CHAPTER

6 Molecular Oncogenesis

DEFINITIONS

Rudolf Virchow (1856) stated that no one, even under torture, can say what cancer is. One century later, Willis (1952) defined cancer as an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissue. Perez-Tamayo (1985) considered cancer as an abnormal new growth of tissue with three unique characteristics: it is uncontrollable, has no expected end point, and is aggressive to the host. All these definitions are merely descriptive of the biologic features of the disease based on clinical observations.

With advances made in molecular pathology, recent definitions are more precise. Thus, Hanahan and Weinberg (2000) described six cardinal features (hallmarks) of cancer, namely:

1. Self-sufficiency of growth signals (autonomy due to activation of protooncogenes).
2. Inhibition of antigrowth signals (inactivation of tumor suppressor genes).
3. Escape from programmed cell death. (anti-apoptotic effect of bcl-2 and IGF) and defects of DNA repair (inactivation of p53 and DNA repair genes).
4. Limitless replicative potential (immortality due to telomerase overexpression).
5. Sustained angiogenesis (VEGF expression).
6. Tissue invasion and metastases.

These 6 essential alterations in cell physiology will collectively dictate the development of malignancy. The present chapter presents the biologic concepts and carcinogenesis mechanisms operable in the development of cancer, as well as, their clinical application (targeted therapy). However, since cancer represents an aberrant change in the normal cellular regulatory mechanisms (homeostasis), a preliminary discussion of this subject is necessary.

HOMEOSTASIS

Homeostasis is defined as the ability of cells to maintain internal stability and resist changes. Three systems are utilized by the cells in this regard. The first is to sense and react to any external or internal signals (signal transduction). The second system is to keep cell population constant (cell population stability), the third is to recognize and repair any DNA damage (genomic stability).

Signal Transduction

Signal transduction describes the mechanisms of transmitting signals from the extracellular microenvironment to the cell interior. The process involves three main steps, namely: (1) a signal (also called a Ligand), (2) a series of receptors and messengers to transmit the signal to the nucleus and (3) transcription factors to induce a genetic response to the signal.

In the classic pathway (Fig 6-1) a growth factor binds to a surface cell membrane receptor leading to its activation (by dimerization and phosphorylation). Signals cross cell membrane to activate membrane-bound receptors, cytoplasmic second messengers and transcription factors. The latter enter the nucleus and finally activate gene transcription which is expressed as differentiation, mitosis, or apoptosis. Membrane receptors may also activate other nearby receptors (cross activation) through the mediation of bridging protein messengers. Steroid ligands can traverse the cell membrane to bind with hormone receptors in the cytoplasm, then translocate into the nucleus to activate transcription.

Growth Factors (Ligands)

Biochemically, ligands may be polypeptides (growth factors) or steroid hormones. Traditionally, growth factors or signals have been classified based on their target cells (Fig 6-2) into those which effect distant cells (endocrine), nearby cells (paracrine) or the same cell (autocrine).

The main growth factors which affect epithelial and stromal cells are presented in Table 6-1, classified according to their target cells and biologic effects. It is obvious that many growth factors are pleiotropic, that is, they have multiple biologic effects on different target cells. Growth factors of the immune cells (cytokines) and their signal transduction (JAK/STAT) are discussed in chapter 8.

Cell Receptors

Receptors, secondary messengers and transcription factors are all protein in nature. The latter have the property to bind to DNA, regulate the activity of RNA polymerase and initiate transcription. All cell receptors are activated by phosphorylation by the enzymes protein kinases using ATP as a source of phosphate groups. Kinases are classified into tyrosine kinases and serine/threonine kinases according to the amino acid residue in the receptor protein. Tyrosine kinases are much more common in signaling pathways than are serine/threonine kinases (e.g. TGF-β/SMADS). Conversely, receptors are deactivated by phosphatases with loss of phosphate groups (e.g. PTEN tyrosine phosphatase of PI-3K/AKT pathway). Receptors are classified according to their cellular localization and class of their activating kinase enzymes (Table 6-2).

Some signal transduction pathways have distinct biologic effects (Fig 6-1). For example:

(1) The PI-3 kinase/AKT pathway which is activated by: IGF, SCF, EGF and RAS, functions to increase cell proliferation and cell survival due to antiapoptotic effect. The latter is the result of inhibition of the proapoptotic protein BAD by AKT.

