Genes that Prevent and Cause Cancer: Tumor Suppressor Genes and Oncogenes (Lecture)

OBJECTIVES
- Describe the normal cellular functions of tumor suppressor genes and oncogenes and explain their roles in cancer.
- Describe the differences between a tumor suppressor gene and an oncogene.
- Describe Knudson’s two-hit hypothesis for the pathogenesis of retinoblastoma.
- Explain why loss-of-heterozygosity of a particular chromosome/chromosomal region in tumor DNA suggests the existence of a tumor suppressor gene in that region.
- Explain why loss of either Rb or p16 results in cell proliferation.
- Name the gene mutated in Li-Fraumeni syndrome and describe two mechanisms by which its loss leads to cancer.
- Name the two genes encoded by oncogenic human papilloma viruses that promote cancer and describe how each of them functions at the molecular level.
- Describe how the HER2/neu oncogene is activated in breast cancer.
- Describe the normal function of Ras and the molecular mechanism by which mutations in Ras genes lead to cancer.
- Describe three mechanisms by which the cyclin D gene is activated in tumors.
- Explain why activation of the Bcl-2 gene promotes cancer.
- Describe the molecular events associated with different stages in the development of colon cancer.

KEY WORDS
- chromosomal inversion
- Li-Fraumeni syndrome
- cyclin D
- multi-step tumorigenesis
- E6
- oncogene
- E6AP
- p16
- E7
- p53
- gain-of-function
- proto-oncogene
- gene amplification
- Ras
- HER2/neu
- Rb gene
- human papilloma virus (HPV)
- retinoblastoma
- loss-of-function
- tumor suppressor gene
- loss-of-heterozygosity (LOH)
- two-hit hypothesis
I. INTRODUCTION
Cancer is caused by the accumulation of mutations that lead to uncontrolled cell growth. It is an evolutionary process in which cells acquire mutations as a result of spontaneous and environmentally-induced DNA damage. Mutations that promote the growth and survival of the cells are selected for in a Darwinian process. Three classes of genes promote tumor formation: genes that regulate cell proliferation, genes that control programmed cell death or apoptosis, and genes required for the repair of damaged DNA. Depending on how they affect each process, they can be grouped into two general categories: 1) tumor suppressor genes and 2) oncogenes. (Genes required for the repair of damaged DNA will be discussed in the lecture titled “How Cancer-Causing Mutations Occur”.)

II. TUMOR SUPPRESSOR GENES
Tumor suppressor genes can be defined as genes which encode proteins that inhibit the formation of tumors. Their normal function is to inhibit cell growth, or act as the “brakes” for the cell cycle. Mutations in tumor suppressor genes contribute to the development of cancer by inactivating that inhibitory function. Mutations of this type are termed loss-of-function mutations. As long as the cell contains one functional copy of a given tumor suppressor gene (expressing enough protein to control cell growth), that gene can inhibit the formation of tumors. Inactivation of both copies of a tumor suppressor gene is required before their function can eliminated. Therefore, mutations in tumor suppressor genes are recessive at the level of an individual cell. As we will see, the inactivation of tumor suppressor genes plays a major role in cancer.

A. Retinoblastoma
Retinoblastoma is a rare childhood tumor of the eye. Most cases are sporadic (as opposed to inherited) and occur unilaterally (affecting one eye). It typically occurs in children 1-4 years of age. However, in 30-40% of cases of retinoblastoma, multiple tumors occur in one or both eyes. These children have a hereditary form of retinoblastoma and tend to acquire tumors earlier than those with sporadic disease. They are also more likely to have multiple tumors, and bilateral tumors (or in both eyes). In families that have multiple cases of retinoblastoma, the disease shows an autosomal dominant inheritance pattern. Approximately 175 cases of retinoblastoma are diagnosed in the US each year.

