growth factor), growth factor receptors on the cell membrane bind to the growth factor, resulting in autophosphorylation of the cytoplasmic portion of the growth factor receptor. This causes recruitment of proteins known as nucleotide exchange factors to the plasma membrane. These nucleotide exchange factors cause Ras to release GDP and bind GTP, thereby activating Ras. The active, GTP-bound form of Ras then sends its signals through cascades of protein phosphorylations in the cytoplasm. The end-point of these cascades is activation of nuclear transcription factors that stimulate expression of genes whose products drive the cell from quiescence into the cell cycle. Once Ras has sent its signals to the nucleus, it h ydrolyzes GTP to GDP and becomes inactive. Mutations that convert the ras proto-oncogene to an oncogene prevent the Ras protein from hydrolyzing GTP to GDP and hence freeze the Ras protein into its “on” conformation, constantly stimulating the cell to divide.

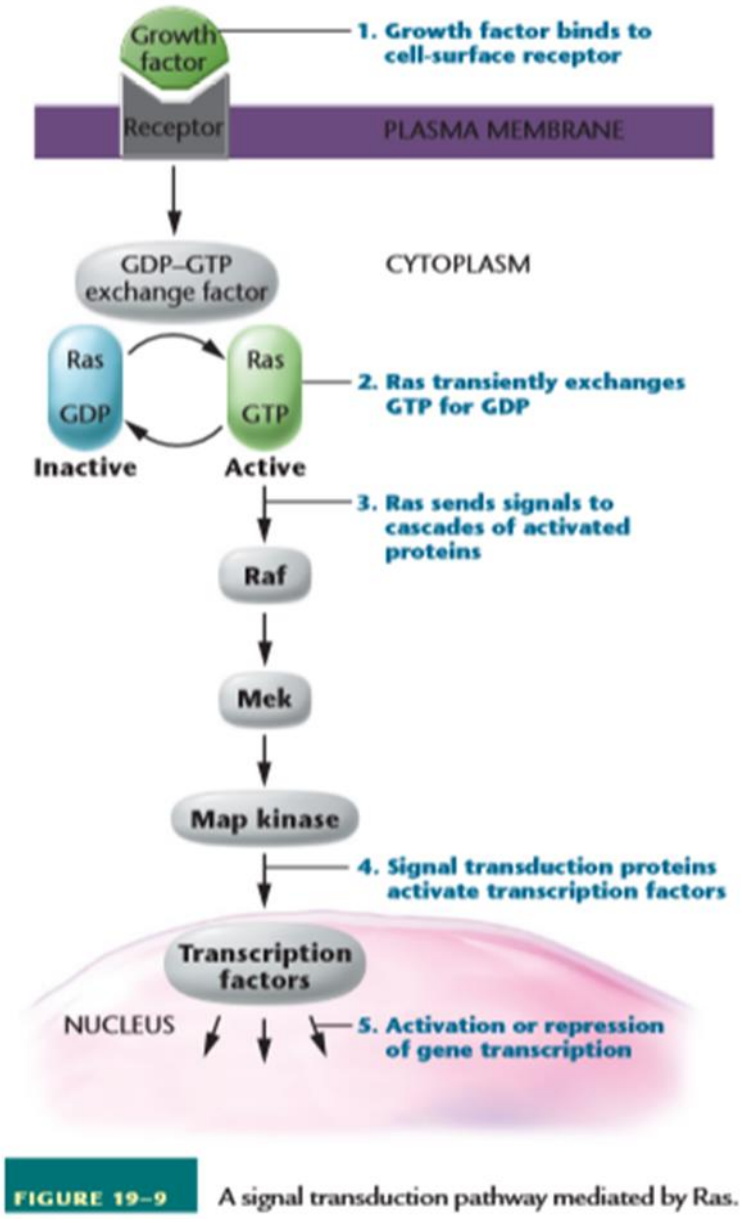


FIGURE 19–9 A signal transduction pathway mediated by Ras.