(2) RAS, a class of small GTP - binding protein, is a down stream target of several protein kinase receptors (e.g. SCF, EGF and PDGF) which transactivate RAS by a bridging protein messenger. The RAS/RAF/MEK pathway is a strong mitogenic signal. RAS also activates PI-3k/AKT pathway.

(3) Transforming growth factor beta (IGF-β/SMAD) pathway inhibits the growth of many epithelial cells.

(4) G-protein receptors which transmit signals through GTP-binding proteins and mainly function with signal transduction of hormones, such as: epinephrine, calcitonin and parathormone.

(5) T-helper lymphocyte (CD4) membrane receptor is involved in cytokine signaling and B-lymphocyte membrane receptors (CD20) involved in production of immunoglobulin in response to antigens (chapter 8).

(6) VEGFR and Notch membrane receptors on endothelial cells are involved in the induction and modulation of angiogenesis in response to tissue anoxia (chapter 7).

(7) WNT and E-Cadherin membrane receptors are involved in the control of cytoplasmic level of β-catenin (chapter 13).

(8) Transcription factors beside binding to DNA, also bind to other proteins with modification of

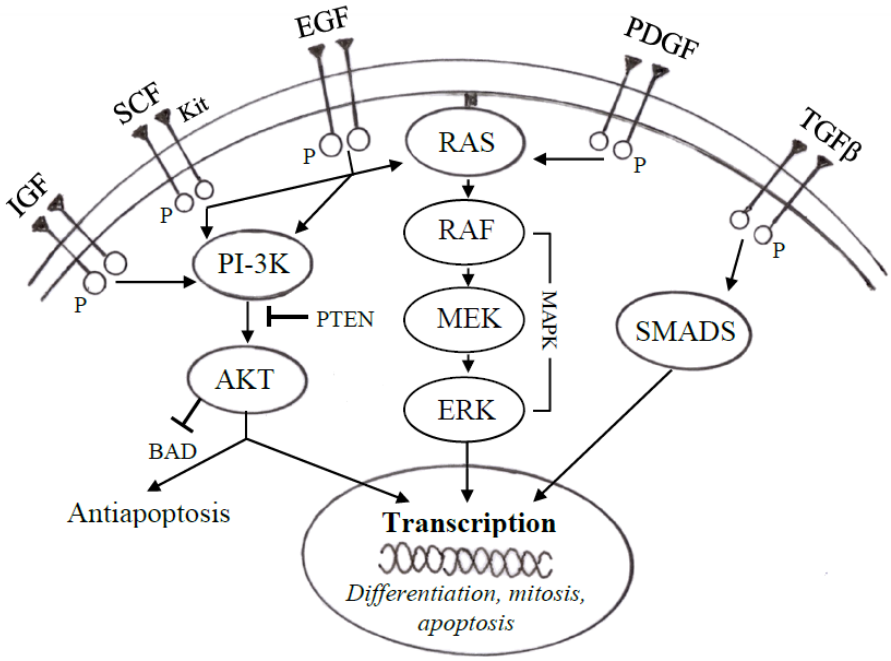

Fig 6-1 *Signal transduction pathways. The binding of growth factors (Ligands) to cell membrane surface receptors initiates a signal which is transmitted through cytoplasmic messengers to the nucleus where it initiates DNA transcription to produce proteins needed for cell differentiation, proliferation or apoptosis (for details refer to Tables 6-1 and 6-2).*

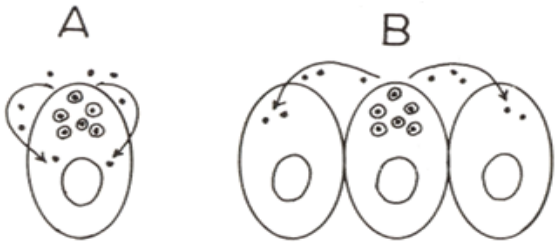

Fig 6-2 *Classification of growth factors according to their target cells. (A) Autocrine secretion affects the same cell, and (B) Paracrine secretion affects neighboring cells.*

