CARCINOGENESIS
THE MOLECULAR BASIS OF CANCER

We know that cancer is a genetic disease and that it is a multi-step process, therefore multiple genetic events (mutations) will occur in tumors. Given that there are about $3 \times 10^9$ nucleotides encoding more than 50,000 genes in the human genome, identifying these mutations is a difficult task.

**Fundamental Principles**

**Nonlethal genetic damage (mutation)**

This may be acquired through environmental agents (chemicals, radiation, or viruses), or inherited in the germ line.

**The genetic hypothesis of cancer** states that a tumor mass results from the clonal expansion of a single progenitor cell that has sustained genetic damage (i.e., tumors are monoclonal). Clonality of tumors is assessed readily in women who are heterozygous for polymorphic X-linked markers, such as the enzyme glucose-6-phosphate dehydrogenase (G6PD) (Fig. 8-25) or X-linked restriction-fragment-length polymorphisms.

**Targets of genetic damage**

**There are four principal targets of genetic damage that involves**

1. growth-promoting proto-oncogenes
2. growth-inhibiting tumor suppressor genes
3. Apoptosis-regulating genes
4. DNA repair genes

Collectively the genetic alterations in tumor cells provide them growth and survival advantages over normal cells.

**Mutant alleles of proto-oncogenes are called oncogenes:** these are dominant because mutation of a single allele can lead to neoplastic transformation. In contrast, typically both normal alleles of tumor suppressor genes must be damaged for transformation to occur (recessive oncogenes). However, some mutant suppressor oncogenes are dominant.

Genes that regulate apoptosis may be dominant, or recessive.

**Tumor suppressor genes are either** promoters or caretakers. Examples of the promoters are RB & p53, where mutation of the gene leads to transformation by unleashing the brakes on cellular proliferation. **Caretaker genes** are responsible for DNA repair & hence the integrity of the genome. Mutation of caretaker genes affects cell proliferation or survival indirectly by interfering with the ability of the cell to repair nonlethal damage in other genes, including proto-oncogenes, tumor suppressor genes, and apoptosis-regulating genes. **Thus, a disability in the DNA repair genes can predispose cells to widespread mutations in the genome and thus to neoplastic transformation.**

**Tumor progression**

Carcinogenesis is a multistep process resulting from the accumulation of multiple mutations. Over a period of time, many tumors become more aggressive by acquiring greater malignant potential. This phenomenon is referred to as tumor progression. Increasing malignancy is often acquired step by step. At the molecular level, tumor progression result from multiple mutations that accumulate independently in different cells, generating subclones with different characteristics such as ability to invade, rate of growth, metastatic ability, hormonal responsiveness, and susceptibility to anti-neoplastic drugs. (Fig. 8-26) Even though most malignant tumors are monoclonal in origin, by the time they become clinically evident; their constituent cells are extremely heterogeneous. (Fig. 8-27)

**CANCER CRITICAL GENES**

These are all the genes whose mutation contributes to the causation and progression of cancer. While we refer to the genes, remember that it is in fact their protein products that are important since they are key regulators of cellular behavior including the cell cycle, apoptosis, DNA repair and other functions. It should be emphasized that these are genes whose products are essential for normal functioning and behavior of the cell (and tissues). It is when they go wrong (mutated) that they are involved in neoplasia.

It is best to consider cancer critical genes in the context of seven fundamental changes in cell physiology that together determine its malignant attributes.

1. Self-sufficiency in growth signals
2. Insensitivity to growth-inhibitory signals
3. Evasion of apoptosis
4. Unlimited replicative potential
5. Sustained angiogenesis
6. Invasive & metastasizing potentials
7. Genomic instability due to defects in DNA repair

Mutations in genes that regulate some or all of the above cellular traits are seen in every cancer, and hence these will form the basis of the molecular origins of cancer.

In writing, gene symbols are italicized but their protein products are not (e.g., *RB* gene and RB protein).

**1. Self-Sufficiency in Growth Signals**

Oncogenes are mutated proto-oncogenes; they promote autonomous cell growth in cancer cells. Their products, oncoproteins, resemble the normal products of proto-oncogenes except that oncoproteins resist regulatory mechanisms, and their production in the transformed cells does not depend on growth factors or other external signals.

**Self-sufficiency involves the following**

**a. Growth Factors**

All normal cells require stimulation by growth factors to undergo proliferation. Most soluble growth factors are made by one cell type and act on a neighboring cell to stimulate proliferation (paracrine action). In contrast, many cancer cells acquire growth self-sufficiency by synthesizing the same growth factors to which they are responsive (autocrine action). Examples include

- Many glioblastomas (high-grade malignant gliomas) secrete platelet-derived growth factor (PDGF) and express the PDGF receptor
- Many sarcomas make both transforming growth factor-α (TGF-α) and its receptor.

