CHAPTER 16

CANCER

OBJECTIVES

- Define the general characteristics and phenotype of cancer cells and the development of malignancy.
- Describe in general the process of metastasis and its significance.
- Outline the general theory behind the use of chemotherapy and radiation to cure cancer.
- ~~List the known causes of or contributors to the development of metastasis.~~
- ~~Discuss the evidence that cancer has to some extent a genetic basis.~~
- ~~Elaborate on the stages in the development of tumorigenesis: initiation and promotion~~.
- Define tumor-suppressor genes and oncogenes and differentiate between them.
- Describe the connection between oncogenes and proto-oncogenes.
- Discuss some of the genes that have been found to be involved in the development of cancer.
- ~~Describe the connection between apoptosis and cancer~~.
- Outline the types of proteins represented by the oncogenes and the functions they perform in the normal cell.
- Emphasize how the normal function of oncogenes can be corrupted, resulting in the development of cancer.

LECTURE OUTLINE

Cancer: General Background

I. What is cancer? - uncontrollable cell proliferation forming malignant tumors that invade surrounding healthy tissue
   A. Cancer is a genetic disease because it can be traced to alterations within specific genes but, in most cases, it is not an inherited disease
      1. In inherited disease, the genetic defect is present in the chromosomes of a parent & transmitted to the zygote
      2. In contrast, the genetic alterations (mutations) leading to most cancers arise mostly in the DNA of somatic cells during the affected individual's lifetime, and are not inherited from parents
   B. Tumors destroy normal tissues & organs in which they appear
      1. If they remain localized, they can usually be treated & cured surgically by removal of tumor
      2. If they metastasize, they are harder to treat & more deadly; can invade surrounding healthy tissue
   C. Metastasis - establishment of secondary tumors
      1. Cells are spawned that break away from the parent mass (promoted by cell surface changes)
      2. They enter lymphatic or vascular circulation
      3. They then spread to distant sites in body where lethal secondary tumors are established (**metastases**); such tumors are often no longer amenable to surgical removal

II. Cancer has been a massive focus of research for decades, but they have had very little impact on either preventing the occurrence of or increasing the chances of surviving most cancers
   A. Current treatments (chemotherapy & radiation) are unable to kill cancer cells selectively
      1. These present treatments lack the specificity needed to kill cancer cells without simultaneously damaging normal cells; this is evidenced by the serious side effects accompanying these treatments
   B. Patients cannot usually be subjected to high enough doses of chemicals or radiation to kill all the tumor cells in their body – researchers are working to develop more effective & less debilitating treatments

Basic Properties of a Cancer Cell

I. Most information on the behavior of human cancer cells has come from cells grown *in vitro* – 2 ways of obtaining cancer cells for culturing
   A. Cancer cells can be obtained by removing a malignant tumor, dissociating the tissue into its separate cells & culturing them *in vitro*
      1. Many different lines of cultured cells originally derived from human tumors have been collected in cell banks & are available for study
   B. Alternatively, normal cells can be converted to cancer cells by treatment with carcinogenic chemicals, radiation or tumor viruses
      1. Cells that have been transformed *in vitro* by chemicals or viruses can generally cause tumors when introduced into a host animal

II. There are many differences in properties from one type of cancer cell to another; at the same time, there are a number of basic properties shared by cancer cells, regardless of their tissue of origin
   A. Cancer cell properties that can be demonstrated *in vitro*, together with their tendency to spread to distant sites in body, are properties that make them a threat to the entire organism's well-being

III. At the cellular level, the most important trait of a cancer cell, whether in the body or the culture dish, is its loss of growth control
   A. Capacity for growth & division is not much different between a cancer cell & most normal cells
      1. When normal cells are grown in tissue culture under conditions favorable for cell proliferation, they grow & divide at rates similar to malignant cells
      2. If normal cells grow to cover culture dish —> their growth rate decreases markedly & they form a single layer (monolayer) of cells covering the dish; this is called **contact inhibition**
      3. Normal cells respond to growth-inhibitory environmental influences (growth factor depletion in culture medium, contact with surrounding cells on dish) with a decrease in their growth rate
      4. Under similar culture conditions, malignant cells keep growing, piling on top of one another to form clumps
   B. Malignant cells are not responsive to the types of signals that cause their normal counterparts to cease growth & division
      1. Cancer cells not only ignore inhibitory growth signals, but they also continue to grow in the absence of stimulatory growth signals that are required by normal cells
      2. Normal cells growing in culture depend on growth factors (epidermal growth factor, insulin) that are present in serum (the fluid fraction of blood), which is usually added to growth medium

3. Cancer cells can proliferate in the absence of serum because their cell cycle does not depend on signals transmitted from growth-factor receptors located at their surface

C. Normal cells growing in culture exhibit a limited capacity for cell division – after a finite number of mitotic divisions, they undergo an aging process that renders them unfit to continue to grow & divide
   1. Cancer cells, on the other hand, are seemingly immortal because they continue to divide indefinitely
   2. The difference in growth potential is often attributed to presence of telomerase in cancer cells & its absence in normal cells
   3. Telomerase is enzyme that maintains telomeres at chromosome ends, thus allowing cells to continue to divide
   4. Absence of telomerase from most types of normal cells is thought to be one of the body's major defenses to protect against tumor growth

D. Chromosomal alterations – the most striking alterations in the nucleus after transformation occur within the chromosomes; probably a result of abnormal growth rather than cause of it
   1. Normal cells maintain their normal diploid chromosome complement as they grow & divide both *in vivo* & *in vitro*; cancer cells often have highly aberrant chromosome complements (**aneuploidy**)
   2. Controversy - does aneuploidy development occur early in tumor formation & is it cause of genetic instability characterizing cancer cells or is it a late event & simply a result of abnormal cancer growth?
   3. Cancer cell growth is much less dependent on normal chromosome content than are normal cells
   4. If normal cell chromosome content is disturbed, a signaling pathway is usually activated —> leads to cell self-destruction (apoptosis) of the cell
   5. This typically doesn't happen in cancer cells; they often cannot do apoptosis at all even if chromosome content is highly deranged
   6. Failure to do apoptosis is another important hallmark distinguishing many cancer cells from normal cells

The Causes of Cancer

I. Percival Pott, British surgeon (1775) made first known correlation between environmental agent & cancer development
   A. Said that high incidence of cancer in chimney sweeps' nasal cavities & scrotal skin was caused by chronic soot exposure
   B. Since then, many chemical carcinogens found in soot & elsewhere (hundreds cause cancer in lab animals); also ionizing radiation, UV light, a variety of DNA & RNA viruses cause cancer in lab animals
   C. All of above have one property in common —> they can alter the genome
      1. Carcinogenic chemicals, like those in soot or cigarette smoke, can either be directly mutagenic or be converted to mutagenic compounds by cellular enzymes
      2. UV radiation, the leading cause of skin cancer, is also strongly mutagenic

~~II. Many viruses can invade mammalian cells growing in culture, transforming them into cancer cells - the viruses are broadly divided into 2 large groups depending on type of nucleic acid in mature virus particles~~

~~A. Two large virus groups - responsible for only a few minor types of cancer; useful in research~~

~~1. DNA tumor viruses - polyoma virus, simian virus 40 (SV40), adenovirus, herpeslike viruses~~

2. RNA tumor viruses (**retroviruses**) – similar in structure to HIV

B. Tumor viruses transform cells since they carry genes whose products interfere with the cell's normal growth-regulating activities

1. Tumor viruses were valuable for identifying genes involved in tumorigenesis, but they are associated with only a small fraction of human cancers
2. Usually these viruses greatly increase a person's risk of developing cancer, rather than being the sole determinant responsible for the disease

C. Example: human papilloma virus (HPV) – transmitted through sexual intercourse; rising in frequency within population; found in ~90% of cervical cancers, indicating its importance in disease development

1. However, the vast majority of women infected with the virus will never develop this malignancy
2. A vaccine against this virus is currently being tested

D. Other viruses linked to human cancers - hepatitis B (liver cancer), Epstein-Barr (Burkitt's lymphoma), a herpes virus, HHV-8 (Kaposi's sarcoma),

1. Some evidence suggests that that SV40 contributes to mesothelioma, a rare cancer of the lining of the lungs that is usually attributed to exposure to asbestos
2. Involvement of SV40 in human cancer is emotionally charged since the virus was a contaminant of the early polio vaccine that was given to millions of people before 1963

E. Certain gastric lymphomas are associated with chronic infection with the stomach-dwelling bacterium *Helicobacter pylori*, which is also responsible for ulcers

1. Unlike any other known cancer, treatment with bacteria-killing antibiotic will usually cure patient the patient of the lymphoma

III. Causes of cancer - epidemiologists try to determine causes; some are obvious, some are not

A. Some obvious causes - smoking –> lung cancer; ultraviolet radiation exposure –> skin cancer

B. But we are still unsure about the causes of most types of human cancers

1. Humans live in complex environments & are exposed to many potential carcinogens in a changing pattern over a period of decades

C. Determining causes of cancer from the huge amount of statistical data on questionnaires has proven difficult, but there have been several important correlations

1. Importance of environmental factors (e.g., diet) is seen most clearly in studies of the children of couples that have moved from Asia to the U. S. or Europe
2. These individuals no longer exhibit a high rate of gastric cancer, as occurs in Asia, but instead are subject to elevated risk of colon & breast cancer, which is characteristic of Western countries

D. Diet can help or hurt - animal fat & alcohol increase risk; certain fruit & vegetable compounds & tea can reduce that risk

E. Nonsteroidal anti-inflammatory drugs (NSAIDs) – aspirin & indomethacin markedly decrease colon cancer risk

1. May do this by inhibiting COX-2, an enzyme that catalyzes synthesis of hormonelike prostaglandins [PGs], which promote intestinal polyp growth, so PG synthesis decreases & so do intestinal polyps
2. Reservatrol (compound in grapes & wine) inhibits cyclooxygenases & exhibits anti-cancer activity

IV. Analysis of the types of mutations caused by specific carcinogens has shed some light on the causes of a few cancers - Ex.: aflatoxin B (produced by certain molds)