Table 6-1 Growth Factors which Affect Epithelial and Stromal Cells

Target cells	Mito-genic	Anti-mito genic	Angio-genic	Fibro-genic
Epithelium				
Epidermal growth factor (EGF)	X			
Transforming growth factor alpha (TGF-α)	X			
Keratinocyte growth factor (KGF)	X			
Hepatocyte growth factor (HGF)	X		X	
Stroma				
Vascular endothelial growth factor (VEGF)			X	
Fibroblast growth factor (FGF)	X		X	X
Stem cell factor (SCF)	X			
Platelet derived growth factor (PDGF)	X		X	X
Both epithelium and stroma				
Insulin growth factor (IGF)	X			
Transforming growth factor beta (TGF-β)		X	X	X

Table 6-2 Cell Receptors and their Related Enzymes

Cellular localization	Receptors
Transmembrane receptors	
Tyrosine kinase associated	IGFR, c-kit, EGFR, PDGFR, FGFR
Inner membrane receptors	
GTP-associated	RAS
Phosphoinositide-3 kinase	PI-3K
Cytoplasmic receptors	
Tyrosine kinase associated	JAK
Serine / threonine kinase	AKT, RAF, ERK, TGFβ/SMADS
Tyrosine phosphatase	PTEN
Transcription factors	
DNA polymerase	C-myc, Jun, Fos, p53, steroid hormone receptors

their action. A classic example is the Myc/Max/Mad network of transcription factors that regulate cell growth and death. Myc/Max heterodimers activate transcription causing cell growth, proliferation and death (apoptosis). Conversely, Mad/Max heterodimers causes differentiation, cell survival, and inhibition of growth and proliferation.

Cell Population Stability

The average human adult is composed of about quadrillion cells (10^{15}). Adult cells are short-lived, about one trillion (10^{12}) cells die each day and must be replaced by an equal number to keep cell population constant. Both cell loss and cell production are under strict genetic and epigenetic control to keep a normal balance.

Cell Loss (Apoptosis)

There are five major types of cell death, namely: Apoptosis, necrosis, autophagy, paraptosis and autoschizis (Fig 6-3). The mode of death is mainly determined by the degree of stress, shifting from autophagy, to apoptosis, to necrosis with the increase of stress. Except for necrosis, all other modes of cell death are genetically programmed and consume energy. A comparison between apoptosis and necrosis is presented in (Table 6-3).

Apoptosis is executed through the action of a family of 12 enzymes, called caspases, which cleave the protein of cytoskeleton. They have been divided into two main classes, namely: initiator procaspases (caspase 1, 2, 4, 5, 8, 9, 11 and 12) and effector caspases (caspase 3, 6, and 7). Initiator caspases initiate and propagate the apoptotic signal, whereas, effector caspases cleave cell proteins. The activation of caspase 3 leads to the activation of other enzymes such as endonucleases and proteases. Endonucleases induce the classic internucleosomal DNA fragmentation of multiple of 180 base pairs producing a characteristic ladder-like pattern by gel electrophoresis. Proteases induce protein cross-linking and cell shrinkage. There are two main pathways to activate caspases, namely: the extrinsic or membranous, and the intrinsic or