1. Two-hit hypothesis
Knudson proposed in 1971 that sporadic cases of retinoblastoma involve the inactivation of both copies of a particular gene, which he called the retinoblastoma gene (Rb). He proposed that this occurs in two steps: A “first hit” inactivates one of the two copies of the Rb gene in one retinoblast. Later a “second hit” inactivates the remaining functional copy of Rb in the same cell or one of its progeny (Figure 1). To explain the inherited form of the disease, he proposed that the affected patients inherited one defective copy of Rb from one parent and a functional copy from the other parent. Because the “first hit” is inherited and is present in all of the cells of the body, a tumor arises when a “second hit” occurs.
in any retinoblast. Because it takes only one additional mutational event, inherited retinoblastoma would be more likely to occur earlier in childhood and more likely to cause bilateral disease. Subsequent research identified the Rb gene on chromosome 13 and confirmed Knudson’s hypothesis.

In both inherited and sporadic retinoblastoma, the second allele of Rb can be inactivated by several mechanisms including point mutation, large deletions that remove Rb and many adjacent genes, or errors in chromosome segregation leading to loss of the entire wild-type chromosome. The latter two mechanisms are more common.

2. Loss-of-heterozygosity (LOH)
How are tumor suppressor genes found? As discussed in the Genetics in Medicine lecture in Prologue, in the genome there are periodic differences in sequences between the two chromosomal homologs (one inherited from each parent). Differences that are commonly studied include variable numbers of tandem repeats (VNTRs) and single nucleotide polymorphisms (SNPs). These variations can be visualized by molecular techniques. If we examine any region of the genome, we will find that most people are heterozygous for some markers. Although these differences often occur between genes rather than in them, they can be used to track the presence of adjacent genes. (This topic will be discussed in more detail in a lecture on Linkage Analysis and Gene Mapping.)
As shown in Figure 2, tumor suppressor genes like Rb can be found by looking for loss-of-heterozygosity (LOH). This example involves a sporadic case of retinoblastoma. Shown is chromosome 13 after the first copy of Rb has been inactivated. In this example, A(a) and B(b) represent two marker loci that lie on either side of the Rb gene. Each has one of two alleles (e.g., A and a). The Rb allele that is inactivated first is flanked by the A and b alleles, whereas the remaining functional copy of Rb is flanked by the a and B alleles. The second hit in Rb often involves deletion of a large region or loss of an entire chromosome. As a result, the functional copy of Rb is lost as are the flanking a and B markers. Consequently, only the A and b alleles are left and the cell is no longer heterozygous for these markers (i.e. only A exists, not A/a and only b exists, not B/b) in the tumor. Loss-of-heterozygosity in a particular region of the genome in a tumor strongly suggests the existence of a tumor suppressor gene in that region.

Rb is also mutated in many other tumor types including breast cancer, osteosarcoma, lung cancer, and genitourinary cancer. How does the loss of Rb promote tumor formation? The Rb protein normally binds to and inactivates the E2F transcription factor. Loss of Rb leads to the activation of E2F, which binds the promoter of the cyclin E gene and in turn causes increased expression of the cyclin E gene and synthesis of Cdk2-cyclin E complexes, which then drives the cell cycle (Figure 3).

**Figure 2.** Loss-of-heterozygosity.

**Figure 3.** How inactivation of Rb leads to proliferation. Loss of both copies of Rb results in the activation of E2F, increased cyclin E transcription, formation of the Cdk2-cyclin E complex and thus cell proliferation.
Surprisingly, children with inherited retinoblastoma are not at higher risk later in life for breast, lung, or genitourinary cancer (even though Rb is often inactivated in sporadic forms of these tumors), although they are at risk of developing osteosarcomas. It may be that the other necessary genetic changes that occur in breast, lung and genitourinary tumors happen at a much lower rate than the rate of inactivation of both copies of the Rb gene. In this scenario, the formation of the tumor would not be limited by loss of Rb, but rather loss of some other tumor suppressor gene or activation of an oncogene (see below). In contrast, the proliferation of retinoblasts in early childhood may be uniquely controlled by Rb such that few if any other genetic changes are required for tumor formation.

