

Tumor Suppressor Genes and Oncogenes: Genes that Prevent and Cause Cancer (Biochemistry/Molecular Biology Lecture)

OBJECTIVES

- Describe the normal cellular functions of tumor suppressor genes and proto-oncogenes and explain their roles in cancer.
- Describe Knudson's two-hit hypothesis for the pathogenesis of retinoblastoma.
- List the common clinical features, evolution and treatment for 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 drives a cell to proliferate.
- Explain how the proteins encoded by the E6 and E7 genes of the oncogenic human papilloma viruses function to promote tumor formation.
- Describe how the HER2/neu oncogene is activated in breast cancer.
- Describe the normal function of Ras proteins and the molecular mechanism by which mutations in Ras genes lead to cancer.
- Describe three pathways 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.
- Describe the six hallmark features of cancer cells and the molecular basis of each.

KEY WORDS

Adenomatous polyposis coli (APC)	multi-step tumorigenesis
Bcl2	mutator phenotype
caretaker	myc
chromosomal inversion	neurofibromatosis (NF1)
chromosome translocation	oncogene
cyclin D	promoters
E6 protein	p16
E7 protein	p53
EMSY	promoters
gain-of-function	proto-oncogene
gene amplification	Ras protein
HER2/neu	Rb gene
human papilloma virus (HPV)	retinoblastoma
loss-of-function	tumor suppressor gene
loss-of-heterozygosity (LOH)	two-hit hypothesis
Li-Fraumeni syndrome	

OPTIONAL READING

Alberts et al. *Molecular Biology of the Cell*; 5th Edition, Garland Science, 2008
Chapter 20; Cancer, pp.1230-1256

Kumar V, Abbas A, Fausto N: *Robbins and Cotran Pathologic Basis of Disease* 7th ed, Elsevier/Saunders, 2005; Chapter 7: Oncogenes and Tumor Suppressor Genes pp. 292-306

I. INTRODUCTION : TYPES OF GENES INVOLVED IN CANCER

Cancer is caused by the accumulation of genetic and epigenetic mutations in genes that normally play a role in the regulation of cell proliferation (as described in the Cell Proliferation lecture), thus leading to uncontrolled cell growth. Cells acquire mutations in these genes as a result of spontaneous and environmentally-induced DNA damage. Those cells with mutations that promote a growth and survival advantage over normal cells are selected for through a Darwinian process, leading to the evolution of a tumor. Genes involved in tumorigenesis include those whose products: 1) directly regulate cell proliferation (either promoting or inhibiting), 2) control programmed cell death or apoptosis, and 3) are involved in the repair of damaged DNA. Depending on how they affect each process, these genes can be grouped into two general categories: **tumor suppressor genes** (growth inhibitory) and **proto-oncogenes** (growth promoting).

Mutant alleles of proto-oncogenes are called **oncogenes**. Since mutation in a single allele of a proto-oncogene can lead to cellular transformation, such mutations are considered dominant. In contrast, typically both alleles of a tumor suppressor gene must be altered for transformation to occur. Genes that regulate apoptosis may be dominant, as with proto-oncogenes, or they may behave as tumor suppressor genes. Tumor suppressor genes may be divided into two general groups: **promoters** and **caretakers**. Promoters are the traditional tumor suppressors, like p53 and RB. Mutation of these genes leads to transformation by directly releasing the brakes on cellular proliferation. Caretaker genes are responsible for processes that ensure the integrity of the genome, such as those involved in DNA repair. Although they do not directly control cell proliferation, cells with mutations in these genes are compromised in their ability to repair DNA damage and thus can acquire mutations in other genes, including proto-oncogenes, tumor suppressor genes and genes that control apoptosis. A disability in DNA repair can predispose cells to widespread mutations in the genome, and thus to neoplastic transformation. Cells with mutations in caretaker genes are therefore said to have a “**mutator phenotype**”. The fact that patients with defects in DNA repair are cancer prone provides one of the most striking pieces of evidence that mutations in DNA lie at the heart of the neoplastic process. (More about genes whose products are involved in DNA repair in the Mutation and Cancer lecture.)

II. TUMOR SUPPRESSOR GENES

Tumor suppressor genes can be defined as genes which encode proteins that normally inhibit the formation of tumors. Their normal function is to inhibit cell proliferation, 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 proliferation), that gene can inhibit the formation of tumors. Inactivation of both copies of a tumor suppressor gene is required before their function can be 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 (RB) is a rare childhood tumor of the eye (see clinical correlate). Most cases (60-70%) are sporadic (as opposed to inherited), occur unilaterally (affecting one eye), and present in children 1-4 years of age. The remaining 30-40% of patients have a hereditary form of retinoblastoma and thus have inherited a germline cancer predisposing mutation (see below). These children tend to acquire tumors earlier than those with sporadic disease and are more likely to have multiple tumors in one (unilateral) or both (bilateral) eyes. In families with the inherited form of retinoblastoma, the disease shows an autosomal dominant inheritance pattern.

CLINICAL CORRELATION OF RETINOBLASTOMA

Retinoblastoma is a malignant tumor of immature neuroectodermal cells of the developing retina that occurs almost exclusively in young children. Approximately 175 cases of retinoblastoma are diagnosed in the US each year. It accounts for 3% of malignant disease in children younger than age 15. It is the most common intraocular tumor in pediatric patients and causes 5% of cases of childhood blindness.

Retinoblastoma may gradually fill the eye and extend through the optic nerve to the brain and less commonly, along the emissary vessels and nerves in the sclera to the orbital tissues. Occasionally, it grows diffusely in the retina, discharging malignant cells into the vitreous or anterior chamber, thereby producing a pseudoinflammatory process that may mimic other ocular inflammatory conditions.