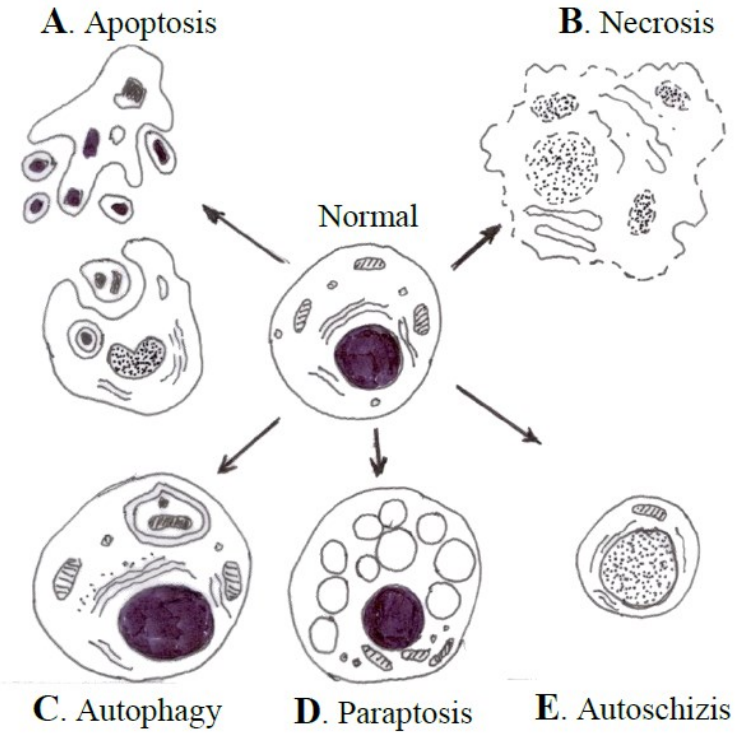

Fig 6-3 *Types of cell death. (A) Apoptosis. The cell shrinks, chromatin is condensed and fragmented, develops blebs, but cell membrane is intact. The resulting apoptotic sacs are phagocytosed by histiocytes. (B) Necrosis. The cell swells, cell membrane rupture and cell organelles are indistinct. (C) Autophagy (self eating). The cell contains a prominent lysosomal vesicle which leads to autophagocytosis. (D) Paraptosis. The cell is normal in size but contains multiple cytoplasmic vacuoles. (E) Autoschizis. The cell shrinks in size mainly due to loss of cytoplasmic mass.*

Table 6-3 Comparison of Apoptosis and Necrosis

	Apoptosis	**Necrosis**
Etiology	Genetically determined (suicidal)	Accidental (homicidal)
Mechanism	Mitochondrial membrane permeability	Cell membrane damage
Affection	Individual cells	Population of cells
Cell size	Shrinkage	Swelling
Cell membrane	Intact	Ruptured
Inflammation	Absent	Present
DNA degradation	Internucleosomal	Random
Degrading enzymes	Caspases	Phospholipase and protease
ATP and protein synthesis	Sustained	Lost

mitochondrial pathways (Fig 6-4).

The extrinsic apoptotic pathway recognizes and eliminates cells with foreign protein on their surface. Examples include: (1) *NK and cytotoxic lymphocytes* expression of perforin and granzyme B, (2) *FAS-mediated* elimination of autoreactive T-cells in the thymus, and (3) *TNF-a and death receptors*. The procaspase 8 is activated by these membrane signals.

The intrinsic pathway functions to eliminate senescent or damaged cells of the organism, and caspases are activated by the liberation of cytochrome c from the mitochondria to the cytoplasm. Telomere shortening or any DNA damage by anoxia, irradiation or mutation is sensed by the tumor suppressor gene TP53 which activates the proapoptotic genes (BAX and BAD) leading to the liberation of cytochrome c from mitochondria to cytoplasm (Fig 6-4). Cytochrome c binds with the adapter protein Apaf-1, and procaspase-9 to form an "apoptosome" complex which activates caspases. It is paradoxical that cytochrome-c, a most useful electron transfer agent in oxidative phosphorylation in mitochondrion, becomes a deadly killer to the cell when released into the cytoplasm.

Apoptosis is under strict control by a variety of genes (Fig 6-4). BAX and BAD gene expressions are proapoptotic, whereas, Bcl-2 and Bcl-x are antiapoptotic. NOXA which inhibits Bcl-2 is considered proapoptotic. These proteins act by affecting the permeability of mitochondrial membrane to cytochrome-c. Thus, BAX in presence of calcium ions increases mitochondrial membrane permeability, a process blocked by Bcl-2.

Apoptosis is considered the physiologic mechanism of choice to remove cells damaged by aging or genetic mutation, hence protection against cancer. Normal adult cells have only a limited capacity of cell division. The ends of chromosomes *(telomeres)* contain many copies of guanine (G)- rich repeats $(TTAGGG)^N$. The enzyme DNA polymerase is unable to replicate telomeres, which shorten by about 100 base pairs with each division. This is referred to as *the end replication problem* (Fig 6-5). When DNA shortening reaches a critical level after 50 divisions, it is sensed by TP53 as a DNA break, resulting in expression of p21 and Bax with permanent arrest of cell cycle *(cell senescence)* and subsequent apoptosis. In this way, telomeres may be considered as death timers. The enzyme *telomerase* can correct telomere shortening (Fig 6-5). Germ cells, stem cells and malignant cells are rich in telomerase, hence they are immortal. Conversely, normal somatic cells lacking this enzyme are short- lived.