The p53 Tumor-suppressor Gene:

The most frequently mutated gene in human cancers— mutated in more than 50 percent of all cancers —is the p53 gene. This gene encodes a nuclear protein that acts as a transcription factor, repressing or stimulating transcription of more than 50 different genes. Normally, the p53 protein is continuously synthesized but is rapidly degraded and therefore is present in cells at low levels. In addition, the p53 protein is normally bound to another protein called MDM2, which has several effects on p53. The presence of MDM2 on the p53 protein tags p53 for degradation and sequesters the transcriptional activation domain of p53. It also prevents the phosphorylations and acetylations that convert the p53 protein from an inactive to an active form. Several types of cellular stress events bring about rapid increases in the nuclear levels of activated p53 protein. These include chemical damage to DNA, double-stranded breaks in DNA induced by ionizing radiation, and the presence of DNA-repair intermediates generated by exposure of cells to ultraviolet light. In response to these signals, MDM2 dissociates from p53, making p53 more stable and unmasking its transcription activation domain. Increases in the levels of activated p53 protein also result from increases in protein phosphorylation, acetylation, and other post-translational modifications (Figure 19–10). Activated p53 protein acts as a transcription factor that stimulates expression of the MDM2 gene. As the levels of MDM2 increase, p53 protein is again bound by MDM2, returned to an inactive state, and targeted for degradation, in a negative feedback loop.

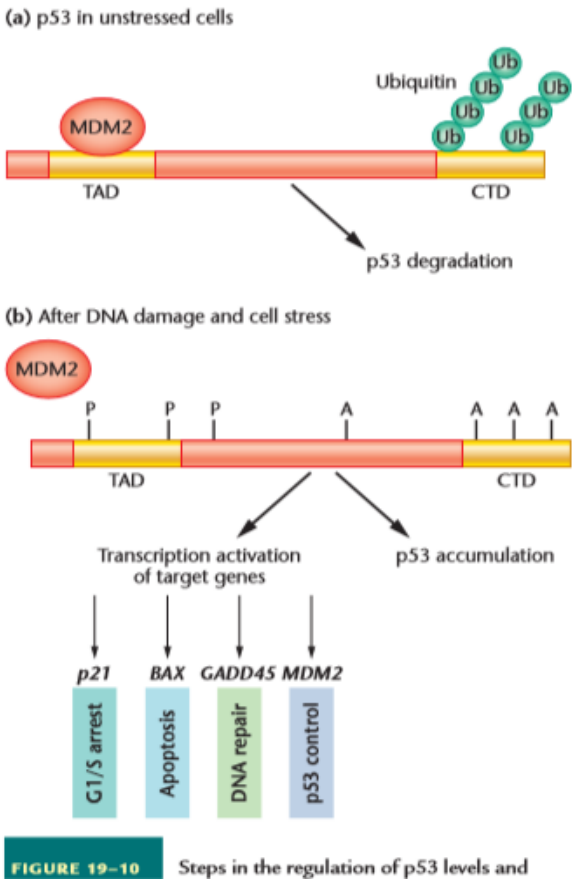


FIGURE 19–10 Steps in the regulation of p53 levels and

The p53 protein initiates several different responses to DNA damage including cell-cycle arrest followed by DNA repair and apoptosis if DNA cannot be repaired. These responses are accomplished by p53 acting as a transcription factor that stimulates or represses the expression of genes involved in each response.

In normal cells, p53 can arrest the cell cycle at the G1/S and G2/M checkpoints, as well as retarding the progression of the cell through S phase. To arrest the cell cycle at the G1/S checkpoint, activated p53 protein stimulates transcription of a gene encoding the p21 protein. The p21 protein inhibits the CDK4/cyclin D1 complex, hence preventing the cell from moving from G1 phase into S phase. Activated p53 protein also regulates expression of genes that retard the progress of DNA replication, thus allowing time for DNA damage to be

repaired during S phase. By regulating expression of other genes, activated p53 can block cells at the G2/M checkpoint, if DNA damage occurs during S phase.