Inheritance

Retinoblastoma occurs in retinal cells that have cancer-predisposing mutations in both copies of the *RBI* gene. About 60-70% of patients have the non-hereditary or “sporadic” form of the disease, in which the initial mutation affecting one copy of the *RBI* gene arises in a somatic retinal cell (or a precursor of a retinal cell). The remaining 30-40% of patients have inherited a cancer-predisposing mutation in one copy of their *RBI* gene from one parent, or have a new germline mutation. Sporadic cases tend to be unilateral (one eye) and unifocal (one tumor), and typically present by 24-30 months of age. Heritable cases tend to be bilateral (both eyes) and multifocal (multiple tumors), and generally present earlier, in the first year of life. Second primary malignant tumors, the most common of which is osteosarcoma, develop in large numbers of survivors of the heritable form of retinoblastoma after a period of many years.

Clinical Presentation

The most common presenting sign is leukocoria (white pupillary reflex). A crossed eye or strabismus is the second most common symptom of retinoblastoma. The child's eye may turn towards the ear (exotropia) or towards the nose (esotropia). Other symptoms may include red painful eye, glaucoma, pseudohypopyon (appearance of purulent material in the anterior chamber of the eye), or poor vision.

Evaluation

The clinical diagnosis of retinoblastoma is usually established by examination of the fundus of the eye using indirect ophthalmoscopy to directly visualize the intraocular tumor(s). Imaging studies (CT or ultrasound) can be used to support the diagnosis by detecting intrinsic calcification within the mass, a finding highly suggestive of retinoblastoma. High-resolution MRI of the orbits can help to stage the tumor, and determine any extraocular spread.

Retinoblastoma usually remains unnoticed until it grows large enough to produce leukocoria or strabismus (inflammation is a much rarer presentation – orbital cellulitis represents about 1% of all presentations of RB) with 90% of cases diagnosed before age 5. All children with poor vision, strabismus, or intraocular inflammation should be evaluated for the presence of retinoblastoma. The earlier the discovery and treatment of the tumor, the better the chance to prevent spread through the optic nerve and orbital tissues. Retinoblastoma can lead to loss of eyesight and, if not detected early enough, death, since systemic and CNS metastasis is almost impossible to treat.

Individuals with a germline mutation in *RBI* are also at increased risk of developing tumors outside the eye over their lifetime. Most of the second primary cancers are osteosarcoma, soft tissue sarcomas or melanomas. These tumors usually manifest in adolescence or adulthood. To detect second non-ocular tumors in individuals with retinoblastoma, physicians and parents should promptly evaluate complaints of bone pain or lumps because of the high risk of sarcomas; however, no specific screening protocols currently exist.

Treatment

Large tumors in eyes with no salvageable vision are often treated by enucleation (surgical removal of the eye). Smaller tumors can be treated with plaque or external beam radiotherapy, cryotherapy (use of liquid nitrogen to freeze and destroy a lesion or growth), or photocoagulation (use of laser to destroy a small tumor). Chemotherapy can be used to reduce initial tumor size prior to applying other modes of therapy. Combined therapy using chemotherapy and coordinated laser treatment can often preserve vision and spare the patient enucleation and radiation that may lead to disfigurement and the induction of secondary tumors. Eradication of tumor before infiltration into the optic nerve or choroid carries an excellent prognosis for survival.

Susan Hung, Curriculum Ambassador 2007

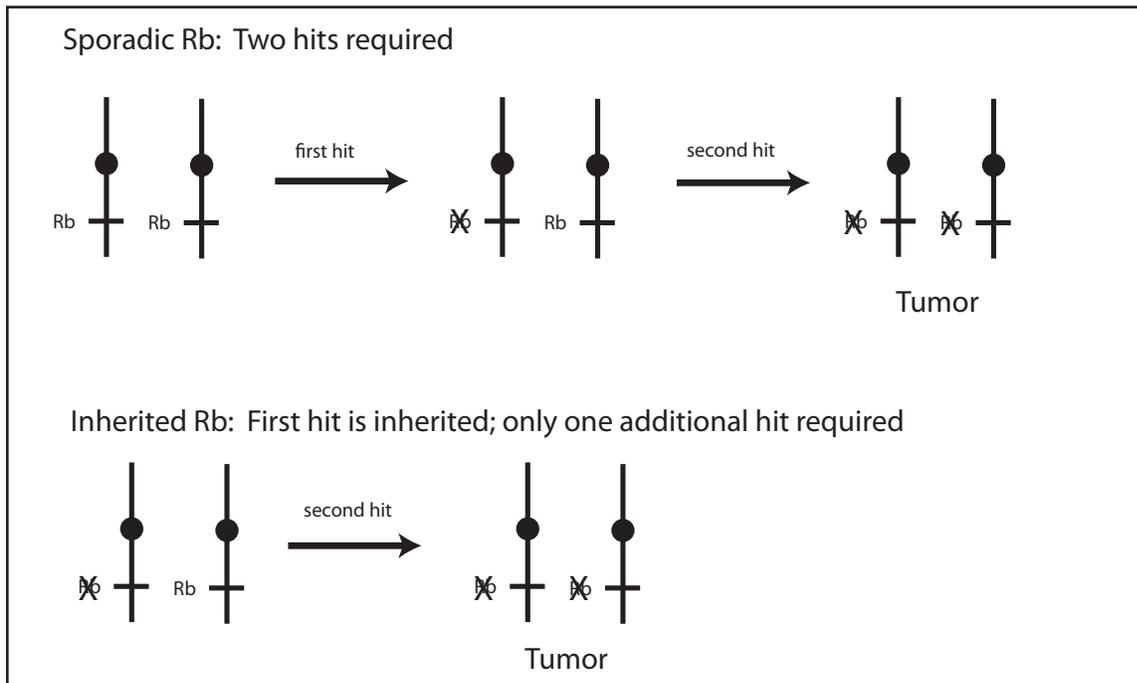


Figure 1. Knudson's two-hit hypothesis for retinoblastoma. In sporadic Rb, both copies of *RBI* (*RBI*) must be inactivated. This requires two mutational events "hits" which each inactivate one copy of *RBI*. In inherited Rb, the first hit is inherited.