A. Aflatoxin B contributes to high incidence of liver cancer in Asia; nuts & grains are stored there under conditions that favor mold growth; can analyze carcinogen effect in population since it is known

B. It causes a characteristic G --> T substitution in tumor-suppressor gene *TP53* (in a codon 249 base pair); allows epidemiologists to make some determination as to the effect of this carcinogen in population

V. Although mutagens in diet & environment are surely a factor in carcinogenesis, most cancer-causing mutations are thought to result from DNA damage caused by normal metabolic reactions

The Genetics of Cancer

I. Cancer - one of the two leading causes of death in Western countries (affects ~1 in every 3 individuals); cells of tumors invariably, when genetically analyzed, are shown to have arisen from a single cell

A. While cancer seems common, that so few cells are changed given the trillions of cells in a human & the billions that divide on a given day, these potential cancer cells are relatively rare events

B. Almost any dividing cell can give rise to a potential cancer cell & grow into a malignant tumor, yet this only occurs in ~1/3 of the human population

II. Cancer results from the uncontrolled proliferation of a single wayward cell (it is **monoclonal**)

A. Malignant transformation (**tumorigenesis**) requires >1 genetic alteration; this explains cancer's rarity

1. It is a multistep process characterized by a progression of permanent alterations in a single cell line
2. Each change makes the cell increasingly less responsive to the body's regulatory machinery
3. They also become more able to invade normal tissues & thus more life-threatening

B. Tumor cells are subjected to a type of natural selection (evolution) that drives the accumulation of cells with properties most favorable for tumor growth; example -- tumor cells with telomerase

1. Only those tumors containing cells that maintain the length of their telomeres will be capable of unlimited growth
2. Any cell appearing within tumor that expresses telomerase will have a tremendous growth advantage over cells that lack it
3. Over time, telomerase-containing cells will flourish, while those lacking it will die off; all of the cells in the tumor will contain telomerase

C. In tumor progression, not all of the changes result from genetic mutation (telomerase is an example)

1. Activation of telomerase expression is considered an epigenetic change, resulting from the activation of a gene that is normally repressed
2. This type of activation process likely involves a change in the structure of chromatin in & around the gene and/or a change in the state of gene methylation
3. Once epigenetic change has occurred, it is transmitted to all progeny of that cell & thus represents a permanent, inheritable alteration
4. Even after they become malignant, cancer cells continue to accumulate mutations & epigenetic changes; making them increasingly abnormal with new properties; get even more dangerous
5. This genetic instability makes the disease difficult to treat by conventional chemotherapy since cells often arise within tumor mass that are resistant to the drug

III. Steps in the development of some malignant tumors
   A. First step in formation of some malignant tumors is often formation of a **benign tumor**
      1. They are made of cells no longer responsive to normal growth controls; they cannot metastasize to distant sites & they lack the ability to invade normal tissues
      2. Some benign tumors pose little threat of becoming malignant, others do (like polyps in colon wall)
   B. Sometimes premalignant cells can be identified by their morphology
      1. Pap smear detects precancerous cervical epithelium cells, which get increasingly abnormal (can happen over a period of >10 years; cells less well differentiated than normal cells, larger nuclei)
      2. If abnormal cells are detected, the site in the cervix is located & destroyed (laser treatment, freezing or surgery)

IV. Genes involved in carcinogenesis are a gene subset; products are involved in growth-related functions
   A. Types of functions these genes affect:
      1. Progression of cell through cell cycle
      2. Adhesion of cell to its neighbors
      3. Repair of DNA damage
      4. Apoptosis
   B. A note on notation conventions most commonly used when referring to human, mouse & viral genes
      1. Human genes are written in capital letters (e.g., *APC*)
      2. Mouse genes are written with the first letter capitalized (e.g., *Brca1*)
      3. Viral genes are written in lower case (e.g., *src*)
   C. Different types of cancers typically have different mutated gene combinations; however, even within a specific type of cancer, there is great variability among the particular genes typically mutated
      1. In genetic terms, the best-defined cancer is colon cancer, where different genes tend to be mutated at different stages of tumorigenesis

V. First detailed model of colon cancer genetic progression
   A. *APC* gene mutations seen in >60% of smallest benign adenomas of colon (probably a first step in the formation of colon cancers)
   B. *TP53* gene mutations tend to occur only at later stages along the path
   C. Cells from metastatic colon cancers, the last stage of cancer progression, exhibit high expression levels of the *PRL3* gene, which encodes a tyrosine phosphatase; its function is not known
      1. *PRL3* is expressed at much lower levels in premetastatic cells

VI. New technology for analyzing gene expression may have considerable impact on cancer diagnosis & treatment has appeared over the past decade – DNA microarrays or DNA chips
   A. Procedure - glass slide prepared with up to 1000s of DNA spots; each one contains DNA corresponding to a single, known gene; can include any particular gene set (growth/division, development, etc.)
      1. Once prepared, the slide is incubated with fluorescently labeled cDNAs made from mRNAs of a particular cell population (e.g., those from a tumor mass or blood cells of leukemia patient)
      2. Fluorescently labeled cDNAs hybridize to complementary DNA spots immobilized on slide

3. Subsequent analysis of fluorescence pattern tells which mRNAs are present in tumor cells & their relative abundance within mRNA population

B. DNA microarray studies have shown that gene-expression profiles can provide invaluable information about the properties of a tumor; for example:
1. Progression of a tumor is correlated with a change in the expression of particular genes
2. Different types of cancers can be distinguished on the basis of their gene-expression profiles
3. The gene-expression profile of a specific tumor can reveal how aggressive (i.e., how lethal) the cancer is likely to be
4. The gene-expression profile of a specific tumor can provide clues as to which type of therapeutic strategy will be most likely to induce tumor regression

C. Example - study of 50 genes transcribed in 2 different leukemias: acute lymphoblastic leukemia (ALL) & acute myeloid leukemia (AML) --> different tumors have characteristic gene-expression profiles
1. Some differences correlate with biological differences between tumors (AML derived from myeloid progenitor & ALL from a lymphoid progenitor), but most differences cannot be explained
2. For example, catalase gene is expressed at high level in AML & low level in ALL
3. Even if results are confusing, they can suggest genes to look at as targets for drug therapy

D. The earlier a cancer is discovered, the more likely is a cure; still a certain percentage of tumors will be fatal, even if discovered & removed at an early stage
1. Some early-stage breast cancers release cells capable of seeding formation of secondary tumors (metastases) at distant locations, while others do not; these differences determine prognosis
2. Recently, it was found that prognosis of a given breast cancer is likely to be revealed in level of expression of ~70 genes out of 1000s studied in DNA microarrays -- important treatment guide
3. Patients with early-stage tumors displaying "poor prognosis" profile of gene expression can be treated aggressively with chemotherapy to maximize the chance of preventing secondary tumors
4. Under current practice, these patients would not get chemotherapy because there would be no indication that the tumor would spread
5. Patients with "good prognosis" profile might be spared debilitating chemotherapy, even if their tumors appear to be more advanced
6. 2003 -- Netherlands Cancer Inst. became first major institute to use gene-expression profiling along with conventional indicators to determine treatment
7. It is hoped that these profiles may eventually be used to improve diagnosis & optimize treatment for individual patients with all types of cancer

VII. Genetic changes are not the only factors of importance in the development of cancer

A. A cell may possess a number of genetic changes that would be expected to cause it to grow into a fully malignant tumor, yet it may remain in a dormant state for the rest of the individual's life

B. Among external influences affecting tumorigenesis are those that promote tumor cell growth & proliferation - example is hormone estrogen (may act as tumor promoter in breast cancer development)
1. There is a correlation between the length of time a woman is exposed to circulating estrogen & breast cancer risk
2. Women, whose ovaries are removed early in life & who get no estrogen replacement therapy, rarely develop breast cancer

3. Estrogen is not mutagenic, it may raise tumorigenesis risk by causing target cell growth & division
4. Compounds that block estrogen action decrease breast cancer risk – tamoxifen is nonsteroidal compound that binds estrogen receptors & blocks estrogen binding
5. This inhibits growth of breast cancer cells, which often depend on estrogen stimulation
6. Tamoxifen is used to help prevent recurrence of breast cancer therapy, but there is a downside; tamoxifen treatment increases a woman's risk of developing uterine cancer & blood clots

C. Recent clinical trials indicate that a new class of safer, more effective drugs called aromatase inhibitors, which will likely replace tamoxifen in the treatment of breast cancer patients
1. Aromatase is an enzyme required for the synthesis of estrogen
2. Aromatase inhibitors are also likely to be prescribed to healthy individuals whose family history suggests that they are at high risk for development of breast cancer

VII. The common carcinomas (breast, colon, prostate, lung) arise in epithelial tissues that are normally engaged in a relatively high level of cell division & leukemias develop in rapidly dividing blood cell precursors

A. These tissues have a stem cell population that divides mitotically, maintaining a relatively constant number of stem cells
1. Stem cells also provide cells that differentiate into short-lived, specialized epithelium or blood cells
2. There is increasing evidence that suggests that these adult stem cells are the primary cells of origin of many types of cancer
3. Unlike other body cells, stem cells have long life & undergo indefinite number of cell divisions, which gives them the time needed to accumulate mutations needed for malignant transformation

B. The adult brain contains small populations of stem cells that recent studies suggest are the source of a number of different types of brain tumors

Tumor-Suppressor Genes and Oncogenes: General Information

I. Genes implicated in tumorigenesis divided into 2 broad categories: tumor-suppressor genes & oncogenes

II. Tumor-suppressor genes - encode proteins that restrain cell growth & prevent malignancy; act as cell brakes
A. Found in late 1960s - fused malignant & normal rodent cells —> some hybrids lost malignant traits —> conclude that normal cells contain something that suppresses uncontrolled cancer cell growth
B. Further evidence - specific regions of particular chromosomes were found to be consistently deleted in cells of certain types of cancers
1. If the absence of such genes correlates with tumor development, their presence normally suppresses tumor formation
2. The missing chromosomal regions contain genes that suppress cancer growth & development
3. Such genes act recessively - both copies must be deleted or mutated before protective function is lost