**b. Growth Factor Receptors**

Mutant genes lead to one of two consequences

1. Formation of mutant receptor proteins that deliver continuous mitogenic signals to cells, even in the absence of the relevant growth factor in the environment.
2. Overexpression of growth factor receptors that render cancer cells hyper-responsive to levels of the growth factor that would not normally trigger proliferation.

Examples include

- Overexpression of the epidermal growth factor (EGF) receptor in 80% of squamous cell carcinomas of the lung.
- HER2/NEU receptor is amplified in 25% to 30% of breast cancers. High level of HER2/NEU protein in breast cancer cells is associated with poor prognosis. Blocking this receptor with anti-HER2/NEU antibodies using the drug trastuzumab (Herceptin) is clinically beneficial because it prevents HER2/neu receptors activation.

**c. Signal-Transducing Proteins**

A common mechanism by which cancer cells acquire growth autonomy is mutations in genes that encode various components of the signaling pathways downstream of growth factor receptors. Many such signaling proteins are associated with the inner leaflet of the plasma membrane, where they receive signals from activated growth factor receptors and transmit them to the nucleus. Two important members in this category are *RAS* and *ABL*.

1. *RAS* is the most commonly mutated proto-oncogene in human tumors in that 30% of all human neoplasms contain mutated *RAS* gene, and the incidence is even higher in some specific cancers (e.g., colon and pancreatic adenocarcinomas). Activated RAS stimulates down-stream regulators of proliferation, which floods the nucleus with signals for cell proliferation. The *RAS* gene mutations (mostly point mutations) interfere with the inactivation of RAS. RAS is thus persists in its activated form, and the cell is forced into a continuously proliferating state.

2. *ABL* is a proto-oncogene with tyrosine kinase activity, which is reduced by internal negative regulatory mechanisms. In chronic myeloid leukemia and certain acute leukemias, this activity is set free because the *ABL* gene is translocated from its normal residence on chromosome 9 to chromosome 22, where it fuses with part of
the breakpoint cluster region (BCR) gene. The BCR-ABL hybrid protein has potent, unregulated tyrosine kinase activity, leading to growth autonomy. Normal ABL protein also localizes in the nucleus to promote apoptosis of cells that suffer DNA damage, but BCR-ABL fusion impairs apoptosis.

d. Nuclear Transcription Factors
Transcription is the synthesis of messenger RNA (mRNA) from a DNA strand; this is a key step in the formation of protein coded by DNA. Transcription factors are proteins necessary for RNA polymerase to initiate transcription of mRNA molecule from its DNA template.

**MYC oncogenes**: oncoproteins of the MYC oncogene are transcription factors regulating the expression of growth-promoting cyclins. In normal cells, MYC levels decline to basal level when the cell cycle begins. In contrast, oncogenic versions of the MYC gene are associated with persistent expression or overexpression, contributing to sustained proliferation. Dysregulation of the MYC gene resulting from a t(8;14) translocation occurs in Burkitt lymphoma. MYC is also amplified in breast, colon, lung, and many other cancers.

**Cyclins and Cyclin-Dependent Kinases (CDKs)**
The orderly progression of cells through the various phases of the cell cycle is orchestrated by CDKs. They are activated by binding to cyclins (so called because of the cyclic nature of their production and degradation). The CDK-cyclin complexes activate the crucial RB protein that drives the cell through the cell cycle. On completion of this task, cyclin levels decline rapidly. Cyclins D, E, A, and B appear sequentially during the cell cycle and bind to one or more CDK. (Fig. 8-28)

Mutations that dysregulate the activity of cyclins and CDKs would favor cell proliferation. The cyclin D genes are overexpressed in many cancers, including carcinoma of the breast & esophagus, melanomas & sarcomas. While cyclins activate the CDKs, their inhibitors (CDKIs), (such as p21, p27, and p57) silence the CDKs and exert negative control over the cell cycle. The CDKIs are frequently mutated or otherwise silenced in many human malignancies. Somatically acquired deletion or inactivation is seen pancreatic & esophageal carcinomas, as well as glioblastomas.

2. Insensitivity to Growth-Inhibitory Signals
Isaac Newton predicted that every action has an equal and opposite reaction. Although Newton was not a cancer biologist, his formulation holds true for cell growth. Whereas oncogenes encode proteins that promote cell growth, the products of tumor suppressor genes apply brakes to cell proliferation. Disruption of such genes renders cells refractory to growth inhibition.

**Retinoblastoma (RB) gene** is the first and prototypic cancer suppressor gene to be discovered. The discovery of cancer suppressor genes was accomplished by the study of retinoblastoma, an uncommon childhood tumor of the eye. Approximately 60% of retinoblastomas are sporadic, and the remaining ones are familial, the predisposition to develop the tumor being transmitted as an autosomal dominant trait. To account for the sporadic and familial occurrence of an identical tumor, Knudson, in 1974, proposed his now famous two-hit hypothesis, which in molecular terms can be stated as follows:

- Two mutations (hits) are required to produce retinoblastoma.
- Both of the normal alleles of the RB locus must be inactivated (two hits) for the development of retinoblastoma.
- In familial cases, children inherit one defective copy of the RB gene in the germ line; the other copy is normal. Retinoblastoma develops when the normal RB gene is lost in retinoblasts as a result of somatic mutation. Because in retinoblastoma families only a single somatic mutation is required for expression of the disease, the familial transmission follows an autosomal dominant inheritance pattern.
- In sporadic cases, both normal RB alleles are lost by somatic mutation in one of the retinoblasts. The end result is the same: a retinal cell that has lost both of the normal copies of the RB gene becomes cancerous. (Fig. 8-29).

