Molecular Techniques in Radiobiology

Chapter 16 (6th edition)

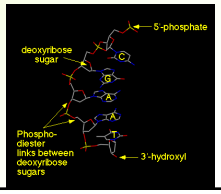
Eric J. Hall., Amato Giaccia,
Radiobiology for the Radiologist

Introduction

- The birth of molecular biology could be ascribed to the discovery of the structure of DNA by Watson and Francis Crick in 1953
- In the late 1940s and early 1950s, Linus Pauling realized that as amino acids were folded into proteins, they formed three-dimensional structures, and that function was related to the structure
- The whole concept emerged that the sequence of bases, which coded for a protein, ultimately determined function leading the way to breaking the genetic code

The structure of DNA

- DNA - deoxyribonucleic acid is a polymer with the monomer units of nucleotides
- Each nucleotide consists of a 5-carbon sugar (deoxyribose), a nitrogen containing base attached to the sugar, and a phosphate group
- There are four different types of nucleotides found in DNA, differing only in the nitrogenous base:
  - A is for adenine
  - G is for guanine
  - C is for cytosine
  - T is for thymine



The structure of DNA

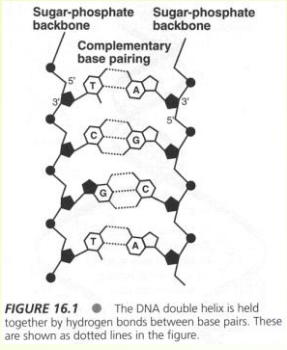


FIGURE 16.1 ● The DNA double helix is held together by hydrogen bonds between base pairs. These are shown as dotted lines in the figure.

- DNA molecule has many deoxyribo-nucleotides (bases) linked in a chain-like arrangement
- Bases are held by hydrogen bonds and are paired complimentary:
  - adenine with thymine
  - cytosine with guanine
- Each half constitutes a template for reconstruction of the other half

RNA and DNA

- RNA – ribonucleic acid, has ribose sugar molecule instead of deoxyribose
- In the cell RNA is usually single-stranded, while DNA is usually double-stranded
- RNA has the base uracil rather than thymine that is present in DNA
- RNA has a much shorter chain of nucleotides
- Unlike DNA, which is located primarily in the nucleus, RNA is found throughout the cell
- There are several types of RNA

Transcription and translation

The flow of genetic information from DNA to protein (gene expression) requires a series of steps:

- In the first step, the DNA code is *transcribed* in the nucleus into mRNA (messanger RNA); transcription is controlled by other DNA sequences (such as promoters), which show a cell where genes are, and control how often they are copied
- During the second step, the RNA copy made from a gene is then fed through a ribosome, which *translates* the sequence of nucleotides (with help of ribosomal RNA and transfer tRNA) in the mRNA into the correct sequence of amino acids and joins these amino acids together to make a complete protein chain
- The new protein then folds up into its active form

Transcription and translation

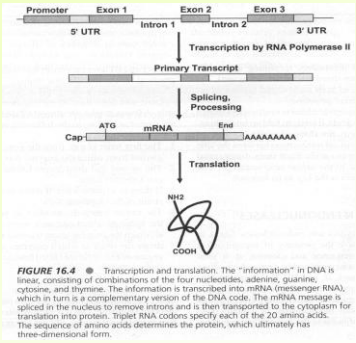


FIGURE 16.4 ● Transcription and translation. The "information" in DNA is linear, consisting of combinations of the four nucleotides, adenine, guanine, cytosine, and thymine. The information is transcribed into mRNA (messenger RNA), which in turn is a complementary version of the DNA code. The mRNA message is spliced in the nucleus to remove introns and is then transported to the cytoplasm for translation into protein. Triplet RNA codons specify each of the 20 amino acids. The sequence of amino acids determines the protein, which ultimately has three-dimensional form.

- Introns and exons are nucleotide sequences within genes
- Typically introns make up the bulk of the gene
- Introns are removed during the process of splicing
- Remaining exons are joint together
- Only the exons of the DNA are translated

Amino acids and proteins

- Each amino acid is specified by triplet mRNA sequence, codon; four bases give a possibility to have 4x4x4=64 combinations
- There are only 20 amino acids; more than one triplet can code each amino acid
- The vast majority of proteins is composed of a mixture of the same 20 amino acids; chains vary from 5 to > 4000 amino acids

Gene manipulation tools

- The most common form of genetic manipulation (engineering) involves the insertion of new genetic material in the host genome at a random or specific location
- Need to have abilities to recognize specific sequences, cleave DNA, replicate the pieces, insert it into a host

Gene manipulation tools

- *Restriction endonucleases*: enzymes found in bacteria that can recognize specific DNA sequence and cleave at or near that site
- Recognition sites are short, 4 to 8 nucleotides, and usually read the same in both directions (palindromic sequences)
- There are types I,II, and III, but only type II cut the DNA without modifications, and are used in molecular biology

Gene manipulation tools

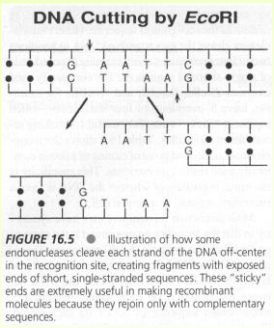


FIGURE 16.5 ● Illustration of how some endonucleases cleave each strand of the DNA off-center in the recognition site, creating fragments with exposed ends of short, single-stranded sequences. These "sticky" ends are extremely useful in making recombinant molecules because they rejoin only with complementary sequences.