III. Oncogenes - encode proteins that promote growth control loss & conversion of cell to malignant state; many of them act as accelerators of cell proliferation & tumorigenesis

A. Oncogenes have other roles as well
   1. They may lead to the generation of genetic instability
   2. They may prevent a cell from becoming a victim of apoptosis
   3. They may promote metastasis

B. Found in RNA tumor virus study
   1. RNA tumor viruses transform a normal cell into malignant cell because they carry a gene that encodes a protein that interferes with the cell's normal cell activities
   2. In 1976, found oncogene *src* in RNA avian sarcoma virus (ASV) & also in uninfected cells
   3. The oncogene (*src*) is really a cellular gene that was incorporated into the viral genome during a previous infection

C. It became clear that cells have genes (**proto-oncogenes**) that can subvert their own activities & push them toward malignant state; they encode proteins that have various functions in cell's normal activities
   1. No disorders linked to them; if in tumor, thought to occur due to somatic mutation
   2. If inherited, they may kill embryo; that's why not associated with inherited cancer predispositions

IV. How can proto-oncogenes be converted into oncogenes (i.e., activated) – several mechanisms
   A. Mutate gene in way that alters gene product properties so it can no longer carry out normal activities
   B. The gene can be duplicated one or more times, resulting in gene amplification & excess production of the encoded protein
   C. Chromosome rearrangement can occur that brings a DNA sequence from a distant site in genome into close proximity to gene, altering gene expression or the nature of the gene product

V. Any of above changes can cause cell to become less responsive to normal growth controls, causing it to behave like malignant cell
   A. Oncogenes act dominantly - one copy of gene makes cell express altered phenotype, regardless of whether or not there is a normal, unactivated copy of the gene on the homologous chromosome
   B. Identify oncogenes by introducing the DNA suspected of containing the gene into cultured cells & looking for altered growth properties

VI. In general, how does cancer develop & why is more than a single genetic alteration required? – most tumors contain alterations in both tumor-suppressor genes & oncogenes
   A. If cells have full tumor-suppressor gene complement, they are protected against oncogene effects - when a cell loses both copies of a tumor-suppressor gene, then you can get transformation
   B. You need both a proto-oncogene converted to oncogene & loss of tumor-suppressor function to get fully malignant tumor
      1. Even then, the cell may not exhibit all of the properties required to invade surrounding tissues or to form secondary colonies by metastasis
      2. May need mutations in genes encoding cell adhesion molecules or extracellular proteases to acquire the full life-threatening phenotype
      3. Colorectal carcinoma studies suggest that mutations in as many as 7 different genes may be necessary for the development of a fully malignant tumor

Tumor Suppressor Genes: Retinoblastoma - The *RB* Gene

I. Normal cell —> cancer cell transformation accompanied by loss of function in ≥1 tumor suppressor genes
   A. ~24 have been implicated as tumor suppressors in humans; among things they encode are:
      1. Transcription factors (*TP53* & *WT1*)
      2. Cell-cycle regulators (*RB* & *p16*)
      3. Components that regulate signaling pathways (*NF1*)
      4. A phosphoinositide phosphatase (*PTEN*)
      5. A protein that regulates RNA polymerase II elongation (*VHL*)
   B. Most tumor-suppressor proteins act as negative regulators of cell proliferation —> if they are gone, uncontrolled cell growth results
      1. Tumor-suppressor gene products also help maintain genetic stability, which may be a primary reason that tumors contain such an aberrant karyotype
   C. Some are involved in development of wide variety of different cancers, others in one or a few types

II. Some cancers run at high frequency in certain families, but at low frequency (rare) in general population
   A. Such cancers are good opportunities to identify tumor-suppressor genes that, when missing, contribute to development of both inherited & sporadic (noninherited) forms of cancer
   B. The first tumor-suppressor gene studied & eventually cloned (retinoblastoma; one of most important)

III. Retinoblastoma is a rare childhood cancer of the retina of eye - gene responsible for this disorder is *RB*
   A. Incidence of retinoblastoma follows 2 distinct patterns:
      1. It occurs at high frequency & young age in members of certain families, so it could be inherited, **and**
      2. It occurs sporadically at an older age among members of the population at large
   B. Since it runs in some families, it likely can be inherited; cells from retinoblastoma patients (kids) have small DNA piece missing from interior portion of 1 member of chromosome 13 homologous pair
      1. Deletion seen in all children with retinoblastoma & all their cells (in tumor, other cells) —> the chromosomal aberration was inherited from one of the parents
      2. Inherited as dominant genetic trait, since children of high-risk families that develop the disease inherit one normal allele & one abnormal allele
      3. Unlike most dominantly inherited conditions (e.g., Huntington's disease) children have only a strong disposition to develop retinoblastoma; they do not necessarily express gene & may not get disease
      4. ~10% who inherit deletion do not develop retinal cancer; with Huntington's & other dominantly inherited conditions, if you have the missing/altered gene, you invariably develop the disorder
   C. Genetic basis first explained - Alfred Knudson (Univ. of Texas, 1971) proposed that retinoblastoma development requires that both *RB* gene copies in retinal cell be eliminated or mutated first
      1. Retinoblastoma is the result of 2 independent hits in a single cell
      2. Sporadic cases have single tumor in one eye; concluded that both *RB* gene copies in that cell had to be either eliminated or mutated (successive spontaneous mutations) to get retinoblastoma

3. Since chance that both alleles of same gene will be target of debilitating mutations in same cell (2 hits) is very unlikely (sporadic is rare), incidence of the cancer in general population is very low
4. Those who inherit a chromosome with an *RB* deletion & genetic predisposition are halfway there
5. People with the inherited deletion have other version of gene mutated in 1 cell, which becomes tumor since cell cannot make a functional *RB* gene product
6. Second mutation fails to occur in ~10% of those inheriting deletion —> no retina tumor; confirmed when patients with inherited predisposition were shown to have 2nd mutation in their cancer cells
7. In contrast, sporadic retinoblastoma patients had normal cells with no mutations & tumor cells where both *RB* genes were mutated

D. People with inherited form of retinoblastoma are at high risk of developing other tumors later in life, especially soft-tissue sarcomas (tumors of mesenchymal, rather than epithelial, origin)
1. *RB* mutation costs are not restricted to those who inherit it; sporadic mutations are seen in other tumors (breast, prostate, lung) in those who inherited 2 normal *RB* genes, but then accumulated mutations
2. If one cultures cells from these tumors after reintroducing wild-type *RB* —> cancer phenotype disappears; thus, loss of this gene function contributes significantly to tumorigenesis

IV. The role of pRb in regulating the cell cycle – pRb, the protein encoded by *RB* gene, helps to regulate the G_1 - S transition in cell cycle (DNA synthesis occurs during S phase) & helps to commit the cell to divide

A. Once a cell enters S phase, it invariably proceeds through the remainder of cell cycle & into mitosis
B. G_1-S transition is accompanied by activation of many different genes that encode proteins from DNA polymerases to cyclins & histones
C. E2F transcription factor (TF) family members are required for S-phase activities & are key pRb targets
1. During G_1, unphosphorylated pRb binds E2F TFs, preventing them from activating a number of genes encoding proteins required for S phase activities (cyclin E, DNA polymerase α)
2. Studies suggest that the E2F-pRb complex is associated with DNA, but acts as gene repressor rather than as a gene activator
3. As the end of G_1 approaches, the pRB subunit of the complex is phosphorylated by cyclin-dependent kinases that control G_1 - S transition at numerous serine & threonine residues
4. After pRb phosphorylation, pRb releases its bound E2F, allowing the E2F TF to activate gene expression; this marks the cell's irreversible commitment to enter S phase

D. If a cell loses pRb activity due to an *RB* mutation —> it cannot inactivate E2F —> this removes certain restraints on the entry into S phase
E. pRb binds to dozens of proteins other than E2F, so it probably has numerous other functions
F. Complexity of pRb interactions is also suggested by the fact that the protein contains at least 16 different serine & threonine residues that can be phosphorylated by cyclin-dependent kinases
1. It is likely that phosphorylation of different combinations of amino acid residues allows the protein to interact with different downstream targets

V. Experimental evidence confirming pRb role

A. Inject excess nonphosphorylated pRb into cells during G_1 —> blocks progression to S

B. Several DNA tumor viruses (adenoviruses, human papilloma virus, SV40) encode a protein that binds pRb, blocking its ability to bind E2F —> division (same result as deletion of *RB* gene)
   1. The ability of these viruses to induce cancer in infected cells depends on their ability to block the negative influence that pRb has on progression of a cell through cell cycle
   2. By using pRb-blocking proteins, these viruses accomplish the same result as when the *RB* gene is deleted, leading to the development of human tumors

Tumor Suppressor Genes: The Role of p53 – The Guardian of the Genome

I. p53 is polypeptide with 53,000 dalton MW; its gene (*TP53)* is now known to be tumor-suppressor gene; it may have more to do with the development of human cancer than any other component of genome
   A. In 1990, *TP53* was recognized as a tumor-suppressor gene that, when absent, causes a rare, inherited disorder, Li-Fraumeni syndrome - very high incidence of some cancers (breast, brain, leukemia)
      1. Like people with inherited form of retinoblastoma, these patients inherit one copy of p53 gene (other is mutated or deleted) —> get cancer if random mutation knocks out other copy of gene
      2. These people are thus very susceptible to cancers resulting from random mutations in normal allele
   B. p53 important - >50% of human cancers have deletions/point mutations of both *TP53* genes in their cells
      1. Tumors with mutations in *TP53* are more virulent, metastasize better, more invasive, poorer survival rate than in those that have a wild-type *TP53* gene
      2. Clearly, loss of *TP53* function is big step in progression of many cancer cells to fully malignant state
      3. >1000 mutations seen in human tumors; proper p53 functioning is very sensitive to even slight changes in amino acid sequence