Activated p53 can also instruct a damaged cell to commit suicide by apoptosis. It does so by activating the transcription of the Bax gene and repressing transcription of the Bcl2 gene. In normal cells, the BAX protein is present in a heterodimer with the Bcl2 protein, and the cell remains viable (Figure 19–8). But when the levels of BAX protein increase in response to p53 stimulation of Bax gene transcription, BAX homodimers are formed, and these homodimers activate the cellular changes that lead to apoptosis. In cancer cells that lack functional p53, BAX protein levels do not increase in response to cell damage, and apoptosis may not occur.

Cells lacking functional p53 are unable to arrest at cellcycle checkpoints or to enter apoptosis in response to DNA damage. As a result, they move unchecked through the cell cycle, regardless of the condition of the cell's DNA. Cells lacking p53 have high mutation rates and accumulate the types of mutations that lead to cancer. Because of the importance of the p53 gene to genomic integrity, it is often referred to as the "guardian of the genome."

The RB1 Tumor-suppressor Gene:

The loss or mutation of the RB1 (retinoblastoma 1) tumorsuppressor gene contributes to the development of many cancers, including those of the breast, bone, lung, and bladder. The RB1 gene was originally identified as a result of studies on retinoblastoma, an inherited disorder in which tumors develop in the eyes of young children. Retinoblastoma occurs with a frequency of about 1 in 15,000 individuals. In the familial form of the disease, individuals inherit one mutated allele of the RB1 gene and have an 85 percent chance of developing retinoblastomas as well as an increased chance of developing other cancers. All somatic cells of patients with hereditary retinoblastoma contain one mutated allele of the RB1 gene. However, it is only when the second normal allele of the RB1 gene is lost or mutated in certain retinal cells that retinoblastoma develops. In individuals who do not have this hereditary condition, retinoblastoma is extremely rare, as it requires at least two separate somatic mutations in a retinal cell in order to inactivate both copies of the RB1 gene.

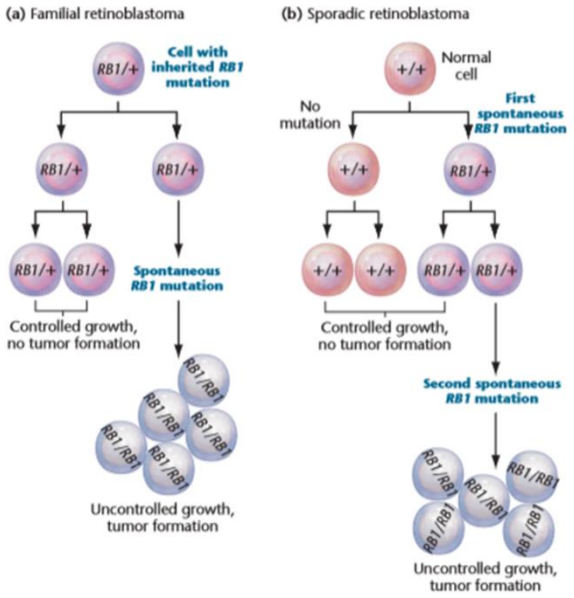


FIGURE 19–11 (a) In familial retinoblastoma, one mutation (designated as *RB1*) is inherited and present in all cells. A second mutation at the retinoblastoma locus in any retinal cell contributes to uncontrolled cell growth and tumor formation. (b) In sporadic retinoblastoma, independent mutations in both alleles of the retinoblastoma gene within a single cell are acquired sequentially, also leading to tumor formation.