1. Two-hit hypothesis

After statistical analysis of many patients with retinoblastoma, Dr. Alfred Knudson proposed in 1971 that sporadic cases of this disease involve the inactivation of both copies of a particular gene, which he called the **retinoblastoma** gene (*RBI*). He proposed that this occurs in two steps: A "first hit" inactivates one of the two copies of *RBI* in one retinoblast. Later a "second hit" inactivates the remaining functional copy of *RBI* 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 *RBI* from one parent and a functional copy from the other parent. Because the "first hit" is inherited and is present in all retinal cells, and in fact 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 (or epigenetic) event for any of these cells to develop into a tumor, inherited retinoblastoma would be more likely to occur earlier

in childhood and more likely to cause bilateral disease (multiple primary tumors). Subsequent research identified *RB1* on chromosome 13 and confirmed Knudson's hypothesis, thus marking the discovery of the first tumor suppressor gene.

In both inherited and sporadic retinoblastoma, the second allele of *RB1* can be inactivated by several mechanisms. In addition to epigenetic silencing (see Mutation and Cancer lecture), the possibilities include point mutation, large deletions that remove *RB1* 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 lectures in Prologue, in the genome there are periodic variations in the DNA sequence between the two homologous chromosomes (one inherited from each parent). Variants that are commonly used as genetic markers include short tandem repeat polymorphisms (STRPs) and single nucleotide polymorphisms (SNPs). These variants can be visualized by molecular techniques. If we examine any region of the genome, we will find that most people are heterozygous for certain polymorphic genetic markers. Although these variants often occur between genes rather than in them, they can be used to track the presence of adjacent genes. (See Genetic Variation lecture in Prologue and Linkage Analysis ILM in Organs CV.)

As shown in Figure 2, tumor suppressor genes like *RB1* can be found by looking for **loss-of-heterozygosity (LOH)** in a tumor. LOH means that *pre-tumor cells* are heterozygous for alleles of a tumor suppressor gene (e.g. one normal and one mutant allele), or alleles of genetic markers that surround the tumor suppressor gene, but the *tumor cells* have lost the normal tumor suppressor allele (and the surrounding marker alleles), so they are no longer heterozygous.

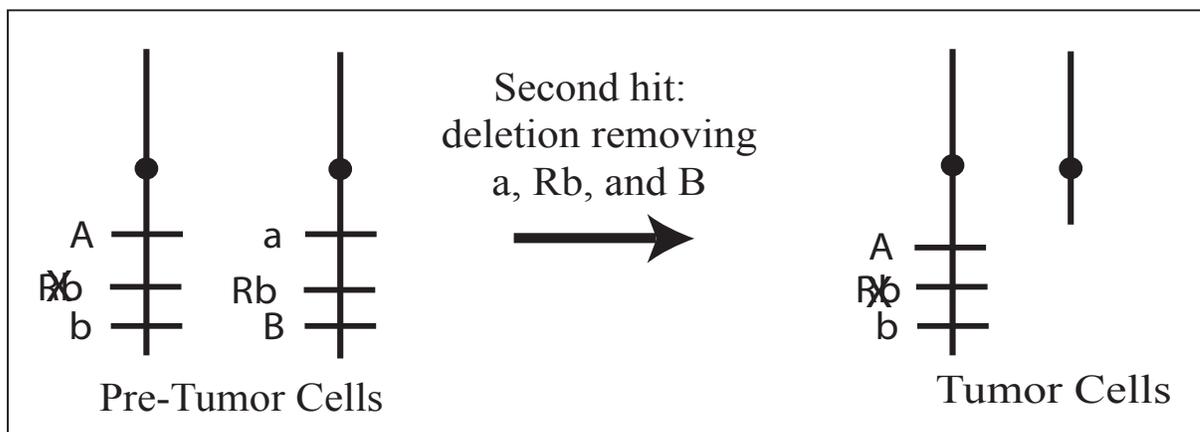


Figure 2: Loss of heterozygosity. The diagram represents chromosome 13 homologs (circle = centromere). The *RB1* locus is indicated between two marker loci, with alleles *A/a* and *B/b*. Either a mutation in *RB1* is inherited, or a sporadic mutation occurs inactivating one copy of *RB1* in a somatic cell. A second mutation, in this case loss of the portion of chromosome 13 that contains *RB1*, occurs in the same cell, resulting in complete lack of a functional *RB1*, leading to tumorigenesis. Thus the pre-tumor cells are heterozygous for a mutation in *RB1*, but tumor cells are no longer heterozygous, having lost the functional copy of *RB1*.

The example illustrated in Figure 2 involves a sporadic case of retinoblastoma. Shown is chromosome 13 after the first copy of *RBI* has been inactivated. In this example, A(a) and B(b) represent two marker loci that lie on either side of *RBI*. Each has one of two alleles (e.g., A and a). The *RBI* allele that is inactivated first is flanked by the A and b alleles, whereas the remaining functional copy of *RBI* is flanked by the a and B alleles. The second hit in *RBI* often involves deletion of a large region or loss of an entire chromosome. As a result, the functional copy of *RBI* 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. A cell that loses both copies of a particular tumor suppressor gene may have a proliferative advantage, and thus be selected for during tumor progression. Therefore, if loss-of-heterozygosity is frequently seen in a specific region of the genome in a particular tumor type, this suggests that a tumor suppressor gene that plays an important role in development of that tumor may be present in that region.