II. How does p53 suppress cancers & why is it so important in suppressing malignancy?
   A. p53 is TF that activates expression of many other genes involved in cell cycle regulation & apoptosis, including the gene encoding p21
      1. p21 inhibits the cyclin-dependent kinase that normally drives cell through G_1 checkpoint
      2. As p53 levels rise in damaged G_1 cell —> *p21* gene is activated & cell cycle progression is arrested
      3. Allows the cell time to repair genetic damage before DNA replication is initiated
      4. If both *TP53* copies are mutated so their product is nonfunctional —> cell makes no p21 inhibitor
      5. There is no feedback control keeping cell out of S phase before it is ready (repairs unfinished) —> failure to repair DNA damage —> get abnormal cells with potential to be malignant
   B. p53 also leads genetically damaged cells along pathway that leads to death by apoptosis —> rids body of cells with malignant potential (thought to do this via several actions)
      1. p53 may do this by activating the expression of the *Bax* gene —> *Bax* gene's encoded product (Bax) initiates apoptosis
      2. If both *TP53* alleles are inactivated —> a cell with damaged DNA is not destroyed even though it lacks the genetic integrity required for controlled growth

3. Reintroduce normal *TP53* gene into cancer cell lacking it —> genetically engineered cell often undergoes apoptosis

III. Control of p53 production – the level of p53 in a healthy G_1 cell is very low
   A. If G_1 cell sustains genetic damage (UV light, chemical carcinogens) —> p53 concentration rises fast
      1. Get same response if you inject cell with DNA preparation containing broken strands
   B. The increase in p53 levels is not due to increased expression of the gene, but to a decrease in the protein's degradation; p53 degradation is facilitated by protein called MDM2,
      1. MDM2 binds p53 & escorts it from nucleus into cytosol
      2. Once in cytosol, MDM2 adds ubiquitin molecules to p53, leading to its destruction by proteasome
   C. How does DNA damage lead to stabilization of p53? – ataxia telangiectasia sufferers lack a protein kinase called ATM & cannot respond properly to DNA-damaging radiation
      1. ATM is normally activated after DNA damage & p53 is one protein it phosphorylates
      2. Phosphorylated version of p53 is no longer able to interact with MDM2 —> stabilizes existing p53 molecules in nucleus —> allows p53 to activate expression of *p21* & *Bax* genes
   D. Some tumor cells have been found that contain wild-type *TP53* gene, but extra copies of *MDM2* gene
      1. Such cells are thought to make excessive amounts of MDM2 —> prevents p53 levels from building to required levels to stop cell cycle or induce apoptosis after DNA damage

IV. Relationship between MDM2 & p53 has also been demonstrated using gene knockout experiments
   A. Mice that lack gene encoding MDM2 die at an early age of development —> their cells probably undergo p53–dependent apoptosis
      1. This interpretation is supported by finding that mice lacking genes encoding **both** MDM2 & p53 (double knockouts) survive to term
      2. Since they cannot make p53, they do not need a protein like MDM2 that facilitates p53 destruction
   B. These results illustrate an important principle in cancer genetics
      1. Even if a crucial gene (*TP53*, *RB*) is not mutated or deleted, its function can be affected as a result of alterations in other genes, whose products are part of same pathway as the crucial gene
      2. In this case, overexpression of MDM2 can have the same effect as the absence of p53
      3. Similarly, presence of pRb can be negated by simultaneous presence of a mutant E2F protein that is not subject to pRb inhibition
      4. As long as tumor-suppressor pathway is blocked, the tumor-suppressor gene itself need not be mutated
   C. Numerous studies suggest that both the p53 & pRb pathways must be inactivated to allow progression of most tumor cells

V. p53 plays pivotal role in cancer treatment with radiation & chemotherapy since it triggers apoptosis
   A. It was long assumed that cancer cells are more sensitive to drug & radiation therapy because they divide more rapidly
      1. But some cancer cells divide more slowly than their normal counterparts, yet they are still more sensitive to drugs & radiation than are normal cells
   B. It may actually be that normal cells are more resistant to drugs or radiation because once they sustain genetic damage, they either arrest cell cycle until repair is complete or undergo apoptosis

1. In contrast, cancer cells that have sustained damage are more likely to become apoptotic as long as they have a functioning *TP53* gene
2. If cancer cells lose p53 function, they often cannot be directed into apoptosis & then become highly resistant to further treatment
3. May explain why tumors that typically lack a functional *TP53* gene (colon, prostate & pancreatic cancers) respond much more poorly to radiation & chemotherapy than those with wild-type copy
4. Tumors with a wild-type copy of the *TP53* gene (testicular cancer, childhood acute lymphoblastic leukemias) respond better

Tumor-Suppressor Genes: Other Examples

I. Mutations in *RB* & *TP53* are associated with a wide variety of human malignancies, other tumor-suppressor gene mutations are seen in only a few types of cancer

II. Colon cancer - 2nd most common cancer in men; 3rd in women; results from mutation accumulation in a number of different genes; identified in genomes of people from families with high colon cancer risk

A. **Familial adenomatous polyposis coli (FAP)** - inherited; individuals develop many (100s or 1000s) premalignant polyps (adenomas) from epithelial cells lining the colon wall

1. If they are not removed, cells within some of these polyps often progress to fully malignant stage

2. Patients have a small chromosome 5 deletion; later found to be site of *APC* tumor-suppressor gene

B. As with *RB*, if a person inherits a mutant *APC* gene & the other one is knocked out (or mutated) in a given cell —> the protective function of the *APC* gene is lost in that cell

1. When the second *APC* gene is lost, growth control in the cell is lost —> the cell proliferates to form a polyp rather than differentiating into a normal intestinal wall epithelial cell
2. Conversion of cells in the polyp to the more malignant state, characterized by the ability to metastasize & invade other tissues, is presumably gained by accumulation of additional mutations
3. One of the additional mutations probably includes *TP53*

C. Mutated *APC* genes are found in up to 80% of sporadic colon tumors in addition to inherited forms of colon cancer; suggests that the *APC* gene plays a major role in the development of this disease

1. The protein encoded by *APC* gene binds many different proteins; its mechanism of action is complex
2. In its best-studied role, APC restrains cell growth by interfering with transcription of genes (e.g., *MYC*) that stimulate cell proliferation
3. APC may also play a role in microtubule attachment to kinetochores of mitotic chromosomes —> thus, loss of APC function could lead directly to abnormal chromosome segregation & aneuploidy

III. Breast cancer - strikes ~1 in 8 women living in U. S., Canada & Europe; 5 - 10% of these cases are traced to inheritance of a gene that predisposes the individual to development of breast cancer

A. Mid-1990s – several labs identified 2 genes (*BRCA1* & *BRCA2*) as being responsible for the majority of inherited breast cancer cases

1. *BRCA* mutations also predispose women to ovarian cancer development (an especially high mortality rate)

B. The precise functions of BRCA1 & BRCA2 remain unclear, but BRCA proteins are part of a large protein complex that responds to DNA damage & activates DNA repair

1. BRCA proteins are part of checkpoint mechanism that halts cell cycle progression after DNA damage
2. Cells with mutant BRCA proteins contain unrepaired DNA along with other abnormalities like an excess number of centrosomes that can lead to abnormal chromosome segregation
3. In cells with a functional *TP53* gene, failure to repair DNA damage leads to the activation of p53, which causes the cell to either arrest the cell cycle or undergo apoptosis
4. Unlike most tumor-suppressor genes, neither of the *BRCA* genes are found to be mutated in sporadic forms of the cancer; the reason for this difference is unresolved

IV. In best-known cell-survival pathway, PIP_3, a phosphoinositide, activates a kinase (called PKB or AKT), this leads to a larger chance that the cell will survive a stimulus that normally leads to its destruction
   A. Whether a cell lives or dies after a particular event depends to a large degree on the balance between proapoptotic & antiapoptotic signals
   B. Mutations that affect this balance (those that contribute to PKB overexpression) can shift the balance toward cell survival —> this can provide a potential cancer cell with a tremendous advantage
   C. The lipid phosphatase, PTEN, can also affect this balance; it removes the phosphate from the 3-position of PIP_3, converting it to $PI(4,5)P_2$, which cannot activate PKB
      1. Cells in which both copies of the *PTEN* gene are inactivated tend to have excessively high levels of PIP_3 —> leads to overactive PKB molecule population
      2. Mutations in *PTEN* gene can cause a rare hereditary disease characterized by an increased risk of cancer; such mutations are also found in a variety of sporadic cancers
   D. Tumor-suppressor genes can often be inactivated by epigenetic mechanisms in addition to mutations like DNA methylation, which silences transcription of gene
      1. When a normal *PTEN* gene is introduced into tumor cells that lack a functioning copy, the cells typically undergo apoptosis as would be expected

Oncogenes and the Function of Oncogene Proteins

I. Oncogenes encode proteins that promote loss of growth control & conversion of cell to malignant state
   A. They are derived from proto-oncogenes, genes that encode proteins having a function in normal cell
      1. Most known proto-oncogenes play a role in control of cell growth, including growth stimulation by external ligands, signal transduction within the cell or progression through the cell cycle
      2. ~100 different oncogenes have been identified; most of them are part of RNA tumor virus genomes
      3. Even though the viral version of each of these genes is derived from cellular versions present in the mammalian genome, only ~12 have been shown to play a role in human carcinogenesis
   B. The oncogene most often mutated in human tumors is *RAS*, encoding the GTP-binding Ras protein; it serves as an on-off switch for a key cell signaling pathway that controls cell proliferation
      1. Oncogenic *RAS* mutants typically encode a protein whose GTPase activity cannot be stimulated
      2. This leaves the protein in an active GTP-bound form, sending continuous proliferation signals along the pathway

3. The human genome actually contains 3 different *RAS* genes & 3 different *RAF* genes that are active in different tissues; of these, *KRAS* & *BRAF* are most often implicated in tumor formation

C. Most of the known oncogenes encode proteins with the following types of functions:
1. Oncogenes that encode growth factors and their receptors
2. Oncogenes that encode cytoplasmic protein kinases
3. Oncogenes that encode nuclear transcription factors
4. Oncogenes that encode products that affect apoptosis
5. Genes that encode proteins involved in DNA repair (mutator phenotype)