- Some endonucleases produce blunt-ended fragments because they cut cleanly through the DNA, cleaving both complementary strands at the same nucleotide position, most often near the middle of the recognition sequence
- Other endonucleases cleave the two strands of DNA at positions two to four nucleotides apart, creating exposed "sticky" ends of single-stranded sequences, very useful for making recombinant molecules

Gene manipulation tools

- *A vector* is a self-replicating DNA molecule that has the ability to carry a foreign DNA molecule into a host cell
- Vectors are typically used to insert fragments of human DNA, containing a gene of interest, into a bacterium that will self-replicate, producing quantities enough for a study
- There are many types of vectors: plasmids, bacteriophages, bacterial artificial chromosomes (BACs), and viruses

Gene manipulation tools

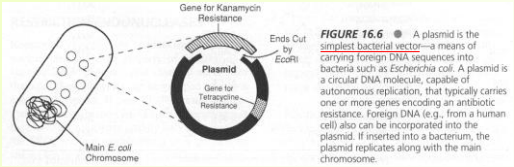


FIGURE 16.6 ● A plasmid is the simplest bacterial vector—a means of carrying foreign DNA sequences into bacteria such as *Escherichia coli*. A plasmid is a circular DNA molecule, capable of autonomous replication, that typically carries one or more genes encoding an antibiotic resistance. Foreign DNA (e.g., from a human cell) also can be incorporated into the plasmid. If inserted into a bacterium, the plasmid replicates along with the main chromosome.

- Plasmids contain genes for resistance to bacteriophages, antibiotics, and some heavy metals
- Only those bacteria that have taken up the plasmid survive and replicate in a culture medium containing the antibiotic
- Useful only for relatively small DNA inserts (~10,000 bps)
- Do not transfect into bacteria with high efficiency

Gene manipulation tools

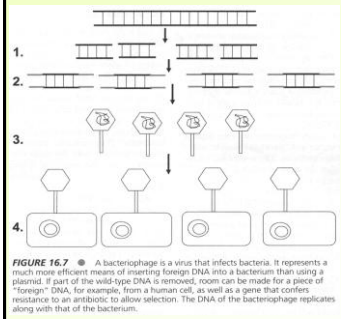


FIGURE 16.7 ● A bacteriophage is a virus that infects bacteria. It represents a much more efficient means of inserting foreign DNA into a bacterium than using a plasmid. If part of the wild-type DNA is removed, room can be made for a piece of "foreign" DNA, for example, from a human cell, as well as a gene that confers resistance to an antibiotic to allow selection. The DNA of the bacteriophage replicates along with that of the bacterium.

- Bacteriophage can infect its host at a much higher efficiency than a plasmid
- It can accommodate a larger range of DNA fragments, from a few to up to 24,000 bp

Gene manipulation tools

- *BACs* (*bacterial artificial chromosomes*) are vectors based on a type of plasmid with sequences encoding self-replication. BACs can accommodate approximately 300 kilobases (kb) of DNA (plasmids are limited to approximately 10-kb insertions)
- BACs were the primary vector used during the genome-sequencing projects, mainly because a BAC carrying a gene of interest is easily acquired
- *Viruses* are highly efficient vectors for introducing foreign genes into mammalian cells
  - **Retroviruses** are a type of RNA virus. The RNA genome of retroviruses is transcribed into DNA, which is then integrated into the host genome. Retroviruses can infect virtually every type of mammalian cell, making them very versatile.
  - **Adenoviruses** are a type of DNA virus

Gene manipulation tools: hosts

- Recombinant DNA molecules can be constructed and manipulated to some extent in the test tube, but amplification and expression ideally require a host:
  - Escherichia coli
  - Yeast
  - Mammalian cells with limited or unlimited lifespan

Gene manipulation tools: hosts

- Mammalian cells do not take up foreign DNA naturally; one of several tricks must be used to bypass natural barriers
  - *Microinjection:* DNA can be injected, cell by cell, directly into the nucleus through a fine glass needle.
  - *Calcium phosphate precipitation:* Cells take up DNA relatively efficiently in the form of a precipitate with calcium phosphate
  - *Cationic lipids:* offer some of the highest transfection efficiencies and expression levels to a wide variety of cells, both in suspension and attached
  - *Electroporation:* Cells in solution are subjected to a brief electrical pulse that causes holes to open transiently in the membrane, allowing foreign DNA to enter
  - *Viral vectors:* The ultimate means of transfection involves the use of a retrovirus. This is analogous to using bacteriophage to get DNA into bacteria. **Oncogenes,** genes that can cause cancer, and their counterpart, **tumor-suppressor genes,** can be studied by incorporating them into retro-viral vectors

Agarose gel electrophoresis

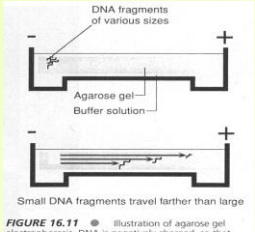
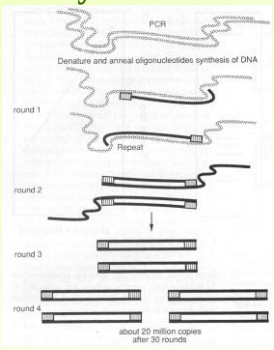


FIGURE 16.11 ● Illustration of agarose gel electrophoresis. DNA is negatively charged, so that under the influence of an electrical field, it migrates toward the anode. During electrophoresis, DNA fragments sort by size, small molecules moving farther than larger molecules. Because smaller molecules move farther than larger molecules in a given time, polyacrylamide gel electrophoresis often is employed to separate smaller DNA fragments with greater resolution than with agarose.