3. Mutation or loss of *RBI* removes a brake on the cell cycle

How does the loss of *RBI* promote tumor formation? Recall that the Rb protein plays a key role in regulating the cell cycle. It is expressed in every cell type, where it exists in an active hypophosphorylated and in active hyperphosphorylated state. In its active state, Rb serves as a brake on the advancement of cells from the G1 to the S-phase of the cell cycle. If Rb is lost or made nonfunctional through mutation, this brake on the cell cycle is released and cells move into S-phase unrestrained. Specifically, Rb normally binds to and inactivates the E2F transcription factor. Loss of Rb results in activation of E2F. E2F 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). Recall that Cdk2-cyclin E activity represents the transition from mitogen-dependent to mitogen-independent cell cycle progression, so inactivation of Rb can lock cells in a proliferating state.

As previously mentioned, individuals with germline mutation of *RBI* are at increased risk of developing second primary non-ocular tumors over their lifetime - most often osteosarcomas, soft tissue sarcomas, or melanomas. In addition, somatically acquired mutations in *RBI* have been described in breast cancers, glioblastomas, small cell lung cancers, and bladder cancers. Since Rb is present in every cell and plays an important role in cell cycle control, a couple questions come to mind. First, why do patients with germline mutation of *RBI* develop primarily retinoblastomas? It is not completely clear why tumors are typically restricted to the retina in patients who inherit a defective allele of *RBI*, though evidence suggests that homozygous loss of *RBI* triggers apoptosis, and that unrestrained action of E2F proteins (as would occur with loss of both *RBI* alleles) not only drives the cell cycle, but also triggers apoptosis. Therefore it is plausible that although in most tissues, homozygous loss of *RBI* induces cell death, the retinoblasts are relatively resistant to the apoptosis-inducing effect. In these cells, therefore, dysregulated E2F gives rise to tumors. In addition, there are likely parallel regulatory pathways in different cell types. While some cell types, e.g. breast, lung or bladder epithelial cells, require additional loss of other tumor suppressor genes or activation of oncogenes, the proliferation of retinoblasts in early childhood may be uniquely controlled by Rb, such that few other genetic changes are required for tumor formation. This may be because retinoblasts terminally differentiate by the age of six years, and thus do not need extensive safeguards

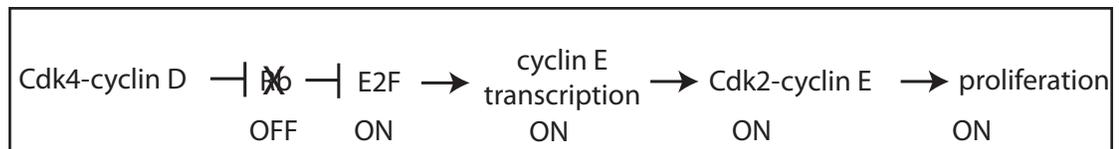


Figure 3. How inactivation of Rb leads to proliferation. Loss of both copies of Rb results in the activation of E2F, increased cyclin E transcription, formation of the Cdk2-cyclin E complex and thus cell proliferation (See “Cell Proliferation” lecture).

against uncontrolled proliferation. Whereas in other cell types, many more safeguards (including triggering apoptosis) have evolved to protect against inappropriate proliferation, and these safeguards must be overcome to develop cancer.

A second question that comes to mind: Why are inactivating mutations of Rb not more commonly seen in human cancer? The answer to this question is more straightforward. Mutations in other genes that control Rb phosphorylation can mimic the effect of Rb loss. Such genes are mutated in many cancers that seem to have normal *RB1* genes. Thus, for example, mutational activation of cyclin D or CDK4 would favor cell proliferation by facilitating Rb phosphorylation, thus maintaining it in an inactivated state. Can you think of other answers to these questions?

B. Genes encoding Cdk inhibitors are tumor suppressor genes

Mutational inactivation of CDK inhibitors also drives the cell cycle by unregulated activation of cyclins and CDKs. One such inhibitor, encoded by the **p16** gene, is a common target of deletion or mutational inactivation in human tumors. Recall that p16 is an inhibitor of Cdk4-cyclin D complexes. Germline mutations of p16 are associated with a subset of hereditary melanomas. Somatic deletion or inactivation of p16 is seen in 75% of pancreatic cancers; 40-70% of glioblastomas; 50% of esophageal cancers; and 20% of non-small cell lung cancers, soft tissue sarcomas, and bladder cancers. The loss of p16 leads to increased Cdk4-cyclin D activity. This results in phosphorylation and inactivation of Rb, leading to activation of E2F and cyclin E transcription. In fact, in cells that harbor mutations in either p16, CDK4, or cyclin D, the function of *RB1* is disrupted even if *RB1* itself is not mutated.

C. p53: a key tumor suppressor

p53, located on chromosome 17p13.1, is the single most common target for genetic alteration in human tumors. In fact, more than 50% of human tumors contain mutations in this gene! Thus it is among the most important “brakes” on tumor formation. Homozygous loss of the p53 gene is found in virtually every type of cancer, including carcinomas of the breast, colon, and lung – the three leading causes of cancer deaths. In most cases, the inactivating mutations affecting both p53 alleles are acquired in somatic cells. In some cases, although it is rare, individuals inherit a mutant p53 allele. As with *RB1*, inheritance of one mutant allele predisposes these individuals to develop malignant tumors because only one additional “hit” is needed to inactivate the second, normal, allele. Inactivation of the second p53 allele leads to increased cell proliferation, decreased apoptosis, and tumor development. These individuals have a rare cancer predisposition syndrome called **Li-Fraumeni syndrome**, and have a 25-fold greater chance of developing a malignant tumor by age 50, compared with the general population. In contrast to patients who inherit a mutant *RB1* allele, the spectrum of tumors that develop in patients with Li-Fraumeni syndrome is quite varied. The most common types of tumors