Although the loss of normal RB genes was discovered initially in retinoblastomas, it is now evident that homozygous loss of this gene (i.e., loss of both alleles) is a fairly common event in several cancers, such as that of the breast, lung, and bladder. Patients with familial retinoblastoma also are at greatly increased risk of developing osteosarcomas and some soft tissue sarcomas.
**p53 Gene: Guardian of the Genome**

The p53 tumor suppressor gene is one of the most commonly mutated genes in human cancers. Normal p53 prevents neoplastic transformation by three interlocking mechanisms:

1. Activation of temporary cell cycle arrest (cell quiescence)
2. Induction of permanent cell cycle arrest (cell senescence)
3. Triggering of programmed cell death (apoptosis).

Damage to the integrity of DNA is a known trigger for p53 response pathways. Thus, p53 has a central role in maintaining the integrity of the genome. In healthy cells, p53 has a short half-life (20 minutes) because of its association with a destructive protein MDM2. When there is an assault on its DNA, p53 releases itself from MDM2 and thus increases its half-life & becomes activated as a transcription factor. Genes whose transcription is triggered by p53 can be grouped into two broad categories:

1. Those that cause cell cycle arrest
2. Those that cause apoptosis.

p53 senses DNA damage and assists in DNA repair by causing G1 arrest and inducing DNA repair genes. A cell with damaged DNA that cannot be repaired is directed by p53 to either enter senescence or undergo apoptosis. In view of these activities, p53 has been called a "guardian of the genome." With homozygous loss of p53, DNA damage goes unrepaird, mutations become fixed in dividing cells, and the cell turns onto a one-way street leading to malignant transformation. More than 70% of human cancers have a defect in p53, and the remaining malignant neoplasms have defects in genes up-stream or down-stream of p53. As with RB protein, normal p53 also can be rendered nonfunctional by certain DNA viruses. Proteins encoded by oncogenic human papilloma viruses (HPVs), hepatitis B virus (HBV), and possibly Epstein-Barr virus (EBV) can bind to normal p53 (& RB) and abolish its protective function. Thus, DNA viruses can abolish two of the well known tumor suppressor genes, RB and p53.

**Transforming Growth Factor-β (TGF- β) Pathway**

In most normal epithelial, endothelial, and hematopoietic cells, TGF-β is a potent inhibitor of proliferation. In many forms of cancer, the growth-inhibiting effects of TGF-β pathways are impaired by mutations in the TGF-β signaling pathway. Mutations affecting the receptor are seen in cancers of the colon. In 100% of pancreatic cancers and 83% of colon cancers, at least one component of the TGF-β pathway is mutated.

**Adenomatous Polyposis Coli-β-Catenin Pathway**

In the rare hereditary disease called adenomatous polyposis coli (APC), patients develop numerous adenomatous polyps in the colon that have an incidence of almost 100% of transformation into colonic cancers. These patients consistently show loss of a tumor suppressor gene called APC (named for the disease). The APC has a dominant function of regulating the intracellular levels of the protein β-catenin. β-catenin is important in the signaling pathway that regulates cell proliferation. With loss of APC, β-catenin degradation is prevented. This leads to transcription of growth-promoting genes, such as cyclin D1 and MYC.

APC behaves as a typical tumor suppressor gene. Individuals born with one mutant allele develop hundreds to thousands of adenomatous polyps in the colon during their teens or 20s, which show loss of the other APC allele. Almost invariably, one or more polyps undergo malignant transformation upon accumulation of other mutations in the cells within the polyp. APC mutations are seen in 70% to 80% of sporadic colon cancers. Colonic cancers that have normal APC genes show mutations of β-catenin that renders them refractory to the degrading action of APC.

### 3. Avoidance of Apoptosis

Accumulation of neoplastic cells may result not only from activation of growth-promoting oncogenes or inactivation of growth-suppressing tumor suppressor genes, but also from mutations in the genes that regulate apoptosis.

There are two distinct programs that activate apoptosis (**Fig. 8-30**)

1. Signaling through the death receptor CD95/Fas (extrinsic pathway)
2. DNA damage (intrinsic pathway).

In the extrinsic pathway CD95/Fas on stimulation recruits the protein procaspase 8 to form the death-inducing signaling complex. Caspase 8 is generated by cleavage of procaspase 8 into smaller subunits. Caspase 8 then activates down-stream caspase 3 that cleaves DNA and other substrates to cause cell death. Activation of the
intrinsic pathway (e.g., by withdrawal of survival factors, stress, and injury) leads to increased permeability of mitochondrial outer membrane, with resultant release of molecules, such as cytochrome c, that initiate apoptosis. Cytochrome c leaks into the cytoplasm activating caspase 9. The latter in turn activates (as caspase 8 of the extrinsic pathway), caspase 3.