- DNA is negatively charged
- Can separate pieces of DNA of different sizes
- After separation is complete ethidium bromide is added, making DNA fluoresce under ultraviolet light to make the position of the DNA visible

Polymerase chain reaction (PCR)



- DNA polymerase is an enzyme that catalyzes the polymerization of nucleotides into a DNA strand
- Primers (short DNA fragments) contain sequences complementary to the target region
- The polymerase "reads" an intact DNA strand as a template and uses it to synthesize the new strand. This process copies a piece of DNA
- As PCR progresses, the DNA generated is itself used as a template for replication, promoting a chain reaction

FIGURE 16.13 The polymerase chain reaction for the amplification of DNA fragments. The number of DNA molecules is doubled in each cycle, which takes about 7 minutes, so that in a matter of several hours, millions of copies of a DNA fragment can be made. (Courtesy of Dr.

Gene analysis: Southern blotting

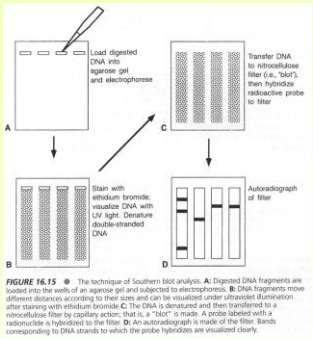


FIGURE 16.15 The technique of Southern blot analysis. **A:** Digested DNA fragments are loaded into the wells of an agarose gel and subjected to electrophoresis. **B:** DNA fragments move different distances according to their sizes and can be visualized under ultraviolet illumination after staining with ethidium bromide. **C:** The DNA is denatured and then transferred to a nitrocellulose filter by capillary action; that is, a "blot" is made. A probe labeled with a radionuclide is hybridized to the filter. **D:** An autoradiograph is made of the filter. Bands corresponding to DNA strands to which the probe hybridizes are visualized clearly.

- Mapping technique, used to detect specific fragments of DNA, named after British biologist Edwin Southern
- Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe
- Can detect mutations, such as insertions, deletions and sequence differences in DNA

Gene analysis: Chromosome walking

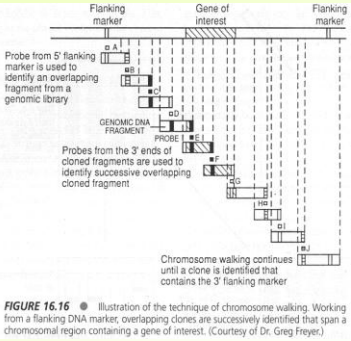


FIGURE 16.16 Illustration of the technique of chromosome walking. Working from a flanking DNA marker, overlapping clones are successively identified that span a chromosomal region containing a gene of interest. (Courtesy of Dr. Greg Freyer.)

- Used when gene has been mapped to a specific arm of a chromosome, but has not been isolated
- Probes are used to identify an overlapping fragment of DNA from the genomic library

Gene knockouts

- Gene knockout technique involves making one of the genes in a living organism inoperative
- Used to learn about a gene that has been sequenced, but has an unknown or incompletely known function

A knockout mouse (left) that is a model of obesity, compared with a normal mouse

- Genes in embryonic stem cells are manipulated and inserted into early embryos
- Gene function is inferred from the difference between the knockout organism and normal individuals

Other blotting techniques

- *Northern blotting* - the technique for separating RNA by gel electrophoresis and is analogous to the Southern blot technique used to study DNA
- *Western blotting* – used to detect specific proteins in a homogenized tissue sample
  - Proteomics - seeks to define the quantities and interactions of the vast number of proteins in a given cell at a given instant in time

Databases and sequence analysis

- GenBank, a database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) for the deposition of individually cloned genomic, cDNA and protein sequences
- Related programs, called search engines, were developed to find similar sequences from the ever-growing list of identified genes contained in the databases
- One of the most commonly used programs for finding related sequences from the genome databases is BLAST, found on the NCBI website

Cancer Biology

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Introduction

- Tissue homeostasis depends on the regulated cell division and self-elimination (programmed cell death) of each of its constituent members except its stem cells.
- A tumor arises as a result of uncontrolled cell division and failure for self-elimination.
- Alterations in three groups of genes are responsible for the deregulated control mechanisms that are the hallmarks of cancer cells: proto-oncogenes, tumor-supressor genes, and DNA stability genes

Proto-oncogenes

- Proto-oncogenes are components of signaling networks that act as positive growth regulators in response to mitogens, cytokines, and cell-to-cell contact
- A gain-of-function mutation in only one copy of a protooncogene results in a dominantly acting oncogene that often fails to respond to extracellular signals