B. Genes encoding Cdk inhibitors are tumor suppressor genes

p16, an inhibitor of Cdk4-cyclin D complexes, is lost in inherited melanoma. It is also mutated in sporadic forms of many cancers including pancreatic cancer, lung cancer, and acute lymphoblastic leukemia. The loss of p16 leads to increased Cdk4-cyclin D activity, phosphorylation and inactivation of Rb, leading to activation of E2F and cyclin E transcription.

C. p53: a key tumor suppressor

p53 is inactivated in the majority of human tumors (as seen by loss-of-heterozygosity), and is therefore among the most important “brakes” on tumor formation. p53 restrains tumor formation by two different mechanisms (Figure 4). p53 activates the p21 Cdk inhibitor gene in response to DNA damage and stress. Loss of p53 in cells prevents the p21 gene from being transcribed, leading to the increased activity of the multiple Cdns normally turned off by p21 and resulting in increased cell proliferation. A second way in which p53 restrains tumor formation is by inducing apoptosis. As with Rb, p53 is also found mutated in a rare inherited cancer. Li-Fraumeni syndrome is an inherited cancer syndrome in which susceptibility to a wide spectrum of tumors is inherited in an autosomal dominant manner. These patients have inherited a defective copy of the p53 gene. As with Rb, a second hit occurs in tumors inactivating the remaining copy.

![Figure 4](image.png)

**Figure 4.** p53 tumor suppressor functions. p53 antagonizes tumor formation by activating the p21 Cdk inhibitor, which blocks proliferation, and by promoting apoptosis.
II. VIRUSES AND CANCER
Viruses are infectious agents that must replicate inside a host cell. Viruses contain their own genome (which can be either DNA or RNA) protected by a coat made up of protein or protein plus lipid. Different viruses use the host cell’s machinery in different ways, but all viruses require the translation apparatus (e.g. ribosomes) of the host cells to translate their mRNAs. Bacteria, in contrast, can generally grow outside the host and have their own translation machinery. Some viruses promote cancer in humans including HIV, Epstein-Barr Virus (EBV), and human papilloma viruses (HPVs). We will discuss how HPVs cause cancer this lecture.

Human papilloma viruses (transmitted through sexual contact) play a role in most cases of cancer of the uterine cervix (see upcoming lecture on cervical cancer). There are at least 77 subtypes of HPV that are distinguished by variations in their DNA sequences. HPV-16 or HPV-18 DNA is found in 70% of cervical tumors. An additional 20% of tumors contain HPV DNA corresponding to one of 20 other cancer-associated subtypes. HPVs have a double-stranded DNA genome. It encodes 7 viral genes that are expressed early during infection (E1-E7) and 2 genes that are expressed late (L1-L2).

Two of the early genes, E6 and E7, promote tumor formation (Figure 5). The E6 protein associates with the cellular protein called E6AP (E6-Associated Protein). The E6-E6AP protein complex catalyzes covalent attachment of a small protein called ubiquitin to p53. The attachment of ubiquitin to proteins targets Figure 5. Inactivation of tumor suppressor genes by proteins encoded by Human Papilloma Virus. The E6 protein of oncogenic HPVs binds to a cellular protein called E6-AP (E6 associated protein), which is a ubiquitin ligase. The E6-E6AP protein complex catalyzes the attachment many copies of the small protein ubiquitin to p53. This leads to the degradation of p53 by a large cytoplasmic protease called the proteasome. The E7 protein promotes cancer by binding directly to the Rb protein, rendering it inactive.
them for degradation in the cytoplasm via a protease called the proteasome. Thus, E6 inactivates p53 by causing the degradation of the protein via the cell’s normal protein degradation machinery. The protein encoded by E7 directly binds to Rb and inactivates it. Thus Rb can be inactivated by loss of the gene (as in retinoblastoma and other tumors) or by inactivation of the protein by a viral protein. As in genetic loss of Rb, inactivation of the Rb protein leads to the activation of the E2F transcription factor and transcription of the cyclin E gene.