Apoptosis is frustrated by cancer cells through
1. Reduced levels of CD95 may render the tumor cells less susceptible to apoptosis.
2. Some tumors have high levels of FLIP, a protein that can bind death-inducing signaling complex and prevent activation of caspase 8.
3. Overexpression of the BCL2 protein protects tumor cells from apoptosis. About 85% of B-cell lymphomas of the follicular type carry a characteristic t(14;18) translocation. At chromosome 14 break occurs at the site where immunoglobulin heavy-chain genes are found, (which is also involved in the pathogenesis of Burkitt lymphoma). Juxtaposition of this transcriptionally active locus with BCL2 (located at 18) causes overexpression of the BCL2 protein. This in turn protects lymphocytes from apoptosis and allowing them to survive for long periods. There is therefore a steady accumulation of B lymphocytes, resulting in lymphadenopathy and marrow infiltration. Because BCL2-overexpressing lymphomas arise in large part from reduced cell death rather than explosive cell proliferation, they tend to be indolent (slow growing) compared with many other lymphomas.

4. Unlimited replicative potential

After about 60 doublings of normal human cells, these cells lose their capacity to divide and enter senescence. This has been ascribed to progressive shortening of telomeres, which is recognized by the DNA repair machinery as DNA breaks. Consequently, there is arrest of the cell cycle mediated by p53 and RB to mend the DNA abnormality. Cells in which the checkpoints are disabled by p53 or RB mutations with the inappropriately activated repair system, show new DNA breaks. The resulting genomic instability from the repetition of such bridge-fusion-breakage cycles eventually produces mitotic catastrophe, characterized by massive cell death. It follows that for tumors to grow indefinitely, as they often do, loss of growth restraints is not enough. Tumor cells must also develop ways to avoid both cellular senescence and mitotic catastrophe. If during crisis a cell manages to reactivate telomerase, the breakage cycles cease and the cell is able to avoid death. However, during this period of genomic instability that precedes telomerase activation, numerous mutations could accumulate, helping the cell march toward malignancy. Passage through a period of genomic instability explains the complex karyotypes frequently seen in human carcinomas. Telomere maintenance is seen in virtually all types of cancers. In 85% to 95% of cancers, this is due to up-regulation of the enzyme telomerase. A few tumors use other mechanisms, termed alternative lengthening of telomeres. Interestingly, in the progression from colonic adenoma to colonic adenocarcinoma, early lesions had a high degree of genomic instability with low telomerase expression, whereas malignant lesions had complex karyotypes with high levels of telomerase activity, consistent with a model of telomere-driven tumorigenesis in human cancer.

5. Sustained angiogenesis

Tumors cannot enlarge beyond 1 to 2 mm in diameter unless they are adequately vascularized. Like normal tissues, tumors require delivery of oxygen and nutrients and removal of waste products; presumably the 1- to 2-mm zone represents the maximal distance across which oxygen, nutrients, and waste can diffuse from blood vessels. Cancer cells can stimulate neo-angiogenesis, during which new vessels sprout from previously existing capillaries, or, in some cases, vasculogenesis, in which endothelial cells are recruited from the bone marrow. Angiogenesis (Neovascularization) is a necessary biologic event in malignancy as it is required for
1. Perfusion that supplies needed nutrients and oxygen
2. Growth of neoplastic cells by newly formed endothelial cells that secrete growth factors (e.g., insulin-like growth factors & PDGF)
3. Access to the blood vessels and hence for metastasis.

Early in their growth, most human tumors remain small or in situ for years until the angiogenic switch. The molecular basis of the angiogenic switch involves increased production of angiogenic factors and/or loss of angiogenesis inhibitors. These factors may be produced directly by the tumor cells themselves, inflammatory macrophages, or tumor stromal cells. Relative lack of oxygen stimulates production of a variety of pro-angiogenic cytokines, such as vascular endothelial growth factor (VEGF), through activation of hypoxia-
induced factor-1α (HIF1α), an oxygen-sensitive transcription factor. Normally, HIF1α is destroyed through binding with the von Hippel-Lindau protein (VHL). When a tumor reaches a critical size, the lack of oxygen prevents HIF1α recognition by VHL, and it is not destroyed. Thus, VHL acts as a tumor suppressor gene, and germ-line mutations of the VHL gene are associated with a variety of tumors such as renal cell carcinoma, hemangiomas of the central nervous system, & retinal angiomas (VHL syndrome). Because of the crucial role of angiogenesis in tumor growth, much interest is focused on anti-angiogenesis therapy. Indeed, anti-VEGF antibody is now approved for the treatment of several types of cancers.