~~II. Oncogenes that encode growth factors or their receptors - first connection between oncogenes & growth factors (1983) – found that cancer-causing simian sarcoma virus has an oncogene (*sis*)~~
~~A. The *sis* oncogene was derived from the cellular gene for platelet-derived growth factor (PDGF), a protein present in human blood~~
~~1. Cultured cells infected with the virus —> secrete large amounts of PDGF into medium —> uncontrolled proliferation of cells & they become cancerous~~
~~2. PDGF overexpression has been implicated in the development of brain tumors (gliomas)~~
~~B. Avian erythroblastosis virus (AEV) is another oncogenic virus – it carries the oncogene (*erbB*); it encodes an altered EGF receptor that is missing part of the extracellular domain that binds the growth factor~~
~~1. Altered receptor constitutively stimulates the cell in growth factor presence & absence, this is the opposite of what would be expected (one would think it could not bind factor, so no signal)~~
~~2. As a result of the constant receptor stimulation, the cells proliferate in an uncontrolled manner~~
~~C. A number of spontaneous human cancers have been found to contain cells with genetic alterations that affect growth factor receptors, including erbB)~~
~~1. Malignant cells usually have a much larger number of receptors in membrane than normal cells~~
~~2. Excess receptor presence makes cells sensitive to much lower-than-normal concentrations of growth factor —> cells are thus stimulated to divide under conditions that do not affect normal cells~~
~~D. Proto-oncogenes encoding growth factor receptors can also be activated by mutations or translocations that cause receptor monomers to dimerize in absence of exogenous ligand~~
~~1. The dimerization activates the protein kinase activities of the receptors~~

III. Oncogenes that encode cytoplasmic protein kinases – include both serine/threonine & tyrosine kinases
A. Raf - serine-threonine protein kinase at head of MAP kinase cascade, the primary growth-controlling cell signaling pathway
1. If *RAF* gene is mutated, altering Raf enzyme action, Raf is ideally placed to wreak havoc within cell
2. Mutate *RAF* so Raf is "on" constitutively —> havoc; contributes to cell's loss of growth control
3. As with growth factor receptors & Ras, a proto-oncogene altered like this is likely to become oncogene
B. First oncogene discovered, *SRC*, codes for a tyrosine protein kinase; in cancer cells transformed by *SRC*-containing tumor virus, a wide variety of proteins is phosphorylated by Src protein, among them:

1. Signal transduction proteins (protein phosphatases, heterotrimeric G proteins)
2. Proteins involved in controlling configuration of cytoskeleton
3. Proteins involved in cell adhesion

C. For unknown reason, *SRC* mutations appear only rarely among genetic changes in human tumor cells

IV. Oncogenes that encode nuclear transcription factors - *MYC* is probably the best studied of these
A. Progression of cells through cell cycle requires the timely activation & repression of a large variety of genes whose products contribute in various ways to cell growth & division
1. Order in which these genes turned on & off is important
2. Thus, changes in proteins controlling expression of these genes could seriously disturb a cell's normal growth patterns
3. If transcription factors are altered, it may change the order of gene expression/repression & alter growth

B. Cells not actively growing & dividing tend to withdraw from cell cycle & enter G_0 from which they can be retrieved
1. *MYC* gene is activated if cells are stimulated by growth factors to reenter cell cycle, leave quiescent G_0 & divide; Myc protein is one of first to appear after stimulation by growth factors to reenter cell cycle
2. Selectively block *MYC* expression --> cell progression through G_1 is blocked
3. *MYC* gene is one of proto-oncogenes found to be altered in human cancers -- it is often amplified within genome or rearranged as a result of chromosome inversion or translocation
4. These chromosomal changes are thought to remove the *MYC* gene from its normal regulatory influences & its level of expression in the cell rises --> results in excess of Myc protein

C. Burkitt's lymphoma (one of Africa's most common types of cancer) - *MYC* gene is translocated to a position adjacent to an antibody gene --> *MYC* gene is activated, initiating malignancy
1. Occurs mostly in people who have also been infected with the herpeslike Epstein-Barr virus
2. In Western world, it is associated only with minor infections (mononucleosis), not tumorigenesis

V. Oncogenes that encode products that affect apoptosis - apoptosis rids the body of cancer cells early in progression toward malignancy
A. Any alteration of the apoptotic process damages cell's ability to self-destruct --> increases chance of that cell giving rise to a tumor

B. *BCL-2* oncogene is the oncogene most closely linked to apoptosis; it encodes a membrane-bound protein that normally acts to inhibit apoptosis
1. *BCL-2* knockout mice - lymphoid tissues, once formed, regress dramatically in these mice via widespread apoptosis
2. *BCL-2* is oncogenic if it is expressed at higher-than-normal levels, like *MYC* gene; the cause of overexpression can be its translocation to an abnormal chromosomal site

C. Follicular B-cell lymphomas (a certain human lymphoid cancer) -- correlated with *BCL-2* gene translocation next to a gene coding for the heavy chain of antibody molecules
1. Overexpression of *BCL-2* may lead to apoptosis suppression in lymphoid tissues
2. Abnormal cells can thus proliferate to form lymphoid tumors

D. *BCL-2* may also play a role in lowering chemotherapy effectiveness, by keeping drug-damaged tumor cells alive & proliferating

1. To combat this cancer cell property, drug companies are developing compounds that make cancer cells more likely to undergo apoptosis

VI. The mutator phenotype: mutant genes involved in DNA repair
- A. Cancer is a disease that results from DNA alterations in somatic cells; thus, any activity that increases the frequency of genetic mutations is likely to raise the risk of developing cancer
- B. Nucleotides that are chemically altered or nucleotides that are incorporated incorrectly during replication are selectively removed from the DNA strand by DNA repair (mismatch repair)
- C. DNA repair processes require the cooperative efforts of a substantial number of proteins, including:
  1. Proteins that recognize the lesion
  2. Remove a portion of the strand containing the lesion, **and**
  3. Replace the missing segment with complementary nucleotides
- D. If any of the proteins involved in mismatch repair are damaged, the mutation rate & cancer risk will rise; this is called a **mutator phenotype**
  1. Cells with the mutator phenotype are likely to incur mutations in both tumor-suppressor genes & oncogenes, greatly increasing their risk of becoming malignant
- E. 1993 - first hard evidence that mismatch repair defect might be a factor in cancer genesis came from hereditary nonpolyposis colon cancer (HNPCC) studies, the most common inherited colon cancer type
  1. It is distinguished from the polyp-forming type of colon cancer (FAP) described earlier
  2. A defective gene responsible for HNPCC is carried by ~ 0.5% of population (accounts for ~5% of all colon cancer cases)
  3. Tumor cell microsatellites (very short, repetitive DNA sequences; genome contains large numbers of them) often have different length than corresponding sequences in normal cells from same patient
  4. Such variation is expected in different individuals, but not in different cells in the same person
- F. Discovery of microsatellite sequence variation in these hereditary (& sporadic) cancers suggested a deficiency in the mismatch repair system is likely at fault – support from HNPCC patient studies
  1. HNPCC tumor cell extracts display DNA-repair deficiencies *in vitro*; normal cell extracts carry out mismatch repair normally *in vitro*
  2. DNA analysis from large number of HNPCC patients has shown deletions or debilitating mutations in any one of a number of genes that encode proteins that form the DNA mismatch repair pathway
- G. Cells with mismatch repair deficiencies accumulate secondary mutations throughout genome
  1. Genes containing long, simple repetitive sequences in their coding region are particularly susceptible to this type of mutation
  2. When these mutations occur in tumor-suppressor genes or oncogenes —> the cells are at greatly increased risk of becoming malignant
  3. In fact, the *APC* gene, which is closely linked to colon cancer, contains a string of adenosines that appear to become mutated in certain familial colon cancers

VII. Concluding remarks on cancer genetics - the war against cancer may ultimately require that the genetic composition of the tumor cells be permanently altered
- A. We now know that many different cancers share the same genetic defects (*TP53, RB, and/or RAS)*
  1. Suggests that many different cancers may be treated by a common approach
  2. A drug that mimics p53 effects or inhibits Ras protein —> could be used to treat many cancers

B. View presented here, that cancer is a gradual multistep progression of individual point mutations, is not universally shared

C. Some argue that mutation rate in humans is not high enough for cells to accumulate the mutations necessary to become fully malignant during an individual's lifetime
   1. They propose that carcinogenesis is initiated by catastrophic events that lead to widespread genetic instability over a relatively small number of cell divisions
   2. For example, mutations in a gene involved in DNA replication or DNA repair might rapidly spawn cells carrying widespread genetic abnormalities

D. Another proposal – cells that have undergone an abnormal cell division & possess aberrant numbers of chromosomes are likely initiators of cancerous growths

E. The best way to decide among these possibilities is to analyze the state of the genome in cells at very early stages of tumor formation
   1. Unfortunately, for researchers & patients, it is virtually impossible to identify tumors when they are composed of a small number of cells
   2. By the time they are detected, the cells already exhibit a high degree of genetic derangement
   3. This makes it difficult to determine whether these genetic alterations are a cause or an effect of tumor growth

New Strategies for Combating Cancer: Background Information and Immunotherapy

I. Conventional approaches to fighting cancer (surgery, chemotherapy, radiation) are often unable to get rid of all cancer cells; new approaches being developed; must be tested & shown effective first in lab animals

A. Most animal studies employ a strain of mutant mice with a compromised immune system –> they cannot reject foreign cells (**xenografts**)
   1. Graft a piece of human tumor tissue under mouse's skin –> follow tumor growth after treatments
   2. Evidence suggests that this may not be as predictive of a treatment's success as previously thought
   3. Successful treatments in mice are very often not as successful in humans (most new clinical studies)
   4. But, there are so many new drugs & therapeutic approaches & also a few notable successes in small clinical trials – reason for optimism