Tumor-suppressor genes

- Tumor-suppressor genes are also components of the same signaling networks as proto-oncogenes, except that they act as negative growth regulators
- They modulate proliferation and survival by antagonizing the biochemical functions of proto-oncogenes or responding to unchecked growth signals
- In contrast to oncogenes, inactivation of both copies of tumor-suppressor genes is required for loss of function in most cases

DNA stability genes

- DNA stability genes form a class of genes involved in both monitoring and maintaining the integrity of DNA.
- Loss of these genes results in defective sensing of DNA lesions as well as improper repair of the damaged template

Mechanisms of carcinogenesis

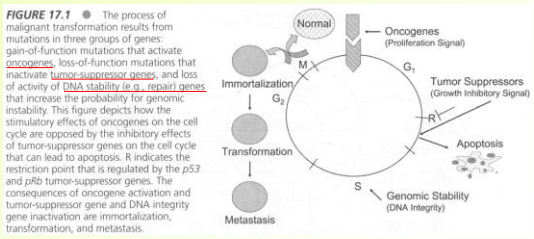


FIGURE 17.1 ● The process of malignant transformation results from mutations in three groups of genes: gain-of-function mutations that activate oncogenes, loss-of-function mutations that inactivate tumor-suppressor genes, and loss of activity of DNA stability (e.g., repair) genes that increase the probability for genomic instability. This figure depicts how the stimulatory effects of oncogenes on the cell cycle are opposed by the inhibitory effects of tumor-suppressor genes on the cell cycle that can lead to apoptosis. R indicates the restriction point that is regulated by the *p53* and *pRb* tumor-suppressor genes. The consequences of oncogene activation and tumor-suppressor gene and DNA integrity gene inactivation are immortalization, transformation, and metastasis.

The malignant progression from normal tissue to tumor to metastasis occurs in a number of discrete "steps" over a period of time

Oncogenes

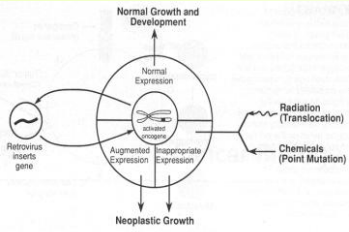


FIGURE 17.2 ● How the concept of oncogenes provides a ready answer for how agents as diverse as viruses, radiations, and chemicals all can induce tumors that are essentially indistinguishable one from another. The retrovirus inserts a gene; a chemical may activate an endogenous oncogene by a point mutation; radiation may do the same by causing, for example, a translocation. (Adapted from Bishop JM: Cellular oncogene retroviruses. *Ann Rev Biochem* 52:301–354, 1983, with permission.)

- First discovered from studies of retroviruses that cause cancer in animals
- Proto-ocogene has to be activated in order to produce tumor

Mechanisms of oncogene activation

- Transcriplional deregulation by overexpression or abnormal expression of the mRNA of a proto-oncogene is a common theme
- It is a dominant gene, mutation in only one copy leads to its activation
- At least four mechanisms exist:
  - Retroviral integration of proto-oncogene sequences in retroviral genomes through recombination
  - DNA mutation of regulatory sites
  - Gene amplification
  - Chromosome rearrangement

Mechanisms of oncogene activation

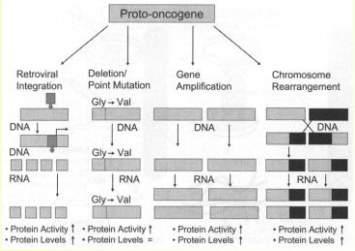


FIGURE 17.3 ● Four basic mechanisms of how a proto-oncogene can become an activated oncogene. Retroviral integration in close proximity to a proto-oncogene results in transcriptional control of the proto-oncogene by the viral promoter, resulting in increased proto-oncogene protein and activity. Deletion or point mutations in the proto-oncogene result in increased activity of the proto-oncogene without necessarily changing transcription or protein levels of the proto-oncogene. Increased copy number of a proto-oncogene by gene amplification results in increased transcription, protein levels, and activity of the proto-oncogene. Chromosome translocation results in an altered proto-oncogene product that can have increased transcription, protein levels, and activity. All of these alterations in a proto-oncogene that occur at the DNA level manifest themselves at the protein level as increased activity and in some

Chromosome translocation

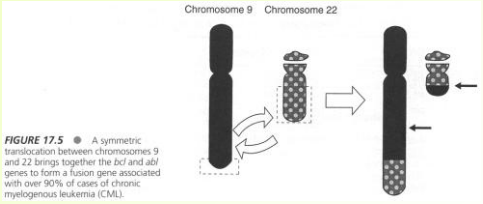


FIGURE 17.5 ● A symmetric translocation between chromosomes 9 and 22 brings together the *bcl* and *abl* genes to form a fusion gene associated with over 90% of cases of chronic myelogenous leukemia (CML).