6. Invasive & metastasizing potentials
The spread of tumors is a complex process involving a series of sequential steps. (Fig. 8-31) A cancer first must breach the underlying basement membrane, then traverse the interstitial connective tissue, and ultimately gain access to the circulation by penetrating the vascular basement membrane. This cycle is repeated when tumor cell emboli extravasate at a distant site.

**Invasion of Extracellular Matrix (ECM)**
Invasion of the ECM is an active process that requires four steps:
1. Detachment of tumor cells from each other
2. Degradation of ECM
3. Attachment to new ECM components
4. Migration of tumor cells
The first step is a loosening of tumor cells. E-cadherin molecules act as intercellular glues that keep the cells together. E-cadherin function is lost in almost all epithelial cancers by mutational inactivation of E-cadherin genes.
The second step is local degradation of the basement membrane and interstitial connective tissue. Tumor cells may either secrete proteolytic enzymes themselves or induce stromal cells to elaborate proteases. Multiple different families of proteases, including matrix metalloproteinases (MMPs) have been implicated in tumor cell invasion. Benign tumors of the breast, colon, and stomach show little type IV collagenase activity, whereas their malignant counterparts overexpress this enzyme. Concurrently, the levels of metalloproteinase inhibitors are reduced so that the balance is tilted greatly toward tissue degradation. Because of these observations, attempts are being made to use protease inhibitors as therapeutic agents.
The third step in invasion involves attachment of tumor cells to another set of ECM proteins. Normal epithelial cells have receptors (such as integrins) for basement membrane laminin and collagens that are polarized at their basal surface. The matrix itself is modified in ways that promote invasion and metastasis. For example, cleavage of the basement membrane proteins collagen IV and laminin by MMPs generates new sites that bind to receptors on tumor cells and stimulate migration.

**Locomotion** is the final step of invasion, propelling tumor cells through the degraded basement membranes and zones of matrix proteolysis. Migration is a complex, multistep process that eventually impinges on the actin cytoskeleton. Such movement seems to be augmented and directed by tumor cell-derived cytokines, such as autocrine motility factors. Stromal cells also produce paracrine effectors of cell motility, such as hepatocyte growth factor/scatter factor (HGF/SCF), which bind to receptors on tumor cells. Concentrations of HGF/SCF are elevated at the advancing edges of the highly invasive brain tumor glioblastoma multiforme, supporting their role in motility.

**Vascular Dissemination and Homing of Tumor Cells**
When in the circulation, tumor cells are vulnerable to destruction by host immune cells. In the bloodstream, some tumor cells form emboli by aggregating and adhering to circulating leukocytes & platelets. Extravasation of free tumor cells or tumor emboli involves adhesion to the vascular endothelium, followed by passing through the basement membrane into the organ parenchyma by mechanisms similar to those involved in invasion.
The site of extravasation and the organ distribution of metastases generally can be predicted by the location of the primary tumor and its vascular or lymphatic drainage. However, in many cases the natural pathways of drainage do not readily explain the distribution of metastases. Some tumors (e.g., lung cancers) tend to involve the adrenals with some regularity but almost never spread to skeletal muscle. Such organ tropism may be related to the expression of adhesion molecules by tumor cells whose ligands are expressed preferentially on the
endothelium of target organs. Another mechanism of site-specific homing involves chemokines and their receptors. Human breast cancer cells express high levels of the chemokine receptors CXCR4. The ligands for these receptor are highly expressed only in those organs where breast cancer cells metastasize. On the basis of this observation, it is speculated that blockade of chemokine receptors may limit metastases. After extravasation, tumor cells are dependent on a receptive stroma for growth. Thus, tumors may fail to metastasize to certain target tissues because they present a nonpermissive growth environment.

**Molecular Genetics of Metastasis**

A long-held theory of tumor progression suggests that, as tumors grow; individual cells randomly accumulate mutations, creating subclones with distinct combinations of mutations. Only a small subpopulation of the tumor cells contains all the mutations necessary for metastasis. However, recent experiments challenge this hypothesis.

7. **Genomic instability due to defects in DNA repair**

Mutations are at the heart of carcinogenesis. Although humans literally swim in environmental agents that are mutagenic (e.g., chemicals, radiation, sunlight), cancers are relatively rare outcomes of these encounters. This results from the ability of normal cells to repair DNA damage. The importance of DNA repair in maintaining the integrity of the genome is highlighted by several inherited disorders in which genes that encode proteins involved in DNA repair are defective. *Individuals born with such inherited defects in DNA repair proteins are at a greatly increased risk of developing cancer*. Defects in three types of DNA repair systems—mismatch repair, nucleotide excision repair, and recombination repair—are discussed below.