B. Four groups of new anticancer strategies:
   1. Those that depend on antibodies or immune cells to attack tumor cells
   2. Those that introduce a gene that either kills tumor cells or causes them to regain normal properties
   3. Those that inhibit the activity of cancer-promoting proteins, **and**
   4. Those that prevent the growth of blood vessels that nourish the tumor

II. Immunotherapy – an approach that tries to get the immune system more involved in fight against cancer

A. William Coley, NY physician (late 1800s) - studied spontaneous remissions of terminal cancer cases
   1. One patient (1891) had inoperable neck tumor; went into remission after streptococcal infection under skin; Coley found the patient & he was still cancer-free
   2. For rest of his life, he tried to develop a bacterial extract that when injected under the skin would stimulate a patient's immune system to destroy the malignancy

3. Coley's approach, called Coley's toxin, worked against some uncommon soft-tissue sarcomas
4. His results confirmed that the body has the capability to destroy a tumor, even after it has become well established

B. In recent years, two broad treatment strategies involving the immune system have been pursued: passive immunotherapy & active immunotherapy

III. Passive immunotherapy – an approach that attempts to treat cancer patients by administering antibodies as therapeutic agents; they recognize & bind specific proteins that play key role in activities of targeted tumor

A. The production of monoclonal antibodies capable of binding to particular target antigens was first developed in mid-1970s; attempts to use them therapeutically have been thwarted for various reasons
1. Most importantly, these antibodies were made by mouse cells & encoded by mouse genes
2. Thus, they were recognized as foreign & cleared from the bloodstream before they could work
3. Over the years, "humanized antibodies", antibodies that are largely human proteins, have been produced; a relatively small part that recognizes the antigen remains "mousey"

B. Presently, ~12 or so monoclonal antibodies have been approved to treat cancer & other medical conditions; >100 others are being tested; example - Herceptin
1. Herceptin is a humanized antibody directed against a cell surface receptor (Her2) that binds a growth factor that stimulates the proliferation of breast cancer cells
2. Herceptin is thought to inhibit activation of the receptor by the growth factor & stimulates receptor internalization
3. ~30% of breast cancers are composed of cells that overexpress the *HER2* gene, which causes these cells to be especially sensitive to growth factor stimulation
4. Herceptin has been shown to be effective either alone or in combination with chemotherapy in slowing the growth of breast cancers in a significant fraction of patients

C. The most effective humanized antibody, to date, is Rituxan, which was approved in 1997 for treatment of non-Hodgkin's B-cell lymphoma
1. Rituxan binds to a cell-surface protein (CD20) that is present on the malignant B cells in ~95% of patients with the disease
2. Binding of antibody to the CD20 protein inhibits cell growth & induces cells to undergo apoptosis
3. The antibody is effective on its own, but even more so when combined with chemotherapy
4. Use of this antibody has completely reversed the prospects for people with this particular cancer; prognosis used to be very grim but now chances for complete remission are excellent

D. A new generation of antibodies is being developed that contain a radioactive atom or toxic compound conjugated to the antibody
1. The antibody targets the complex to the cancer cell & the associated atom or toxic compound kills the targeted cell

E. Others are working to produce antibodies that have a completely human amino acid sequence – mice have been genetically engineered so that their immune system produces human antibody molecules

IV. Active or adoptive immunotherapy – an approach that tries to get a person's own immune system more involved in the fight against malignant cells

A. The immune system has evolved to recognize & destroy foreign materials, but cancers are derived from a person's own cells

1. Many tumor cells have proteins (like telomerase) not usually seen in normal cells or mutated proteins (like Ras) different from those in normal cells, they are still basically host proteins in host cells
2. Thus, the immune system typically fails to recognize these proteins as inappropriate
3. Even if a person does have immune cells (T cells) that recognize tumor-associated antigens, tumors evolve mechanisms that allow them to escape immune destruction

B. Many strategies have been used to stimulate the immune system to mount a more vigorous response against tumor cells
1. Immune cells are isolated from patient, stimulated in one way or another *in vitro*, allowed to proliferate in culture & then reintroduced into the patient
2. Early such trials were disappointing but recent results provide reason for cautious optimism – many patients have recently shown positive responses to treatment
3. Their tumors have disappeared, shrunk in size or extent, or at least stopped growing

C. Why does one person do well as others do poorly? - need to learn more about properties of tumors that respond to the treatment and those that do not
1. DNA microarrays may help, since they can tell us which genes are being expressed in a given tumor
2. Many of these trials are difficult to interpret because these patients often have advanced disease & no other options
3. Immunotherapy is more likely to give positive results in individuals with less advanced cancers, yet these people tend not to be enrolled in these studies
4. As we learn more, things will get better

D. The ultimate goal is to produce immunopreventative treatments in which people would be vaccinated with antigens that would prevent them from ever developing life-threatening cancers

New Strategies for Combating Cancer: Gene Therapy, Inhibition of Cancer-Promoting Proteins and Inhibition of the Formation of New Blood Vessels (Angiogenesis)

I. Gene therapy - treatment in which patient's genotype is changed by addition, deletion or alteration of specific gene; first considered for inherited diseases (cystic fibrosis, muscular dystrophy); now applied to cancer
A. Muscular dystrophy & cystic fibrosis were presumed to be likely targets for gene therapy because they could be corrected by a normal gene introduced into the cells of affected tissues & organisms
B. Replace missing/damaged copies of tumor-suppressor genes (like *TP53*) with wild-type copy – > tumor cells should return to nonmalignant state by acquisition of a wild-type tumor-suppressor gene
1. Deliver wild-type p53 to tumor cells through infection with virus engineered to carry it
2. Such viruses were shown to restore normal growth to malignant cells both in culture & lab animals
C. In two phase II trials, a p53-containing adenovirus (Adnexin) was injected directly into tumors of patients with recurrent head & neck cancers

1. There was a significant increase in average survival time of patients without evident toxicity, but no permanent cures

II. Inhibiting the activity of cancer-promoting proteins - cancer cells behave as they do because they have proteins that either are present at abnormal concentrations or display abnormal activity
   A. If one selectively blocks these proteins —> it should stop uncontrolled growth & invasive properties of malignant cells; many small MW molecules that inhibit cancer-promoting proteins have been made
      1. Some were custom-designed to inhibit a particular protein; whereas others were identified by randomly screening large numbers of compounds synthesized by pharmaceutical companies
      2. A number have shown some promise in halting growth of various tumors, one has had unparalleled success in clinical trials on patients with chronic myelogenous leukemia (CML)
   B. CML is caused by a specific chromosomal translocation that brings a proto-oncogene (*ABL*) into contact with another gene (*BCR*) to form a chimeric gene (*BCR-ABL*)
      1. Blood-forming cells carrying this translocation make a protein that expresses a high level of Abl tyrosine kinase activity, causing the cells to proliferate uncontrollably & initiating tumorigenesis
      2. A compound (Gleevec) was identified; it selectively inhibits Abl kinase by binding to the protein's inactive form & preventing its phosphorylation by another kinase (needed for Abl activation)
   C. Initial clinical trials on Gleevec showed that nearly all CML patients who got the drug at a sufficiently high dose went into remission; also it had only minor adverse side effects
      1. The drug was rapidly approved & has been in use for several years; patients with advanced CML, when initially treated with Gleevec, generally develop resistance to the drug within a few months
      2. Resistance results either from mutations in ABL portion of fusion gene or from ABL gene amplification
      3. In contrast, patients treated at early disease stage (smaller tumor cell population) appear to remain in remission
   D. CML study confirms that oncogenes are valid targets for therapeutic drugs & that "old-fashioned" enzyme inhibitors may play an important role in cancer treatment
      1. This is especially true if inhibitors are given in combinations that act on several aberrant proteins simultaneously

III. Inhibiting new blood vessel formation (**angiogenesis**) - as tumors grow in size, they stimulate angiogenesis supplying nutrients & O_2, removing wastes from fast-growing tumors & provides conduits for cancer spread
   A. Judah Folkman (Harvard, 1971) - suggested that solid tumors might be destroyed by inhibiting their ability to form new blood vessels; after 25 years, it is now a promising anti-cancer strategy
   B. Cancer cells promote angiogenesis by secreting growth factors, like VEGF, that act on endothelial cells of surrounding blood vessels, stimulating them to proliferate & develop into new vessels
      1. Angiogenesis inhibitors also exist; a number of naturally occurring inhibitors (endostatin & thrombospondin) have been identified, but most have been developed by biotechnology companies
      2. These include antibodies & synthetic compounds directed against integrins, growth factors, growth-factor receptors & the drug thalidomide (infamous for causing birth defects in the 1950s)

C. Preclinical studies on mice & rats suggested that angiogenic inhibitors might be effective in stopping tumor growth; tumors treated with these inhibitors did not become resistant to repeated drug application

D. Tumor cells develop resistance to the usual chemotherapy agents because they are genetically unstable & can evolve into resistant forms, **but…….**
   1. Angiogenesis inhibitors target normal, genetically stable, endothelial cells, which continue to respond to the presence of these agents
   2. In addition, angiogenesis is not a required activity in a mature adult, so these inhibitors should not interfere with normal physiologic activities

E. Inhibiting angiogenesis in human tumors is not as easy as was expected based on mouse studies – ~80 anti-angiogenic agents are currently being tested in clinical trials on >10,000 patients

F. Most promising results to date have been obtained with a humanized antibody (Avastatin) that is directed against VEGF
   1. Several Avastatin phase II studies (on patients with lung, breast, kidney & colon cancers) suggested that the antibody might significantly prolong lives of patients with advanced forms of these diseases
   2. Larger phase III results (2003) – results mixed; patients with advanced breast cancer – Avastatin had no survival value
   3. In metastatic colon cancer (800 patients) – increased survival value from 15.6 months in controls (got standard chemotherapy & placebo) to 20.3 months (standard chemotherapy & Avastatin)
   4. The above is not a cure, but it is a significant achievement in patients with advanced colon cancer & suggests that further exploration would be wise

IV. Presently, the best anticancer strategy is early detection

A. Screening procedures are in place – mammography (breast cancer); Pap smears (cervical cancer); PSA determinations (prostate cancer); colonoscopy (colorectal cancer)