- The first real breakthrough in identifying tumor-specific chromosome alterations occurred in the late 1950s when Dr. Peter Nowell found a consistent shortened version of chromosome 22 in individuals afflicted with chronic myelogenous leukemia (CML)

Tumor-suppressor genes

- Recessive gene, both copies of tumor-suppressor gene have to be inactivated in order to suppress malignant transformation
- First discovered through family history studies of patients with hereditary cancers, such as retinoblastoma (*Rb* gene) or Li-Fraumeni syndrome (*p53* gene)

Retinoblastoma studies

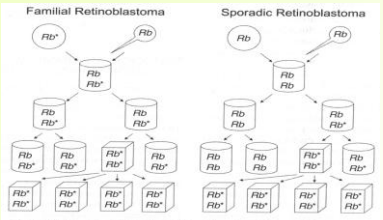


FIGURE 17.6 ● *Rb* mutations in familial and sporadic retinoblastoma. In familial retinoblastoma, one normal allele (*Rb*) and one mutated allele (*Rb**) are inherited from either parent, resulting in a heterozygous individual containing *Rb/Rb** retinal cells. Subsequent mutation in any retinal cell inactivates the remaining normal *Rb* allele, leading to loss of growth control and expansion of the homozygous mutant *Rb*/Rb** retinal cells that leads to retinoblastoma. In sporadic retinoblastoma, two normal *Rb* alleles are inherited from each parent. First, a mutation inactivates one copy, resulting in heterozygous *Rb/Rb** retinal cells. A subsequent mutation within the same retinal cell inactivates the remaining copy of normal *Rb*, leading to loss of growth control and expansion of homozygous *Rb*/Rb** retinal cells that leads to retinoblastoma. (Illustrating the concepts proposed by Knudson AG: Mutation and cancer: Statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68:820–823, 1971.)

Li-Fraumeni syndrome studies

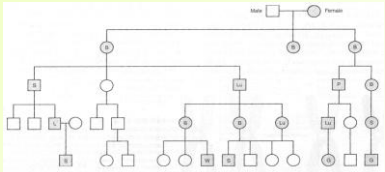


FIGURE 17.7 ● Pedigree analysis of familial cancer history of Li–Fraumeni syndrome. Symbols: B = breast cancer; G = glioblastoma; L = leukemia; Lu = lung cancer; P = pancreatic cancer; S = sarcoma; W = Wilms' tumor. (From LJ FP, Fraumeni JF. Prospective study of a family cancer syndrome. *J Amer Med Assoc* 247:2692–2694, 1982.)

- Extremely rare syndrome, predisposes to development of multiple cancers by young adulthood
- Led to discovery of *p53* gene, its suppression results in a number of tumors

Tumor-suppressor genes

TABLE 17.2

Examples of Cancer Predisposition Genes and Their Associated Syndromes

Tumor-Suppressor Gene	Syndrome	Tumor
Rb	Retinoblastoma	Retinoblastoma
WT1	Familial Wilms' tumor	Wilms' tumor
NF1	Neurofibromatosis type 1	Neurofibroma, sarcoma
NF2	Neurofibromatosis type 2	Schwannoma, meningioma
APC	Familial adenomatosis polyposis	Tumor of colon, stomach, intestine
p53	Li-Fraumeni Syndrome	Breast, lung, brain tumors, sarcoma
VHL	von Hippel-Lindau disease	Tumor of kidney, adrenal
E-CAD	Familial gastric cancer	Tumor of stomach, breast
PTCH	Gorlin syndrome	Basal cell carcinoma
PTEN	Cowden syndrome	Hamartoma
MEN1	Multiple endocrine neoplasia	Tumor of pituitary, pancreas, parathyroid

Tumor-suppressor genes

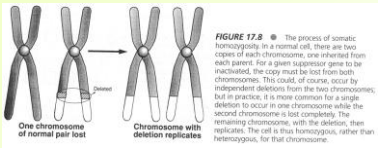


FIGURE 17.8 ● The process of somatic homozygosity. In a normal cell, there are two copies of each chromosome, one inherited from each parent. For a given suppressor gene to be inactivated, the copy must be lost from both chromosomes. This could, of course, occur by independent deletions from the two chromosomes; but in practice, it is more common for a single deletion to occur in one chromosome while the second chromosome is lost completely. The remaining chromosome, with the deletion, then replicates. The cell is thus homozygous, rather than heterozygous, for that chromosome.

- Often tumor-suppressor gene is lost through somatic homozygosity: one chromosome of a pair is lost, a deletion occurs in the remaining chromosome, and the chromosome with the deletion replicates
- This process has been documented for a number of tumors

The multi-step nature of cancer

- Carcinogenesis is a multi-step process: a number of distinct events that may be separated in time have to occur
- Genetic analysis of cells from solid tumors suggests alterations, mutations, or deletions in multiple signaling genes, either oncogenes or suppressor genes. For example, 6 to 12 mutations have been suggested for the formation of a carcinoma
- The following stages can be identified in tumor development: *initiation, promotion, and progression*

The multi-step nature of cancer

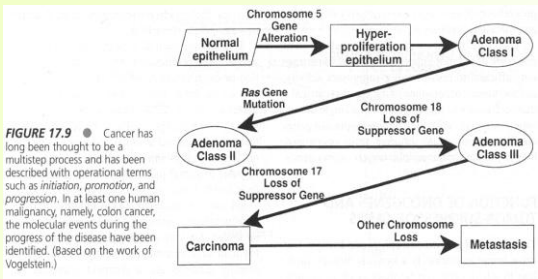


FIGURE 17.9 ● Cancer has long been thought to be a multistep process and has been described with operational terms such as *initiation*, *promotion*, and *progression*. In at least one human malignancy, namely, colon cancer, the molecular events during the progress of the disease have been identified. (Based on the work of Vogelstein.)