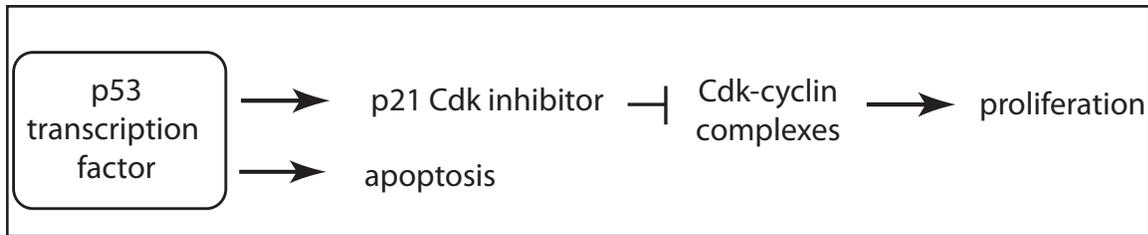


Figure 4. p53 tumor suppressor functions. *p53 antagonizes tumor formation by activating the p21 Cdk inhibitor (which blocks proliferation) and by promoting apoptosis.*

are sarcomas, breast cancer, leukemia, brain tumors, and carcinomas of the adrenal cortex. As compared with sporadic tumors, those that afflict patients with Li-Fraumeni syndrome occur at a younger age, and a given individual may develop multiple primary tumors.

p53 restrains tumor formation by two different mechanisms (Figure 4). In the first, 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 Cdks normally turned off by p21 and resulting in increased cell proliferation. A second way in which p53 restrains tumor formation is by inducing apoptosis.

D. Caretaker Genes that Function as Tumor Suppressors

BRCA1, located on 17q21, and *BRCA2*, located on 13q12, are tumor suppressor genes associated with breast and ovarian cancer, along with several other cancers. About 10% of all cases of breast and ovarian cancer are hereditary cancers, and most of these cases are due to inheritance of a germline mutation in either *BRCA1* or *BRCA2* (see *Familial and Hereditary Cancer Syndromes lecture*). As with other tumor suppressor genes, the remaining allele is inactivated or lost during the course of tumor formation (LOH). The proteins encoded by *BRCA1* and *BRCA2* are expressed in most tissues and cell types (indicating that gene expression does not account for the restricted phenotype of breast and ovarian cancer), and share a number of functional similarities.

BRCA1 and *BRCA2* function as “caretaker” genes, like p53, which serve to maintain genomic integrity. The gene products encoded by *BRCA1* and *BRCA2* are nuclear proteins that co-localize with RAD-51 at sites of DNA damage, and play a role in homologous recombination repair of double-stranded breaks (see *Mutation and Cancer lecture*). There is also evidence that *BRCA1* and *BRCA2* interact with the p53-mediated DNA damage checkpoint (see *Mutation and Cancer lecture*). Loss of *BRCA1* or *BRCA2* leads to the accumulation of other genetic defects, which can then lead to cancer formation. In addition to their roles in DNA repair, *BRCA1* and *BRCA2* have been implicated in a variety of cellular processes, including DNA synthesis, regulation of gene transcription (similar to p53, one target of *BRCA1* transcriptional activation is the Cdk inhibitor p21), cell cycle checkpoint control, centrosome duplication and ubiquitination.

Most *BRCA1* and *BRCA2* mutations lead to frameshifts resulting in missing or non-functional protein, or, in the case of *BRCA2*, to nonsense mutations leading to premature truncation of the protein. These mutations are all consistent with the loss of function expected with tumor suppressor genes.

A puzzle in breast cancer has been why mutations in *BRCA1* and *BRCA2* are not found as often in sporadic (i.e. non-familial) cases of breast cancer. Recent studies suggest partial answer. A protein called **EMSY** has been found that binds and inhibits BRCA2. Remarkably, EMSY is frequently overexpressed in sporadic breast cancers due to gene amplification, and this is apparently a much more likely event than the loss of the two normal copies of either the *BRCA1* or *BRCA2* genes. Increased EMSY expression correlates with poor clinical outcome.

E. Regulators of Signal Transduction

Another potential way that products of tumor suppressor genes may operate is by down-regulating growth-promoting signals. The products of the APC gene (5q21) and the NF-1 gene (17q11.2) fall into this category. Germline mutations of these genes are associated with benign tumors that are precursors of carcinomas that develop later.

In the case of the **APC (adenomatous polyposis coli)** gene, individuals born with one mutant allele invariably develop hundreds or even thousands of adenomatous polyps in the colon during their teens or 20s (familial adenomatous polyposis, or FAP; see Familial and Hereditary Cancer Syndromes lecture). Almost invariably, one or more of these polyps undergo malignant transformation, giving rise to cancer. As with other tumor suppressor genes, both copies of the APC gene must be lost for tumor development. When this occurs, adenomas formed. In addition to cancers arising in the setting of FAP, the majority (70-80%) of non-familial colorectal carcinomas and sporadic adenomas also show homozygous loss of the APC gene, thus firmly implicating APC loss in the pathogenesis of colonic tumors. (See lecture on Colon Cancer.) As described in the Cell Proliferation lecture, an important function of the APC protein is to cause degradation of beta-catenin, thus maintaining low levels in the cytoplasm. Inactivation of the APC gene, and consequent loss of the APC protein, increases the cellular level's of beta-catenin, which, in turn, translocates to the nucleus and up-regulates cellular proliferation. Thus APC is a negative regulator of beta-catenin signaling. Beta-catenin itself functions as a proto-oncogene (see below). Interestingly, cases of colorectal cancer that do not show loss of APC may in fact have activating mutations in beta-catenin. Dysregulation of the APC/beta-catenin pathway is not restricted to colon cancer; mutations in either APC or beta-catenin have also been found in nearly 30% of melanoma cell lines.