**Defective mismatch repair**

*Hereditary Nonpolyposis Colon Cancer Syndrome (HNPCC) (Lynch syndrome)*

This disorder is characterized by familial carcinomas of the colon that affect predominantly the cecum and proximal colon. It results from defects in genes involved in DNA mismatch repair. When a strand of DNA is being repaired, these genes correct faulty pairing of say G with T instead of the normal A with T. Without these mismatch repair genes, errors gradually accumulate in several genes, including proto-oncogenes and cancer suppressor genes. One of the hallmarks of patients with mismatch repair defects is **microsatellite instability (MSI)**. Microsatellites are repeats of one to six nucleotides found throughout the genome. In normal people, the length of these microsatellites remains constant. However, in patients with HNPCC, these satellites are unstable and increase or decrease in length. MSI is not limited to this syndrome as it can be detected in 15% of sporadic cancers.

**Defective nucleotide excision repair system**

*Xeroderma Pigmentosum*

Patients with this inherited disorder are at increased risk for the development of cancers of the skin exposed to the sun (UV) light. The basis of this disorder is defective DNA repair. UV light causes cross-linking of pyrimidine residues & thus preventing normal DNA replication. Such DNA damage is repaired by the nucleotide excision repair system. Several proteins are involved in nucleotide excision repair, and an inherited loss of any one can give rise to xeroderma pigmentosum.

**Defective recombination repair**

*Diseases with Defects in DNA Repair by Homologous Recombination*

Mutations in two genes, *BRCA1* and *BRCA2*, account for 80% of cases of familial breast cancer. In addition to breast cancer, women with *BRCA1* mutations have a substantially higher risk of epithelial ovarian cancers. Likewise, mutations in the *BRCA2* gene increase the risk of breast cancer in both men and women as well as cancer of the ovary, prostate, pancreas, etc. Cells that lack these genes develop chromosomal breaks. Indeed, both genes seem to function, at least in part, in the homologous recombination DNA repair pathway. Similar to other tumor suppressor genes, both copies of *BRCA1* and *BRCA2* must be inactivated for cancer to develop. Although linkage of *BRCA1* and *BRCA2* to familial breast cancers is established, these genes are rarely inactivated in sporadic cases of breast cancer. In this regard, *BRCA1* and *BRCA2* are different from other tumor suppressor genes, such as *APC* and *p53*, which are inactivated in both familial and sporadic cancers.
ETIOLOGY OF CANCER: CARCINOGENIC AGENTS

Genetic damage lies at the heart of carcinogenesis. Three classes of gene damaging or carcinogenic agents can be identified:

1. Chemicals
2. Radiant energy
3. Microbial agents.

Chemicals and radiant energy are documented causes of cancer in humans, and oncogenic viruses are involved in at least some human tumors.

Chemical Carcinogens

More than 200 years ago, the London surgeon Sir P. Pott correctly attributed scrotal skin cancer in chimney sweeps to chronic exposure to soot. Based on this observation, the Danish Chimney Sweeps Guild ruled that its members must bathe daily.

Direct-Acting Carcinogens

These require no metabolic conversion to become carcinogenic. They are in general weak carcinogens but are important because some of them are cancer chemotherapeutic drugs (e.g., alkylating agents) that have successfully cured, controlled, or delayed recurrence of certain types of cancer (e.g., leukemia & lymphoma), only to evoke later a second form of cancer, usually leukemia. This situation is even more tragic when their initial use has been for non-neoplastic disorders, such as rheumatoid arthritis. The risk of induced cancer is low, but its existence dictates cautious use of such agents.

Indirect-Acting Agents

This designation refers to chemicals that require metabolic conversion to an ultimate carcinogen. Examples include

1. The polycyclic hydrocarbons e.g. benzopyrene and other carcinogens are some of the most potent indirect chemical carcinogens. They are formed in the high-temperature combustion of tobacco in cigarette smoking. These products are implicated in the causation of lung cancer in cigarette smokers. Polycyclic hydrocarbons may also be produced from animal fats during the process of broiling meats. The principal active products in many hydrocarbons are epoxides, which form combine with molecules in the cell, principally DNA, but also with RNA and proteins.

2. The aromatic amines and azo dyes are another class of indirect-acting carcinogens. β-naphthylamine was responsible for a 50-fold increased incidence of bladder cancers in heavily exposed workers in the aniline dye and rubber industries. Because indirect-acting carcinogens require metabolic activation for their conversion to DNA-damaging agents, much interest is focused on the enzymatic pathways that are involved, such as the cytochrome P-450-dependent monooxygenases.

3. Aflatoxin B₁ is of interest because it is a naturally occurring agent produced by some strains of Aspergillus, a mold that grows on improperly stored grains and nuts. There is a strong correlation between the dietary level of this food contaminant and the incidence of hepatocellular carcinoma in some parts of Africa and the Far East.

4. Vinyl chloride, arsenic, nickel, chromium, & insecticides are potential carcinogens in the workplace and about the house.

5. Nitrites used as food preservatives have caused concern, since they cause nitrosylation of amines contained in the food. The nitrosoamines so formed are suspected to be carcinogenic.