The multi-step nature of cancer

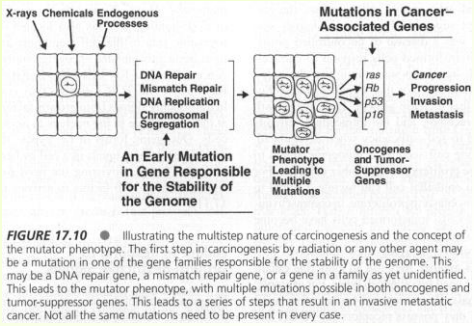


FIGURE 17.10 ● Illustrating the multistep nature of carcinogenesis and the concept of the mutator phenotype. The first step in carcinogenesis by radiation or any other agent may be a mutation in one of the gene families responsible for the stability of the genome. This may be a DNA repair gene, a mismatch repair gene, or a gene in a family as yet unidentified. This leads to the mutator phenotype, with multiple mutations possible in both oncogenes and tumor-suppressor genes. This leads to a series of steps that result in an invasive metastatic cancer. Not all the same mutations need to be present in every case.

Functions of oncogenes and tumor-suppressor genes

- Several categories of cell functions are perturbed by mutations in oncogenes and tumor-suppressor genes
- Mostly these are functions related to regulation of proliferation, growth-restriction and apoptosis signals
- Combination in deregulations of these functions lead to tumor initiation, invasion and metastasis

Deregulated proliferation

- Normal cells rely on extracellural growth signals; typically one cell secretes a mitogenic signal to stimulate the proliferation of another cell type
- Signal is initiated at the cell membrane (receptors) and is transduced to the nucleus via a cascade of proteins affecting regulatory functions
- In contrast to untransformed cells, transformed cells become autonomous in regulating their growth by responding to the mitogenic signals they themselves produce

Deregulated proliferation

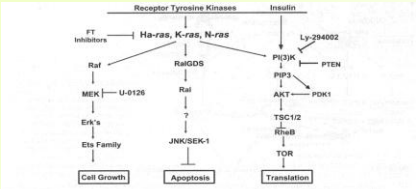


FIGURE 17.11 ● The Ras family (H-Ras, K-Ras and N-Ras) are proteins that act as GDP/GTP-regulated binary switches that reside at the inner surface of the plasma membrane and act to relay extracellular ligand-stimulated signals to cytoplasmic signaling cascades. However, oncogenic forms of Ras lose their responsiveness to growth receptor tyrosine–kinases and constitutively activate cascades of serine–threonine kinases that control cell proliferation, inhibit apoptosis, and increase protein translation. These pathways are depicted in a linear fashion, but are more complex and are regulated by positive and negative feedback loops. This illustration depicts three direct effectors of Ras that have been shown to mediate these events. Ras stimulates proliferation by activating the Raf–MEK–Erk pathway that phosphorylates and activate the Ets transcription factor that leads to increased levels of cyclin D_1, mRNA, and protein. Increased levels of cyclin D_1 result in increased Rb phosphorylation and transition of cells from G_1 to S phase of the cell cycle. A second direct effector of Ras, RalGDS, is a guanine nucleotide exchange factor that activates Ral A and B GTPases and promotes tumor cell survival through the JNK/SEK (Jun kinase/stress kinase) pathway. The third major pathway that Ras directly regulates is the PI(3)K pathway. This pathway has long been known to be important in regulating cell growth through insulin signaling. Like Raf and RalGDS, oncogenic forms of Ras increase PI(3)K activity that leads to increased phosphorylation of the lipid kinase PIP3, which in turn inhibits the negative regulation of TOR by TSC1/2 and RheB. Increased TOR leads to increased translational initiation of mRNAs that possess a structured 5′ region known as a "cap." This is an oversimplified illustration, because there are at least three other signaling pathways that lie downstream of Ras. The Ras pathway can be disrupted using pharmacologic inhibitors of farnesyltransferases (FT), PI(3)K (Ly-294002), or MEK (U-0126). (Adapted from Marianne Powell with permission.)

Failure to respond to growth-restrictive signals

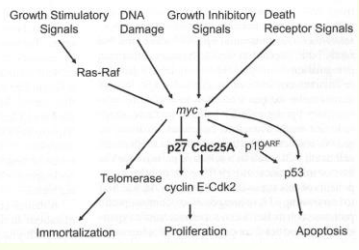
- The oncogenic activation of nuclear oncogenes stimulates the cell into the synthetic phase (S phase), where it duplicates its genetic material before cell division
- Nuclear proto-oncogenes can work as transcription factors by binding to DNA in a sequence-specific manner and forming complexes with themselves or other proteins that will increase mRNA transcription of genes such as *cyclin D* that promotes cell division

Failure to commit suicide (apoptosis)