The retinoblastoma protein (pRB) is a tumor-suppressor protein that controls the G1/S cell-cycle checkpoint. The pRB protein is found in the nuclei of all cell types at all stages of the cell cycle. However, its activity varies throughout the cell cycle, depending on its phosphorylation state. When cells are in the G0 phase of the cell cycle, the pRB protein is nonphosphorylated and binds to transcription factors such as E2F, inactivating them (Figure 19–12). When the cell is stimulated by growth factors, it enters G1 and approaches S phase. Throughout the G1 phase, the pRB protein becomes phosphorylated by the CDK4/cyclin D1 complex. Phosphorylated pRB r eleases its bound regulatory proteins. When E2F and other regulators are released by pRB, they are free to induce the expression of over 30 genes whose products are required for the transition from G1 into S phase. After cells traverse S, G2, and M phases, pRB reverts to a nonphosphorylated state, binds to regulatory proteins such as E2F, and keeps them sequestered until required for the next cell cycle. In normal quiescent cells, the presence of the pRB protein prevents passage into S phase. In many cancer cells, including retinoblastoma cells, both copies of the RB1 gene are defective, inactive, or absent, and progression through the cell cycle is not regulated.

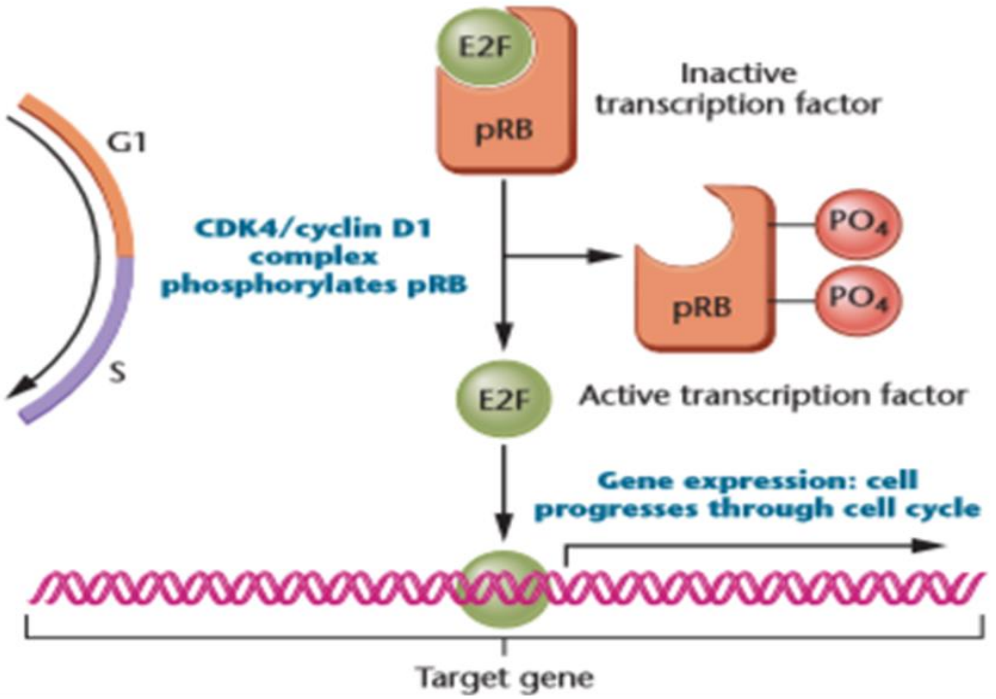


FIGURE 19–12 During G0 and early G1, pRB interacts with and inactivates transcription factor E2F. As the cell moves from G1 to S phase, a CDK4/cyclinD1 complex forms and adds phosphate groups to pRB. As pRB becomes phosphorylated, E2F is released and becomes transcriptionally active, allowing the cell to pass through S phase. Phosphorylation of pRB is transitory; as CDK/cyclin complexes are degraded and the cell moves through the cell cycle to early G1, pRB phosphorylation declines, allowing pRB to reassociate with E2F.

Adenomatous Polyposis Coli(APC):

The tumor suppressor gene, APC, is mutated in almost 90% of human colon cancers and 30% of melanoma skin cancers. The inherited loss of APC tumor suppressor function results in familial adenomatous polyposis (FAP). FAP patients develop hundreds to thousands of colon polyps by their second or third decade of life. By age 40, one or two of these polyps usually develops into a malignant carcinoma, and thus, many of these patients choose to have a colectomy to prevent carcinoma formation. Mutations in APC occur in the majority of sporadic colon cancers too.