III. ONCOGENES

Cells contain many normal genes that are involved in regulating cell proliferation. Some of these genes can be mutated to forms that promote uncontrolled cell proliferation. The normal forms of these genes are called proto-oncogenes, while the mutated, cancer-causing forms are called oncogenes. In contrast to tumor suppressor genes, which put the breaks on cell proliferation, oncogenes actively promote proliferation (analogous to the gas petal of the cell cycle). Mutations that convert proto-oncogenes to oncogenes typically increase the activity of the encoded protein or increase the expression of the normal gene. Such mutations are dominant or gain-of-function mutations. Therefore, only one copy of the gene needs to be mutated in order to promote cancer.

Oncogenes were first identified in oncogenic retroviruses that had picked up a cellular oncogene (c-onc) and incorporated it into the viral genome to produce a viral oncogene (v-onc). J. Michael Bishop and Harold Varmus of UCSF were awarded the 1989 Nobel Prize in Medicine for the discovery of the cellular origin of the viral oncogenes.

A. HER2/neu and breast cancer

The HER2/neu gene encodes a receptor tyrosine kinase closely related to the epidermal growth factor (EGF) receptor. This gene is overexpressed in ~25% of breast cancers. Breast cancers that overexpress HER2/neu tend to be more aggressive clinically. The mechanism by which HER2/neu is overexpressed is gene amplification which results in multiple copies of the gene accumulating in tumor cells. (Gene amplification will be discussed further in the lecture on How Cancer-Causing Mutations Occur). The increase in the levels of the HER2/neu tyrosine kinase receptor on tumor cells results in increased signaling via the Ras-MAPK pathway, resulting in cellular proliferation. The discovery of HER2/neu amplification in breast cancer has led to the development of the anticancer drug Herceptin. Herceptin is a monoclonal antibody that binds to HER2/neu on the cell surface resulting in receptor down-regulation (decreased numbers of HER-2/neu at the cell surface) and killing of cells by the immune system. It was approved in 1998 for use in treatment of breast cancer (more about Herceptin in the Frontiers of Cancer Chemotherapy lecture).

B. Activation of ras: the oncogene most commonly activated in human tumors

The human genome encodes three Ras genes: H-ras, K-ras, N-ras. A large fraction of tumors contain mutations in one of these three genes. For
example, 70-90% of pancreatic carcinomas contain a mutation in the K-ras gene. ras oncogenes are activated by point mutations that result in proteins unable to hydrolyze GTP (Figure 6). These mutant Ras proteins are therefore “locked” in the GTP-bound (active) form which therefore continually activates the MAP kinase pathway, which in turn leads to cell proliferation.

C. **The Myc oncogene is often amplified or overexpressed in cancers**
The Myc protein acts in the nucleus as a signal for cell proliferation through several mechanisms. One mechanism, as discussed in the Cell Proliferation lecture, is as a transcription factor for the cyclin D gene. Myc is encoded by a proto-oncogene that is overexpressed or amplified in many cancers. Excess quantities of Myc can cause cells to proliferate in circumstances that normal cells would halt. In some cancers, rather than being amplified, Myc is made active by chromosomal translocation. (See Clinical Cytogenetics lecture for description of chromosomal abnormalities.) As a result of this chromosomal rearrangement, a strong promoter sequence is place inappropriately next to the Myc protein coding sequence, producing unusually large amounts of the Myc mRNA. For example, as you will learn later in this course, in Burkitt’s lymphoma a translocation brings the Myc gene under the control of sequences that normally drive the expression of antibodies in B cells. As a result, mutant B cells proliferate to excess and form a tumor.