B. It is hoped that proteomics will lead to new screening tests based on relative levels of various proteins in blood

C. Advances in genomics may help as well – screening will warn us of the types of cancer to which we may be most susceptible
   1. Such tests are already available for persons whose family history suggests that they might carry *BRCA1* mutations & thus be at risk for breast cancer

D. The earlier a cancer is discovered the greater is the chance for survival so screening procedures could have significant impact in lowering the death rates from cancer

Experimental Pathways: The Discovery of Oncogenes

I. Peyton Rous (Rockefeller Inst. for Medical Research, 1911) – published short paper with farsighted observation on same page as a syphilis treatment; had virtually no impact on scientific community

A. Worked with chicken sarcoma that could be propagated from hen to hen by inoculating a host of the same strain with pieces of tumor tissue
   1. Rous described experiments that strongly suggested that tumor could transmitted to another organism by a filterable virus
   2. A filterable virus was a term coined about a decade earlier to describe pathogenic agents that were small enough to pass through filters that were impenetrable to bacteria

B. He took hen breast tumors, ground cells in mortar with sterile sand, centrifuged particulate material into pellet, forced "supe" fluid through many filters, including those small enough to prevent bacteria passage
   1. He injected filtrate into breast muscle of recipient hen —> significant percentage of injected animals developed tumor
   2. The virus discovered by Rous in 1911 is an RNA-containing virus; by end of 1960s, similar viruses were found to be associated with mammary tumors & leukemias in rodents & rats

C. Certain mouse strains that developed certain tumors with very high frequency had RNA-containing viral particles within the tumor cells & budding from cell surface
   1. It was apparent that gene(s) causing tumors in these inbred strains are transmitted vertically through fertilized egg from mother to offspring so that adults of each generation invariably develop tumor
   2. Provided evidence that viral genome can be inherited through gametes & subsequently transmitted from cell to cell by means of mitosis without having any obvious effect on cell behavior
   3. Not an inbred lab strain peculiarity – wild (feral) mice treated with chemical carcinogens develop tumors that often contain antigens characteristic of RNA tumor viruses & exhibit virus particles in EM

II. One question concerning vertical transmission of RNA tumor viruses was whether the viral genome is passed from parents to progeny as free RNA molecules or is somehow integrated into the host cell DNA

A. Evidence indicated that infection & transformation by these viruses required DNA synthesis – Howard Temin (Univ. of Wisconsin) suggested that RNA tumor virus replication occurs via a DNA intermediate
   1. The DNA intermediate was called a **provirus**; it then serves as a template for synthesis of viral RNA

B. This process, however, required a unique enzyme, an RNA-dependent DNA polymerase, which had never been found in any type of cell

III. David Baltimore (MIT, 1970) and Howard Temin & Satoshi Mizutani – an enzyme having the above activity was discovered independently by the two groups

A. Baltimore examined virions (mature viral particles) from two RNA tumor viruses: Rauscher's mouse leukemia virus (R-MLV) & Rous sarcoma virus (RSV)
   1. He incubated purified virus preparation in conditions that promote DNA polymerase action: Mg^{2+} (or Mn^{2+}), NaCl, dithiothreitol (prevents oxidation of enzyme –SH groups)
   2. The mixture included all 4 deoxyribonucleoside triphosphates with TTP radioactively labeled
   3. Under these conditions, the preparation incorporated the labeled DNA precursor into an acid-insoluble product that exhibited the properties of DNA

B. As is characteristic of DNA, the reaction product was rendered acid soluble (converted to low-MW products) by treatment with pancreatic deoxyribonuclease or micrococcal nuclease
   1. The reaction product was unaffected by pancreatic ribonuclease or by alkaline hydrolysis (to which RNA is sensitive)
   2. The DNA-polymerizing enzyme was found to co-sediment with the mature virus particles, suggesting that it was part of the virion itself & not an enzyme donated by the host cell
   3. Product was insensitive to pancreatic ribonuclease (RNase) treatment, but template was very RNase-sensitive, especially if virions were RNase-pretreated before addition of other reaction mix components

C. These results strengthened the suggestion that viral RNA was the template for synthesis of DNA copy, which presumably served as a viral mRNA synthesis template needed for infection & transformation
   1. Results suggest that cell transformation by RNA tumor viruses occurs through DNA intermediate,
   2. They also overturned Francis Crick's Central Dogma, which stated that information in cell always flows from DNA to RNA to protein
   3. RNA-dependent DNA polymerase became known as **reverse transcriptase**

IV. During the 1970s, attention turned to identification of tumor virus genes that were responsible for transformation & the mechanism of action of their gene products
   A. Evidence from genetic analyses indicated that mutant virus strains could be isolated that retained the ability to grow in host cells, but were unable to transform the cell into one with malignant properties
      1. Thus, the capacity to transform a cell resided in a restricted portion of the viral genome
   B. Harold Varmus, J. Michael Bishop, Dominique Stehelin, et al. (Univ. of Calif. – San Francisco) – isolated mutant strains of avian sarcoma virus (ASV) carrying deletions of 10 – 20% of genome
      1. These deletions rendered the virus unable to induce sarcomas in chickens or to transform fibroblasts in culture
      2. Gene responsible for transformation (missing in these mutants) was referred to as *src* (for sarcoma)
   C. To isolate DNA corresponding to deleted regions of these mutants (presumably carrying genes needed for transformation), the following strategy was adopted
      1. RNA from genomes of complete (oncogenic) virions was used as template for formation of radioactively labeled, single-stranded, complementary DNA (cDNA) using reverse transcriptase
      2. Labeled cDNA (present as fragments) was then hybridized to RNA from one of deletion mutants
      3. DNA fragments that did not hybridize to RNA represented genome parts deleted from transformation-defective mutant & thus were presumed to contain gene needed by virus for transformation
      4. DNA fragments that did not hybridize to RNA were separated from those that were part of DNA-RNA hybrids by column chromatography
      5. A DNA sequence called $cDNA_{sarc}$ was isolated; it corresponded to ~16% of viral genome (1600 nucleotides out of a total genomic length of 10,000 nucleotides)
   D. Once isolated, $cDNA_{sarc}$ was a very useful probe – it was first shown that this labeled cDNA hybridized to DNA extracted from cells of a variety of avian species (chicken, turkey, quail, duck, emu)
      1. This indicated that cellular genomes of these birds contain a DNA sequence closely related to *src*
      2. This was first strong evidence that a gene carried by tumor virus that causes cell transformation is actually present in DNA of normal, uninfected cells & is presumably part of cells' normal genome
      3. The transforming genes of the viral genome (oncogenes) are not true viral genes, but rather cellular genes that were picked up by RNA tumor viruses during a previous infection
      4. Possession of this cell-derived gene endows the virus with the power to transform the very same cells in which this gene is normally found
      5. The fact that the *src* is found in all avian species tested suggests that the sequence has been conserved during avian evolution & thus probably governs a basic activity of normal cells

E. Subsequent studies show that $cDNA_{sarc}$ binds to DNA from all vertebrate classes including mammals, but not to DNA from sea urchins, fruit flies or bacteria, **thus……**

1. The *src* gene is not only present in RNA of the ASV genome & the genome of chicken cells it can infect, but a homologous gene is also seen in DNA of distantly related vertebrates
2. This suggests that the *src* gene plays some critical function in the cells of all vertebrates

V. What is the function of the *src* gene product? – the product was initially identified by Ray Erikson et al. (Univ. of Colorado) by two independent procedures

A. Procedures used to identify the *src* gene product

1. Precipitation of the protein from extracts of transformed cells by antibodies prepared from RSV-infected animals
2. Synthesis of the protein in cell-free protein-synthesizing system using isolated viral gene as a template

B. The *src* gene product was found to be a protein of 60,000 daltons (named $pp60^{src}$)

1. When $pp60^{src}$ was incubated with [^{32}P]ATP, radioactive phosphate groups were transferred to the heavy chains of the associated antibody (IgG) molecules used in the immunoprecipitation
2. This suggested that the *src* gene codes for an enzyme that possesses protein kinase activity
3. When cells infected with ASV were fixed, sectioned & incubated with ferritin-labeled antibodies against $pp60^{src}$, the antibodies were found to be localized on the plasma membrane inner surface
4. This suggests a concentration of the *src* gene product in this part of the cell

C. A protein kinase is the type of gene product that might be expected to have potential transforming activity, since it can regulate the activities of numerous other proteins

1. Each protein phosphorylated by such a protein kinase might serve a critical function in one or another activity related to cell growth

D. Analysis of the *src* gene product's role turned up an unexpected finding

1. Unlike all other protein kinases whose function had been studied, $pp60^{src}$ transferred phosphate groups to tyrosine residues on substrate protein rather than to serine or threonine residues
2. Existence of phosphorylated tyrosine residues had not been detected earlier since phosphorylated serines & threonines are ~3000 times more abundant in cells than phosphotyrosine
3. Also phosphothreonine & phosphotyrosine residues are difficult to separate from one another by traditional electrophoretic procedures

E. Not only did the product of the viral *src* gene (v-*src*) code for a tyrosine protein kinase, so too did c-*src*, the cellular version of the gene

1. However, the number of phosphorylated tyrosine residues in proteins of RSV-transformed cells was ~8 times higher than that of control cells
2. This suggested that the viral version of the gene may induce transformation because it functions at a higher level of activity than the cellular version
3. The results of the RSV study provided preliminary evidence that increased activity of an oncogene product could be a key to converting a normal cell into a malignant cell

F. Soon there was evidence that the malignant phenotype could also be induced by an oncogene with an altered nucleotide sequence – Robert Weinberg et al. (MIT) used technique of DNA transfection

1. He obtained 15 different malignant cell lines that were derived from mouse cells that had been treated with a carcinogenic chemical –> these cells had been made malignant without exposure to viruses