The **NF1** gene behaves similar to the APC gene. Individuals who inherit one mutant allele develop numerous benign neurofibromas, presumably as a result of the inactivation of the second copy of the NF1 gene. This condition is called neurofibromatosis type 1, or NF1. Some of the neurofibromas may undergo malignant transformation into neurofibrosarcomas. Children with NF1 also are at increased risk for developing acute myeloid leukemia. The function of neurofibromin, the protein product of the NF1 gene, is to regulate signal transduction via the Ras protein. Neurofibromin is a GTP-ase activating protein that facilitates conversion of active Ras (GTP bound) to inactive Ras (GDP bound). With loss of NF1, Ras is trapped in an active, signal-emitting state.

III. 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)**. The fundamentals of viral tumorigenesis, including some of the history, were covered in the I3 block. Here we will merely review how HPVs cause cancer in order to connect to two of the main tumor suppressor genes discussed in this section.

Human papilloma viruses (transmitted through sexual contact) play a pathogenic role in most cases of cervical cancer (see information on cervical cancer in the Pathological Characteristics of Benign and Malignant Neoplasms lab, the Cancer Screening lecture, and the Clinical and Translational Research online module). 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. There are 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 a 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 a polyubiquitin chain to a protein targets it to the proteasome for degradation. 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), by mutations in genes that control Rb Phosphorylation, 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. Without the safeguards of both p53 and Rb, the cell is strongly driven toward cancer.

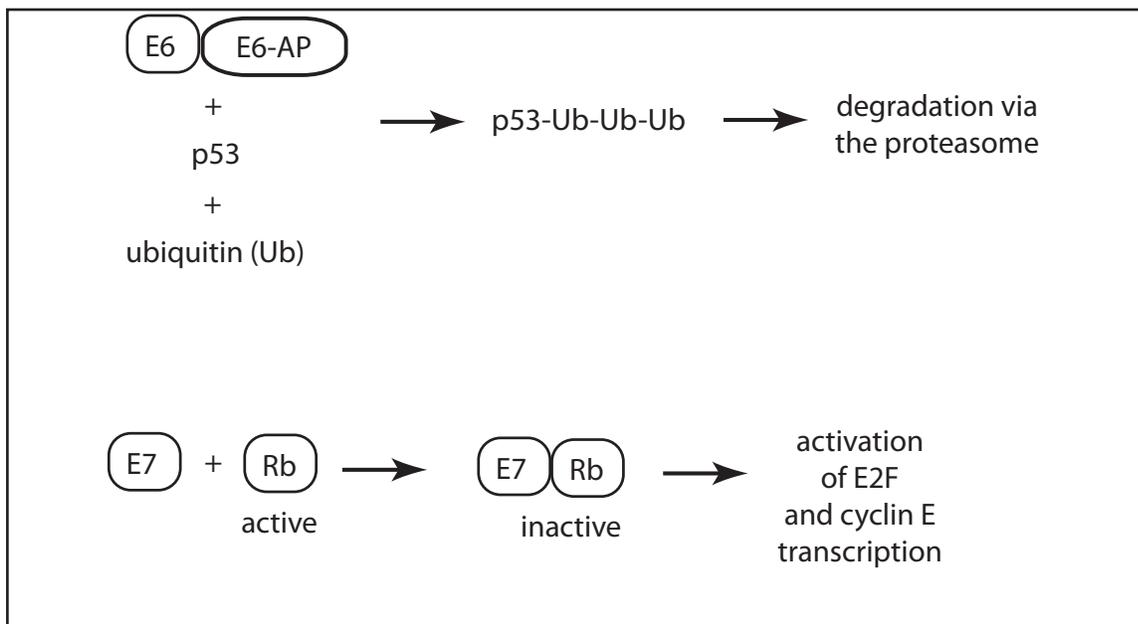


Figure 5. Inactivation of tumor suppressor genes by proteins encoded by Human Papilloma Virus. The E6 protein of oncogenic HPVs binds to a cellular protein called E6-AP (E6 associated protein), which is a ubiquitin ligase. The E6-E6AP protein complex catalyzes the attachment many copies of the small 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.

IV. 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 brakes on cell proliferation, oncogenes actively promote proliferation (analogous to the gas pedal 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 (they both belong to the ErbB family of receptors). This gene is overexpressed in ~25% of breast cancers. Breast cancers that overexpress HER2/neu tend to be more aggressive clinically (more on this in upcoming lectures on Breast Cancer and Cancer Treatment). The mechanism by which HER2/neu is overexpressed is **gene amplification**, which results in multiple copies of the gene accumulating in tumor cells. The increase in the levels of the HER2/neu tyrosine kinase receptor on tumor cells results in increased signaling via the Ras-MAPK pathway, driving 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 Drug Development 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.