D. **Cyclin D can be activated through several different mechanisms**
As discussed in earlier lectures, cyclin D forms part of the G1 Cdk which normally functions to inactivate the Rb tumor suppressor protein by phosphorylating it (Figure 7). Cyclin D is often overexpressed in cancers leading to reduced activity of Rb. In breast cancer, the gene is often amplified. As with HER2/neu amplification, this results in more cyclin D protein.

Cyclin D also plays a role in parathyroid adenomas, tumors of the parathyroid gland that result in uncontrolled calcium mobilization from bones (Figure 8). (The normal function of parathyroid hormone is to induce calcium release from bones). In these tumors, cyclin D can be activated
by a different mechanism. A **chromosomal inversion** places the strong transcriptional control region of the parathyroid hormone gene adjacent to the cyclin D gene. This results in the continuous expression of the cyclin D gene, which in turn promotes proliferation.

In chronic lymphocytic leukemias, cyclin D is deregulated by yet another mechanism. In these tumors, cyclin D is activated via a **chromosomal translocation** that places the cyclin D gene under control of the immunoglobulin heavy chain transcription control region. (We will discuss how chromosomal rearrangements occur in the lecture on How Cancer Causing Mutations Occur.)

### E. Bcl-2: apoptosis proteins and cancer

A translocation between chromosomes 14 and 18 (t(14:18)(q32;q21)) is the most common translocation in human lymphoid malignancies. It is especially common in follicular cell lymphomas (80-90% of cases have it). The translocation results in the activation of bcl-2 because the gene is brought into proximity with potent transcriptional regulatory sequences from the immunoglobulin heavy chain gene. The antiapoptotic activity of bcl-2 results in the increased survival of the cells that overexpress bcl-2. This in turn gives the cells the opportunity to acquire additional mutations that lead to cancer. Thus, programmed cell death is an important mechanism that normally restricts tumor formation.
IV. MULTISTEP TUMORIGENESIS: MOLECULAR EVENTS ASSOCIATED WITH THE GENESIS OF COLON CANCER

Colon cancer is unusual in that the precancerous lesions can be identified early via colonoscopy. Molecular analysis of the various stages of colon cancer is therefore possible. These studies have identified a rough sequence of molecular events that characterize tumor development in colorectal cancer (Figure 9). Because we now know the molecular function of many tumor suppressor genes and oncogenes, we can now understand the genetic changes that occur during cancer formation.

The earliest stage of colon cancer formation is the appearance of a hyperproliferative epithelium (Figure 9). This is associated with the loss of the APC tumor suppressor. As discussed in the previous lecture, APC limits Wnt signaling in the gut epithelium where it is expressed at high levels in nondividing cells and low levels in the stem cells of the crypts. Loss of the APC gene results in hyperactive Wnt signaling and proliferation of cells beyond the crypts. Subsequent to loss of APC, there appears genetic instability, which permits the cells to acquire mutations more rapidly. Progression of adenomas is associated with activation of the mitogenic Ras-MAPK pathway through the acquisition of activating mutations in the K-ras oncogene. Later, a further blow

![Figure 9](image_url)

**Figure 9.** Multistep model for colorectal cancer. Shown is the sequence of pathological events that take place during the development of sporadic colon cancer and the genetic changes that occur during this sequence. The sequence of events is not absolute – this diagram describes what happens on average.
to growth control occurs via the loss of a SMAD gene required for signaling through the growth inhibitory TGF-beta signaling pathway occurs. Finally, the progression from late adenomas to malignant carcinomas is associated with the loss of the p53 tumor suppressor, which releases the cells from DNA damage and stress induced apoptosis and cell cycle arrest. (Cancer nomenclature will be explained in the Introduction to Cancer Pathology lecture.) There are likely many more changes in tumor suppressor genes and oncogenes that occur during tumor progression that have yet to be identified. Nonetheless, the study of colon cancer has given us a picture of the multi-step evolution of a tumor and the genetic changes that drive them. Importantly, an understanding of the specific genetic changes that occur during this multi-step process allows for the identification of key steps for intervention and the development of specific therapeutic strategies.