- Two major pathways that mediate cell death emanate either from the cell membrane or from the mitochondria
- The signals transmitted by each pathway results in the activation of intracellular proteins, termed caspases, that cleave a diverse number of proteins at specific sites
- Cell lines deficient in Caspases 3 and 9 exhibit substantially reduced levels of apoptosis during development and in response to exogenous stress-inducing stimuli
- Tumor suppressor gene *p53* in an important modulator of oncogene-induced apoptosis

Oncogene *myc*

FIGURE 17.14 ● The *myc* oncogene can stimulate cell proliferation and primes the cell for apoptotic cell death. Depending on the cellular context, *myc* can stimulate proliferation through the activation of cyclin E–cdk2, which phosphorylates pRb and drives cells into S phase in response to mitogenic signals, and *myc* can prime cells for apoptosis by increasing the levels of p53 through ARF (p19). Also, *myc* can promote the inactivation of the cyclin–cdk inhibitor p27 as well as increase Cdc25A phosphastase activity. Also *myc* can induce immortalization through regulating telomerase activity. Therefore, *myc* is the prototypical example of how one oncogene can affect cell proliferation, cell death, and immortalization.



Myc codes for a protein that binds to the DNA of other genes and is therefore a transcription factor. When a gene like *Myc* is altered to cause cancer, the cancerous version of the gene is called an oncogene. The healthy version of the gene that it is derived from is called a proto-oncogene

Escaping senescence

- A telomere is a region of DNA (repeat sequence of TTAGGG) at the end of a chromosome, protecting it from deterioration
- Each time a normal somatic cell divides, the terminal end of the telomere is lost; successive divisions lead to progressive shortening, and after 40 to 60 divisions, vital DNA sequences are lost. At this point, the cell cannot divide further and undergoes senescence
- Cancer cells avoid this process of aging by activating the enzyme telomerase, which is a reverse transcriptase that polymerizes TTAGGG repeats to offset the degradation of chromosome ends that occurs with successive cell divisions; in this way, the cell becomes immortal
- Mutation in tumor-suppressor gene p53 is involved

Angiogenesis

- Angiogenesis, the recruitment of new blood vessels to regions of chronically low blood supply, is essential for the progression of solid tumors to malignancy
- A number of proangiogenic growth factors have been identified, VEGF was the first growth factor isolated that could stimulate proliferation and migration of blood vessel cell lining
- Studies have shown that blocking the binding of VEGF to its receptor inhibits tumor angiogenesis and tumor growth. These findings have led to the development of new antibody approaches for antiangiogenesis therapy for clinical use

Invasion and metastasis

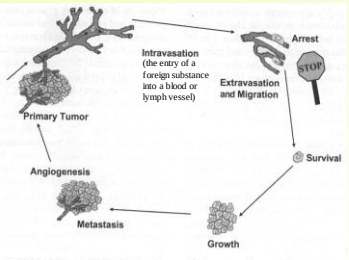


FIGURE 17.16 ● Critical steps in the metastatic process. Tumor cells acquire the ability to disrupt the extracellular matrix of the basement membrane, allowing them to intravasate into local blood or lymph vessels. To form metastases at remote locations, they must migrate and extravasate through the host tissue, blood or lymph supply and survive and proliferate in the new soil. All of these processes are depicted in this figure. (From Le Q, Denko NC, Giaccia AJ: Hypoxic gene expression and metastasis. *Cancer and Metastasis Rev* 23:293–310, 2004.)

- The genetic circuits that regulate metastasis remain mainly undiscovered
- Decreased expression or impaired function of adhesion molecules, and regulatory alterations in chemical agents for digestion of extracellular matrix (*ECM*) have been implivated

Gatekeepers and caretakers

- It appears that most tumor-suppressor genes can be broadly divided into two classes that have been called "gatekeepers" and "caretakers."
- *Gatekeepers* are genes that directly regulate the growth of tumors by inhibiting cell division or promoting cell death, rate limiting for tumor growth. Both alleles (maternal and paternal) must be lost or inactivated for a tumor to develop. The identity of gatekeepers varies with each tissue
- Inactivation of *caretaker* genes does not directly promote the growth of tumors, but leads instead to genomic instability that only indirectly promotes growth by causing an increase in mutation rate. The targets of the accelerated mutation rate that occurs in cells with defective caretakers are the gatekeeper tumor-suppressor genes, oncogenes, or both

Mismatch repair genes

- Mismatch repair (MR) is responsible for correction of errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides
- The primary function of mismatch repair genes is to scan the genome as it replicates and to spot errors of mismatch
- Mutations in MR genes were found responsible for the mutator phenotype associated with a predisposition for hereditary nonpolyposis colon cancer (HNPCC) and possibly other familial cancers

Radiation-induced signal transduction

- Ionizing radiation can regulate the expression of early-response genes, resulting in the stimulation of signal transduction pathways and activation of transcription factors
- It may also enhance the response of the cell to radiation in terms of repair and cell-cycle arrest; and provide a mechanism for secondary stimulation of various late-response genes
- Understanding of these defense mechanisms can help exploiting them for treatment of cancer

Gene therapy

Chapter 26 (6th edition)