Mechanisms of Action of Chemical Carcinogens

Most chemical carcinogens are mutagenic. Indeed, all direct and ultimate carcinogens contain highly reactive groups that combine DNA (as well as proteins and RNA). Oncogenes and tumor suppressors (such as RAS and p53), are important targets of chemical carcinogens. Indeed, specific chemical carcinogens, such as aflatoxin B₁, produce characteristic mutations in the p53 gene, such that detection of the "signature mutation" within the p53 gene establishes aflatoxin as the causative agent. These associations are proving useful tools in epidemiologic studies of chemical carcinogenesis.

Carcinogenicity of some chemicals is augmented by subsequent administration of promoters (e.g., hormones, phenols, and drugs) that by themselves are nontumorigenic. In the initiation-promotion sequence of chemical carcinogenesis; promoters are not mutagenic but induce cell proliferation. It seems that the application of an
initiator may cause the mutational activation of an oncogene; subsequent application of promoters leads to clonal expansion of initiated (mutated) cells. Forced to proliferate, the initiated clone of cells accumulates additional mutations, developing eventually into a malignant tumor. Indeed, the concept that sustained cell proliferation increases the risk of mutagenesis, and hence neoplastic transformation, is also applicable to human carcinogenesis. For example, pathologic hyperplasia of the endometrium and increased regenerative activity that accompanies chronic liver cell injury are associated with the development of cancer in these organs.

**Radiation Carcinogenesis**

Radiation, whatever its source (UV rays of sunlight, x-rays, nuclear fission, radionuclides) is an established carcinogen. Unprotected miners of radioactive elements have a 10-fold increased incidence of lung cancers. Follow-up of survivors of the atomic bombs dropped on Hiroshima and Nagasaki disclosed a markedly increased incidence of leukemia—principally acute and chronic myeloid leukemia—after an average latent period of about 7 years, as well as an increased mortality rate from thyroid, breast, colon, and lung carcinomas. The nuclear power accident at Chernobyl in the former Soviet Union continues to be associated with high cancer incidence in the surrounding areas. Therapeutic irradiation of the head and neck can give rise to papillary thyroid cancers years later.

The oncogenic properties of ionizing radiation are related to its mutagenic effects; it causes chromosome breakage, translocations, and, less frequently, point mutations. Biologically, DNA breaks seem to be the most important form of DNA damage caused by radiation. Natural UV radiation derived from the sun can cause skin cancers (melanomas, squamous cell carcinomas, and basal cell carcinomas). UV light has several biologic effects on cells. Of particular relevance to carcinogenesis is the ability to damage DNA by forming pyrimidine dimers. This type of DNA damage is repaired by the nucleotide excision repair pathway. With extensive exposure to UV light, the repair systems may be overwhelmed, and skin cancer results. As mentioned above, patients with the inherited disease *xeroderma pigmentosum* have a defect in the nucleotide excision repair pathway. As expected, there is a greatly increased predisposition to skin cancers in this disorder.

**Viral and Microbial Oncogenesis**

Only a few viruses have been linked with human cancer.

**Oncogenic RNA Viruses**

*Human T-cell leukemia virus-1 (HTLV-1)* is the only retrovirus that has been demonstrated to cause cancer in humans. HTLV-1 is associated with a form of T-cell leukemia/lymphoma that is endemic in certain parts of Japan and the Caribbean but is found sporadically elsewhere. Similar to the human immunodeficiency virus (HIV), HTLV-1 has tropism for CD4+ T cells, and this subset of T cells is the major target for neoplastic transformation. The molecular mechanisms of transformation are not clear. Human infection requires transmission of infected T cells via sexual intercourse, blood products, or breastfeeding. Leukemia develops only in about 3% to 5% of infected individuals after a long latent period of 20 to 50 years. HTLV-1 does not contain a viral oncogene.

**Oncogenic DNA Viruses**

Four DNA viruses are strongly associated with human cancer; these are

1. *human papillomavirus (HPV)*
2. *Epstein-Barr virus (EBV)*
3. *Kaposi sarcoma herpesvirus (KSHV, also called human herpesvirus 8 [HHV8])*
4. *hepatitis B virus (HBV)*

**Human Papillomavirus**

HPVs are associated with the following

1. *Benign squamous papillomas (warts);* these are caused by some types (e.g., HPV1, 2, 4, and 7).
2. *Squamous cell carcinoma of the cervix and anogenital region;* these are caused by high-risk HPVs (e.g., HPV 16 and 18).
3. *Oropharyngeal carcinomas:* at least 20% of these are associated with HPV.
4. Genital warts (having low malignant potential); these are associated with low-risk HPVs predominantly HPV-6 and HPV-11.

The oncogenic potential of HPV can be related to products of two early viral genes, E6 and E7. Infection with HPV itself is not sufficient for carcinogenesis. Association with a mutated RAS gene results in full malignant transformation. These data strongly suggest that HPV acts in concert with other environmental factors. However, the primacy of HPV infection in the causation of cervical cancer is attested to by the near complete protection from this cancer by anti-HPV vaccines.