APC mutation is an early event in colon carcinogenesis, and is therefore, considered to be the initiating event. Loss of this tumor suppressor gene results in constitutive activity of the oncogene, c- myc, through an intricate collection of protein-protein interactions. Briefly, APC interacts with other cellular proteins, including the oncogene β-catenin. APC regulates the rates of proliferation and apoptosis by several different mechanisms. Wild-type APC is important for cytoskeletal integrity, cellular adhesion, and Wnt signaling. APC plays a role in the G1/S transition of the cell cycle by modulating expression levels of c-myc and cyclin D1. Wildtype, full length APC is also important in maintaining intestinal cell migration up the crypt and inducing apoptosis.

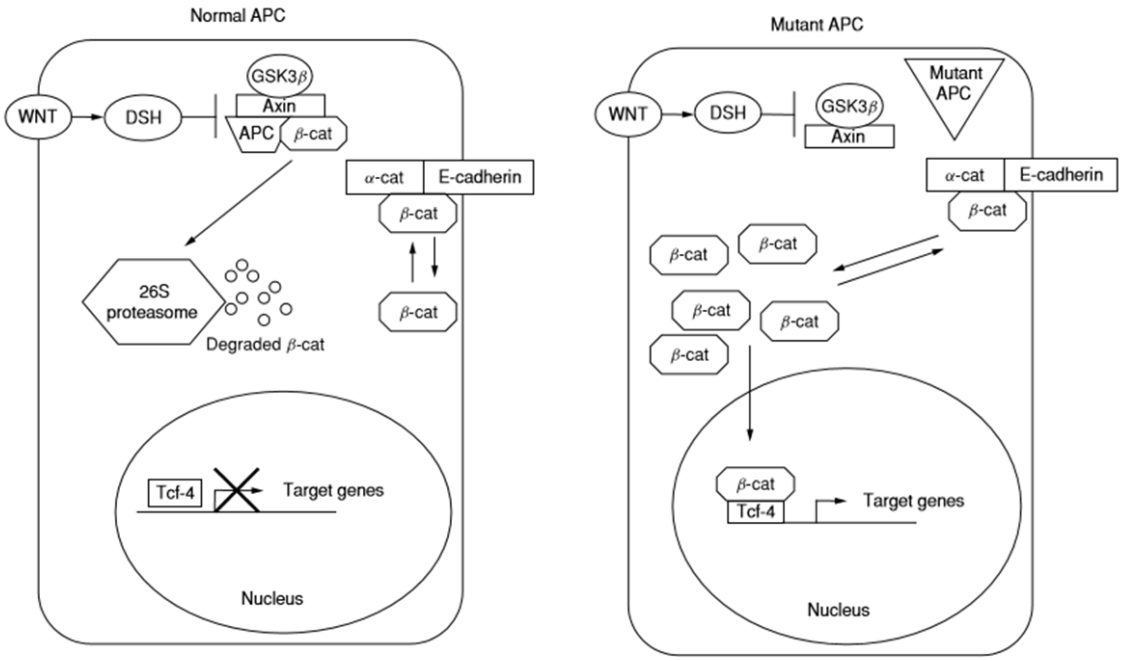


Figure 1.11. The APC signaling pathway. In a normal cell, APC forms a complex with axin, GSK-3β, and β-catenin. This promotes proteosomal degradation of β-catenin and prevents transcription of β-catenin/Tcf4 target genes. When APC is mutated, the multi-protein complex cannot form and β-catenin is not degraded. Instead, β-catenin is translocated to the nucleus where it binds with Tcf4 to activate transcription of various target genes. Some of the known target genes, like c-myc and cyclin D1, play important roles in cell proliferation.