Eric J. Hall., Amato Giaccia,
Radiobiology for the Radiologist

Approaches to gene therapy

- Genes are introduced into tumor cells using viral vectors: retrovirus, adenovirus, and herpesvirus
- There are al least 6 different approaches
  - Suicide-gene therapy
  - Cytotoxic virus targeted to p53-deficient cells
  - Molecular immunology (cancer vaccines)
  - Tumor-suppressor gene therapy
  - Radiation-inducible gene linked to a cytotoxic agent
  - Targeting signal transduction pathways

Suicide-gene therapy

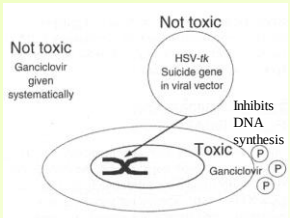


FIGURE 26.1 ● The principle of suicide-gene therapy. The thymidine kinase gene from the herpes simplex virus (HSV-*tk*), contained in a viral vector (adenovirus, so that dividing and nondividing cells can be infected), is injected into the tumor. Ganciclovir is administered systemically. This is a prodrug that is in itself nontoxic. In cells containing the thymidine kinase gene, the prodrug is activated to become a toxic agent.

- Suicide-gene therapy is based on transducing cells with a gene that converts a prodrug into a cytotoxic agent
- There is a substantial bystander effect; that is, more cells are killed than transduced initially
- This therapy has produced growth delay and some cures in animal models
- Because of the limited efficiency of gene delivery, suicide-gene therapy needs to be combined with conventional radiotherapy
- Phase I/I I clinical trials have shown promise

Targeted p-53 deficient cells

- A cytotoxic virus can be constructed that is engineered to replicate and kill only in cells with mutant *p53*
- To the extent that mutant *p53* is a hallmark of cancer, this treatment differentiates between normal cells and cancer cells
- Growth arrest has been observed in model animal tumors and in early clinical trials by targeting mutant *p53*

Molecular immunology (cancer vaccines)

- The approach is to provoke a cellular immune response against the cancer by injecting a vaccine genetically engineered to express immune stimulatory molecules or tumor-specific antigens
- Molecular immunology shows some promise in animal models but is generally only effective against small tumor burdens
- Developing strategy is to combine molecular immunology with suicide-gene therapy

Tumor-suppressor gene therapy

- Tumor-suppressor gene therapy is the replacement, with a correct copy, of the mutated gene that initiates or contributes significantly to the malignant phenotype
- The gene *p53* has received the most attention of any gene because it is so commonly mutated in human cancers
- Phase I/I I clinical trials show some promise in the treatment of non-small-cell lung cancer
- The therapy is limited by a lack of information on the target genes that are essential for maintaining the malignant phenotype and the fact that multiple genetic changes are involved

Radiation-inducible gene linked to a cytotoxic agent

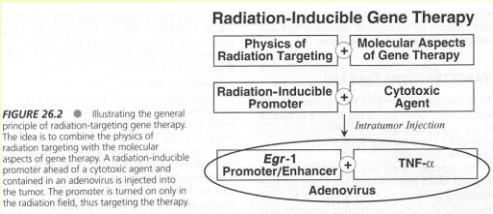


FIGURE 26.2 ● Illustrating the general principle of radiation-targeting gene therapy. The idea is to combine the physics of radiation targeting with the molecular aspects of gene therapy. A radiation-inducible promoter ahead of a cytotoxic agent and contained in an adenovirus is injected into the tumor. The promoter is turned on only in the radiation field, thus targeting the therapy.

- Combination of the physics of radiation-targeting technology with molecular gene therapy

Radiation-inducible gene linked to a cytotoxic agent

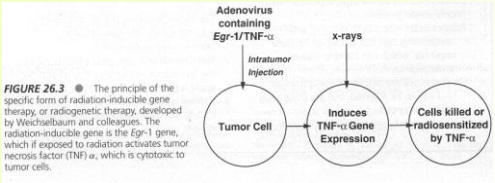


FIGURE 26.3 ● The principle of the specific form of radiation-inducible gene therapy, or radiogenetic therapy, developed by Weichselbaum and colleagues. The radiation-inducible gene is the *Egr*-1 gene, which if exposed to radiation activates tumor necrosis factor (TNF) α, which is cytotoxic to tumor cells.

- There is the potential to use a more radiation-specific promoter gene and a more effective toxic agent
- There is the possibility of including a promoter that is specific for a particular tumor, for example, prostate or breast cancer (in some human cancers, advancing to phase II trials)

Targeting signal transduction pathways

A hallmark of the malignant cell is the dysregulation of growth and signal transduction pathways that often result in resistance to radiotherapy. Several potential targets have been identified:

- –The epidermal growth factor receptor (EGFR) mediates growth regulation in a wide spectrum of human cancers, and tumors expressing high levels of EGFR appear to be radioresistant
- –Raf-1 is a kinase that plays an important role in cell proliferation, differentiation, survival, and angiogenesis and is therefore a prime target for novel cancer therapies
- –NF/cB is a cellular transcription factor that plays a central role in the cellular stress response

Summary

- Development of molecular techniques, such as gene identification and manipulation tools greatly advanced identification of specific genes and understanding of genetic pathways responsible for tumor proliferation
- There is a number of approaches to gene therapy; the winning approach will be a synergistic combination of several treatment modalities