**Epstein-Barr Virus (EBV)**

This virus has been implicated in the pathogenesis of several human tumors:

1. Burkitt lymphoma
2. B-cell lymphomas in patients with immunosuppression including AIDS
3. A subset of Hodgkin lymphoma
4. A subset of nasopharyngeal carcinoma.

Except for nasopharyngeal carcinoma, all others are B-cell tumors. **Burkitt lymphoma** is endemic in certain parts of Africa and is sporadic elsewhere. In endemic areas, tumor cells in virtually all patients carry the EBV genome. EBV uses the complement receptor, CD21, to attach to and infect B cells. Such infection leads to polyclonal B-cell proliferation and generation of B-lymphoid cell lines. One of the EBV-encoded genes, called LMP-1, acts as an oncogene. LMP-1 promotes B-cell proliferation & prevents apoptosis by activating BCL2. Another EBV-encoded gene, EBNA-2, transactivates several host genes, including cyclin D.

In immunologically normal individuals, EBV-driven polyclonal B-cell proliferation is readily controlled, and the individual at most develops a self-limited infectious mononucleosis. Evasion of the immune system seems to be a key step in EBV-related oncogenesis. In regions of the world where Burkitt lymphoma is endemic, concomitant (endemic) malaria impair immune competence, allowing sustained B-cell proliferation. Interestingly, although LMP-1 is the primary transforming oncogene in the EBV genome, it is not expressed in EBV-derived Burkitt lymphoma, because it is recognized by the immune system. Presumably, infected cells expressing viral antigens such as LMP-1 are kept in check by the immune system. **Lymphoma cells emerge only when additional mutations, such as the t(8;14) translocation,** a consistent feature of this tumor, activate the MYC oncogene. MYC activation may substitute for LMP-1 signaling, allowing the tumor cells to down-regulate LMP-1 and evade the immune system. In nonendemic areas, 80% of tumors do not harbor the EBV genome, but all tumors possess the specific t(8;14) translocation. This suggests that, although non-African Burkitt lymphomas are triggered by mechanisms other than EBV, they develop cancer by similar pathways.

**Hepatitis B and Hepatitis C Viruses**

It is estimated that 80% of hepatocellular carcinomas worldwide are due to infection with HBV or HCV. However, the mode of action of these viruses in tumorigenesis is not fully understood. The HBV and HCV genomes do not encode any viral oncoproteins. Indeed, the oncogenic effects of HBV and HCV are multifactorial, but the dominant effect seems to be immunologically mediated chronic inflammation with hepatocyte death leading to

1. Regeneration
2. Exposure to genotoxic agents causing genomic damage.

As with any cause of hepatocellular injury, chronic viral infection leads to the compensatory proliferation of hepatocytes. One key molecular step seems to be activation of the NF-κB pathway in hepatocytes caused by mediators derived from the activated immune cells. Activation of the NF-κB pathway within hepatocytes blocks apoptosis, allowing the dividing hepatocytes & to accumulate mutations. Both HBV and HCV also contain proteins (HBx) within their genomes that may more directly promote the development of cancer. In addition, viral integration can cause secondary rearrangements of chromosomes, including multiple deletions that may harbor unknown tumor suppressor genes.

Though not a DNA virus, HCV is also strongly linked to the pathogenesis of liver cancer. The molecular mechanisms used by HCV are less well defined than are those of HBV. In addition to chronic liver cell injury
and compensatory regeneration, components of the HCV genome, such as the HCV core protein may have a
direct effect on tumorigenesis.

Helicobacter pylori
H. pylori is the first bacterium classified as a carcinogen. H. pylori infection is implicated in the genesis of both
gastric adenocarcinomas and gastric lymphomas.
The scenario for the development of gastric adenocarcinoma is similar to that of HBV- and HCV-induced liver
cancer. It involves increased epithelial cell proliferation in a background of chronic inflammation. As in viral
hepatitis, the inflammatory scene contains numerous genotoxic agents, such as reactive oxygen species. There is
an initial development of chronic inflammation/gastritis, followed by gastric atrophy, intestinal metaplasia of
the lining cells, dysplasia, and cancer. Like HBV and HCV, the H. pylori genome also contains genes directly
implicated in oncogenesis. Strains associated with gastric adenocarcinoma have been shown to contain
cytotoxin-associated A (CagA) gene. Although H. pylori is noninvasive, CagA is injected into gastric epithelial
cells, where it causes initiation of a signaling cascade that mimics unregulated growth factor stimulation.
H. pylori are also associated with an increased risk for the development of gastric lymphomas. Their molecular
pathogenesis is incompletely understood but seems to involve strain-specific H. pylori factors, as well as host
genetic factors. It is thought that H. pylori infection leads to the formation of H. pylori-reactive T cells, which in
turn cause polyclonal B-cell proliferations. In time, a monoclonal B-cell tumor emerges in the proliferating B
cells, perhaps as a result of accumulation of mutations in growth-regulatory genes. In keeping with this, early in
the course of disease, eradication of H. pylori "cures" the lymphoma by removing antigenic stimulus for T cells.