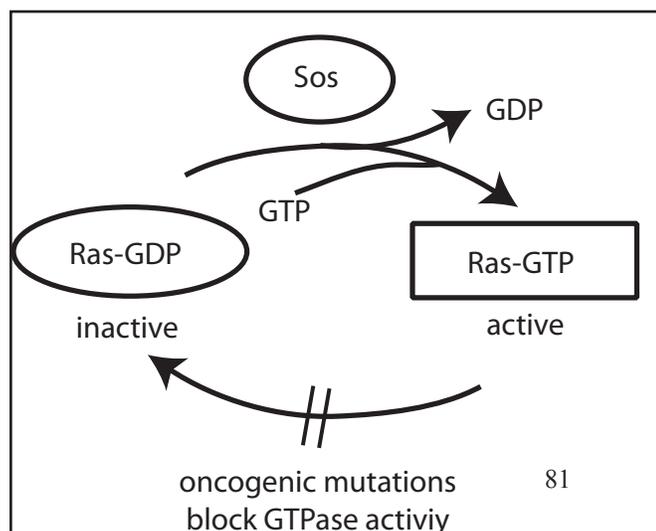


Figure 6. How mutations in the Ras oncogenes promote cancer. *Oncogenic mutations in ras genes prevent the protein from hydrolyzing GTP to GDP. As a result, the protein remains always in its active GTP-bound form, continually activating the MAP kinase cascade, leading to proliferation.*

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 where normal cells would halt. In some cancers, rather than being amplified, Myc is made active by chromosomal translocation. (See the Cancer Cytogenetics section of the Mutation and Cancer lecture for a description of chromosome abnormalities in cancer.) As a result of this chromosomal rearrangement, a strong promoter sequence is placed 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 large amounts of antibodies in B cells. As a result, mutant B cells produce large amounts of the Myc protein, proliferate to excess, and form a tumor.

D. Cyclin D can be activated through several different mechanisms

As discussed in the Cell Proliferation lecture, 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 cyclin D gene is often amplified. As with HER2/neu amplification, this results in more cyclin E protein being produced.

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 **chromosome inversion** places the strong transcriptional control region of the parathyroid hormone gene adjacent to the cyclin D gene on chromosome 11q. 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 chromosome translocation that places the cyclin D gene under control of the immunoglobulin heavy chain transcription control region. (Chromosome rearrangements are described in the Cancer Cytogenetics section of the Mutation and Cancer lecture.)

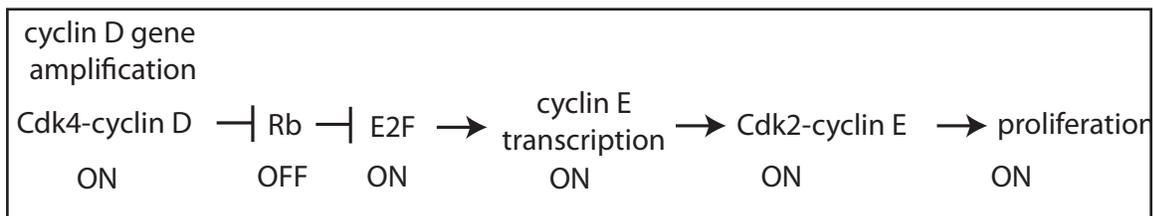


Figure 7. How cyclin D gene amplification leads to proliferation. Increased numbers of cyclin D genes leads to increased levels of Cdk4-cyclin D complexes which activate cyclin E transcription via the Rb-E2F pathway.

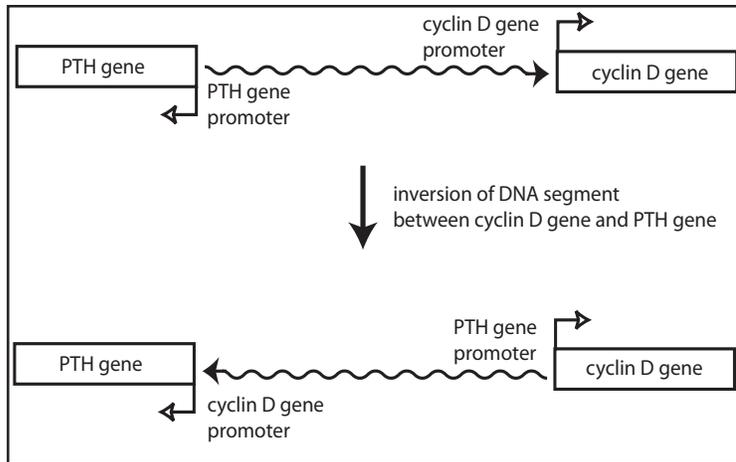


Figure 8. Activation of cyclin D in parathyroid adenomas. *In parathyroid adenomas, the cyclin D gene is activated by a DNA inversion. The inversion places the promoter of the parathyroid hormone gene (PTH gene) next to the cyclin D coding region.*

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.

V. 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 many tumor suppressor genes and oncogenes, we are beginning to 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. Recall that APC limits Wnt signaling in the gut epithelium where it is expressed at high levels in non-dividing 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 to be a period of genomic 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 to growth control occurs via the loss of a SMAD gene required for signaling through the growth inhibitory TGF-beta signaling pathway. 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. (For description of cancer nomenclature, see the Introduction to Cancer Pathology lecture.)

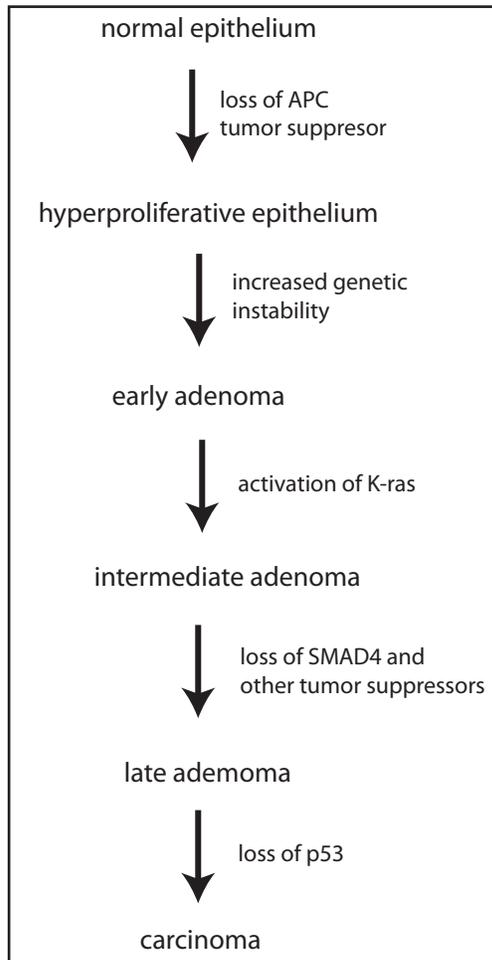


Figure 9. Multistep model for colorectal cancer. Shown is the sequence of pathological events that take place during the development of sporadic colon cancer and the genetic changes that occur during this sequence. The sequence of events is not absolute – this diagram describes what happens on average.