2. DNA from each was extracted & used to transfect a nonmalignant mouse fibroblast (NIH3T3) cell; it takes up exogenous DNA with high efficiency & can easily transform into malignant cells in culture
3. After transfection with tumor cell DNA, the fibroblasts were grown *in vitro* & the cultures were screened for formation of clumps (foci) that contained cells transformed by the added DNA
4. Of the 15 cell lines tested, 5 yielded DNA that could transform recipient NIH3T3 cells; DNA from normal cells lacked this capability
5. Demonstrated that carcinogenic chemicals produced nucleotide sequence alterations in genes that gave the altered genes the ability to transform other cells
6. Thus, cellular genes could be converted into oncogenes in 2 different ways: by becoming incorporated into genome of virus or by becoming altered by carcinogenic chemicals

VI. Attention turned to human cancer when it was shown that DNA isolated from human tumor cells can transform mouse NIH3T3 cells after transfection (1981)
A. Of 26 human tumors studied, 2 provided DNA that was capable of transforming mouse fibroblasts
1. DNA was extracted from bladder carcinoma cell lines (identified EJ & J82) – extensive efforts were undertaken to determine if the genes were derived from tumor virus; no viral DNA evidence detected
2. The first evidence that some human cancer cells contain an activated oncogene that can be transmitted to other cells, causing their transformation
B. Discovery of cancer transmission from cell to cell by DNA fragments provided basis for determining which genes in a cell, when activated by mutation or another mechanism, cause malignancy in a cell
1. To do this, one starts by isolating the foreign DNA causing the transformation & then analyzing it for the presence of cancer-causing alleles
2. 1982 – 3 different labs simultaneously reported the isolation & cloning of an unidentified gene from human bladder carcinoma cells that can transform mouse NIH3T3 fibroblasts
C. Does the gene from bladder cancer cells bear a relationship to oncogenes carried by RNA tumor viruses?
1. 3 labs soon reported that the oncogene from human bladder carcinomas that transforms NIH3T3 cells is the same oncogene (named *ras*) carried by Harvey sarcoma virus, a rat RNA tumor virus
2. Preliminary comparisons of the viral & cellular versions of *ras* failed to show any differences, indicating that the two genes are either very similar or identical
3. This suggests that cancers that develop spontaneously in the human population are caused by a genetic alteration similar to the changes in cells that have been virally transformed in the lab
4. Interestingly, cancers induced by the Harvey sarcoma virus (sarcomas & erythroleukemias) are quite different from the bladder tumors, which have an epithelial origin
5. This was the first indication that alterations in the same human gene – *RAS* – can cause a wide range of different tumors
D. By the end of 1982, reports on the precise changes in the human *RAS* gene that leads to its activation as an oncogene appeared
1. Nucleotide sequence analysis of the large transformation-causing DNA fragment showed that the malignant bladder cell DNA is activated by a single base substitution within the gene-coding region
2. Both human bladder carcinomas studied (EJ & T24) had DNA with precisely the same alteration: a G-containing nucleotide at specific site in proto-oncogene DNA converted to T in activated oncogene

3. The base substitution results in the replacement of a valine for a glycine as the 12th amino acid residue of the polypeptide

E. Determination of the nucleotide sequence of v-*ras* gene carried by Harvey sarcoma virus revealed a change in base sequence affecting precisely the same codon altered in human bladder carcinoma DNA
   1. The change in the viral gene substitutes an arginine for the normal glycine
   2. This particular glycine residue must play a critical role in the structure & function of this protein
   3. Human *RAS* gene is a proto-oncogene that, like *SRC*, can be activated by linkage to viral promoter
   4. The *RAS* gene can be activated to induce transformation by 2 totally different pathways: either by increasing its expression or by altering the amino acid sequence of its encoded polypeptide

F. In summary, research on RNA tumor viruses stemmed from the belief that they might be an important causal agent in the development of human cancer
   1. The search for viruses as a cause of cancer led to the discovery of the oncogene
   2. The discovery of oncogenes led to the realization that the oncogene is a cellular sequence that is acquired by the virus
   3. This ultimately led to the discovery that an oncogene can cause cancer without the involvement of a viral genome

LECTURE HINTS

The Biology of Cancer

Define cancer as a disease and list the traits necessary for malignant tumor formation: uncontrollable cell proliferation and the development of the ability of cells to metastasize. Mention the current treatments used to treat cancers: chemotherapy and radiation.

Explain the philosophy of this kind of treatment, which essentially involves killing cells that are dividing. This, of course, affects tumor cells that divide uncontrollably. It also affects normal cells that divide as part of their normal function. Consequently, the goal of chemotherapy and radiation has been to kill off as many cancer cells as possible without killing the patient. Anti-cancer drugs are essentially poisons. The hope is that they will poison a large number of cancer cells in addition to normal cells. Once one course of chemotherapy has ended, the patient is allowed to recover and is then treated with another course of chemotherapy using a different drug that will knock out most of the cells that had survived the first part of the treatment. This is continued until the cancer is eliminated. The symptoms of chemotherapy and radiation: the nausea, hair loss, etc. are the result of the damage to the normal cells that also divide. Also, stress that the initial philosophy above may not be entirely correct. It seems likely that drug treatments with chemotherapeutic agents initiate apoptotic reactions in cells, a slight variation from the previous view.

Outline the phenotype of cancer cells: the chromosomal alterations, the reduced and disorganized nature of the cytoskeleton, the changes that occur at the cell surface and less dependence on the presence of serum. Remind the class about contact inhibition. Summarize the causes of cancer that have been catalogued thus far.

The Genetics of Cancer

Outline what is known about the steps in the process of tumorigenesis: the formation of a benign tumor followed by the development of the ability to metastasize. Distinguish between tumor initiation and promotion. A description of the Berenblum experiment would help illustrate the difference. Berenblum repeatedly applied a mutagen, coal tar, to the skin of his mice. This led to malignant growths. A single, low-dose treatment was not enough. If a single treatment with coal tar were followed with treatment by another agent (croton oil), skin tumors were induced. Most were benign papillomas, but a small percentage became malignant. Croton oil, by itself, was not carcinogenic, but it promoted tumor development that was initiated by coal tar, even if the croton oil treatment was delayed until several months after the coal tar treatment. However, the croton oil treatment had to be preceded by the coal tar treatment. This suggests that the change wrought by coal tar is permanent & inheritable.

List some of the information that suggests the involvement of genes in cancer development. For example, you can mention the presence of *APC* gene mutations in more than 60% of the smallest benign colon adenomas; this is a mutation that may act as an initiator for colon cancers. You may also wish to mention the inherited predisposition to breast cancer.

Tumor Suppressor Genes and Oncogenes: General Information

Start your discussion of the genes that have been identified as influential in cancer development by describing the experiments that demonstrated tumor suppression by the cytoplasm of normal cells. This experiment suggested that normal cells contain some substance(s) that can suppress cancer cell traits, hence the name of the genes that apparently code for these proteins. Make sure that you point out the recessive nature of tumor-suppressor gene action and how in the future what we are learning about tumor-suppressor genes may be useful in cancer treatment.

Describe oncogenes and their demonstrated involvement in conversion to the malignant state. Point out that they encode proteins that lead to the loss of growth control and that they act in a dominant fashion so that one copy of the gene in a cell makes that cell express the altered phenotype. Describe the experiments that are used to identify such genes. DNA suspected to be an oncogene candidate is inserted into cells. If the cells are transformed, there is a good chance that the DNA in question contains an oncogene. Furthermore, emphasize the finding that the oncogenes originally discovered in RNA viruses were found to have nearly identical correlates serving as normal genes in normal cells. These genes have come to be called proto-oncogenes. Describe how proto-oncogenes are thought to be converted into oncogenes. The normal genes may mutate in a way that alters the properties of their gene products so that they may not be able to carry out normal activities. On the other hand, a mutation in the regulatory region of the gene may alter the gene's expression so that too little or too much of the gene product may be made. The latter could occur due to chromosome rearrangement.
Lastly, describe the current thinking on cancer development. For instance, it appears that a fully malignant tumor may require mutations in as many as 7 genes, including both tumor-suppressor genes and oncogenes.

Tumor Suppressor Genes

I think it may be wise to resist going into a great deal of detail on different tumor-suppressor genes. This depends to some extent on the level of the course you are teaching. I teach what is considered to be an introductory level Cell Biology course to predominantly a group of

sophomores and some juniors. Sometimes too much information can overload students, especially those at the sophomore level. If your class can grasp the general concepts surrounding cancer, the greater detail may be counterproductive. If you wish to cover tumor-suppressor genes in greater detail, discuss, to any depth you feel is appropriate, retinoblastoma and the *RB* gene and *TP53* and its relationship to apoptosis, in addition to the relationship between these two genes.

Oncogenes and the Function of Oncogene Proteins

Once again, you may wish to add to your discussion of oncogenes a brief description of the types of gene products typically made by oncogenes. Remind your students about the normal functions of each type of enzyme and ask them how mutating the gene might lead to tumorigenesis. You may find it useful to mention examples of each type, especially emphasizing those that have already been mentioned either earlier in this lecture or in previous lectures. This section may also be as detailed or as brief as you wish.

~~New Strategies for Combating Cancer~~

~~If time permits, discuss the new approaches being developed for cancer treatment. Give examples of each one. The examples in the book are excellent and relatively interesting to consider. They are also useful because they tie in information covered earlier in the course and thus provide an opportunity to integrate various topics already covered.~~

~~Explain the four general approaches presently being taken in new cancer treatments: immunotherapy, gene therapy, inhibition of the activity of cancer-promoting proteins, and inhibition of angiogenesis. The treatments described in the text provide concrete examples of how such approaches might work and illustrate the kinds of studies that must be carried out before such treatments can be generally approved for humans.~~

~~You might consider recommending to your class a recent book about Judah Folkman and his efforts to develop antiangiogenesis as a cancer therapy. The book is entitled Dr. Folkman's War: Angiogenesis and the Struggle to Defeat Cancer by Robert Cooke. When he first suggested this approach to cancer therapy, many of his colleagues thought it to be preposterous and bemoaned the waste of his talents, time and intellect in such a fruitless search. Now it is at the center of a multi-billion-dollar business and shows great promise in the treatment of cancer and other diseases. Folkman's story is one that encourages persistence and the courage of one's convictions.~~