There are additional changes in tumor suppressor genes and oncogenes that occur during tumor progression that are different in different colon cancers, some of which have yet to be identified. Nonetheless, the study of the molecular basis 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

VI. THE HALLMARKS OF CANCER

If we consider the hallmark features of cancer cells that were introduced in the introductory lecture on Cancer Biology, we can begin to map the molecular defects in the genes and processes we've discussed in the last two lectures to one of the six acquired capabilities of cancer cells. Let's consider the first four acquired capabilities here (the last two will be covered in later lectures). A couple examples of molecular defects that can result in each acquired capability are included below. Can you think of others?

1. Self-Sufficiency in Growth Signals

Normal cells cannot proliferate in the absence of stimulatory signals, but cancer cells can. Many oncogenes act by mimicking normal growth signaling through one of several mechanisms.

- While most mitogenic growth factors are made by one cell type in order to stimulate

proliferation of another (heterotypic signaling), many cancer cells acquire the ability to synthesize their own growth factors, creating a positive feedback signaling loop (autocrine stimulation), and obviating the dependence on growth factors from other cells. The production of PDGF (platelet derived growth factor) and TGF- α (tumor growth factor α) by glioblastomas and sarcomas are two examples.

- Cell surface receptors that transduce growth-stimulatory signals into the cell, for example EGFR and HER2/neu, may be over expressed or structurally altered, leading to ligand-independent signaling.
- Downstream targets of the signaling pathway can also be altered. For example, Ras is found mutated about 25% of human tumors, thus leading to ligand-independent activation of the Ras-Raf-MAPK signaling pathway.

2. Insensitivity to antigrowth signals

Antigrowth signals can block proliferation by two mechanisms: 1) forcing cells out of the active proliferative cycle into the quiescent (G_0) state, until appropriate growth signals put them back into the cell cycle; or 2) inducing differentiation, which permanently removes their proliferative potential. Cancer cells evade these antiproliferative signals. At the molecular level, many and perhaps all anti-proliferative signals are funneled through the Rb protein (and its two relatives, p107 and p130). Disruption of the Rb pathway liberates E2Fs and thus allows cell proliferation, rendering cells insensitive to anti-growth factors that normally block advance through G1. The Rb signaling pathway (regulated by TGF- β anti-proliferative signaling and other extrinsic factors) can be disrupted in a variety of ways in different types of human tumors, for example:

- Loss of TGF β (normally acts to prevent phosphorylation that inactivates Rb)
- Loss of Smad4 (normally transduces signals from ligand-activated TGF β receptors to downstream targets)
- Loss of CDK inhibitors – p16, p21, p53
- Inactivation of Rb directly by hyperphosphorylation or inactivating mutation

Tumor cells also use various strategies to avoid terminal differentiation. Overexpression of cMyc is one such strategy.

3. Evading Apoptosis

As discussed earlier, the ability of tumor cell populations to expand in number is determined not only by the rate of cell proliferation but also by the rate of cell attrition. Programed cell death, or apoptosis, represents a major source of this attrition. Resistance to apoptosis as can be acquired by cancer cells through a variety of strategies. Examples include;

- Loss of p53 (normally activates pro-apoptotic proteins; represents the most common loss of a proapoptotic regulator)
- Activation or upregulation of anti-apoptotic Bcl2

4. Limitless Replicative Potential

The first three acquired capabilities – lack of requirement for growth signals, insensitivity to antigrowth signals, and resistance to apoptosis – all lead to an uncoupling of the cell's growth program from signals in its environment. However, it has been shown that this uncoupling alone does not ensure expansive tumor growth. Most types of mammalian cells carry an intrinsic mechanism that limits their proliferation by keeping track of the number of cell generations. This mechanism uses the number of telomere repeats at the ends of chromosomes, which erode through successive cycles of replication - eventually

leading to cell death. A small percentage of tumor cells avoid this by obtaining a mutation that upregulates expression of the telomerase enzyme, thus acquiring limitless replicative potential by maintaining their telomeres. (More on telomeres and telomerase in the Mutation and Cancer lecture.)

5 and 6. The 5th and 6th acquired capabilities, Sustained Angiogenesis and Tissue Invasion and Metastasis, will be covered in detail in later lectures.

References

Alberts et al. *Molecular Biology of the Cell*; 5th Edition, Garland Science, 2008.

GeneReviews on the NCBI GeneTests website:

<http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests> And references therein.

- BRCA1 and BRCA2 Hereditary Breast and Ovarian Cancer
- Retinoblastoma

Hanahan, D. and Weinberg, R. (2000). The Hallmarks of Cancer. *Cell* 100, 57-70.

Mendelsohn et al. *The Molecular Basis of Cancer*, 3rd Edition, Elsevier/Saunders, 2008

OMIM (Online Mendelian Inheritance in Man) <http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim> And references therein.

- BRCA1, BRCA2

Acknowledgements:

Significant contributions to this lecture were made by

Hiten Madhani, PhD, Department of Biochemistry and Biophysics, who originally developed this lecture (lecturer 2002 – 2006);