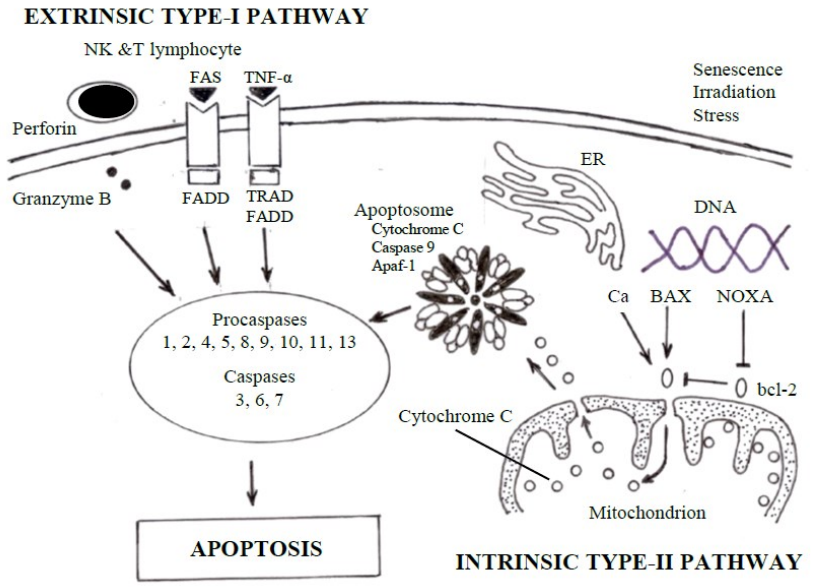

Fig 6-4 *The two pathways of apoptosis. The extrinsic pathway is a reaction to foreign protein, whereas, the intrinsic pathway is a programmed cell death to eliminate senescent or DNA damaged cells. The enzymes caspases play a major role in both pathways.*

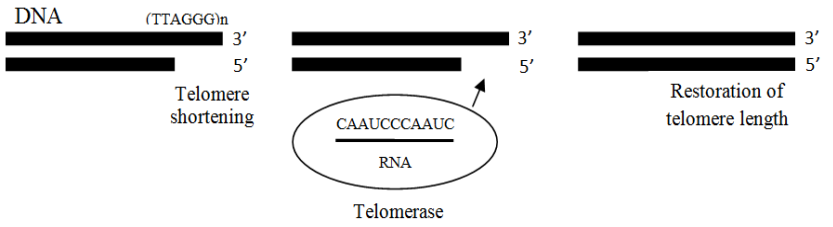

Fig 6-5 *Telomere shortening due to failure of replication at chromosomal ends. It is corrected by the telomerase enzyme equipped with its own RNA molecule.*

Cell Renewal

All tissues of the body contain a limited number *of adult or somatic stem cells* which have retained their ability to reproduce themselves (self renewal), as well as, to give rise to differentiated cell of their tissue of origin (organ specificity). Thus, an adult stem cell may produce through *asymmetric division*, one differentiated cell and another stem cell; or divide by *symmetrical division* (in case of need or neoplasia), to produce two stem cells (Fig 6-6). This capacity of stem cells to double modes of cell division assures immortality of stem cells which act as a constant reserve store for cell renewal according to needs. This property is under strict *genetic control* (genes: WNT, Notch, Bmi-1, oct-4, Sox -2 and Nanog). Moreover, *epigenetic mechanisms* also play important role. Thus, according to the *stem cell niche hypothesis*, signals from the local microenvironment (or niche) control stem cell renewal or differentiation. Under normal conditions, niche cell signals keep stem cells in quiescent state (G0) until they are called upon by mitogenic signals to produce new cells. Contrary to adult stem cells, progenitor cells are incapable of self renewal and produce differentiated cells which ultimately die by apoptosis.

Tissues of the body are classified according to their capacity of self renewal (turnover rate) into three main groups: *(1) Non-renewal or stable cells*; including oocytes (non-dividing end cells), skeletal muscle and nerve cells. Recently, adult stem cells were identified in skeletal muscle and neural stem cells were detected in certain regions of brain

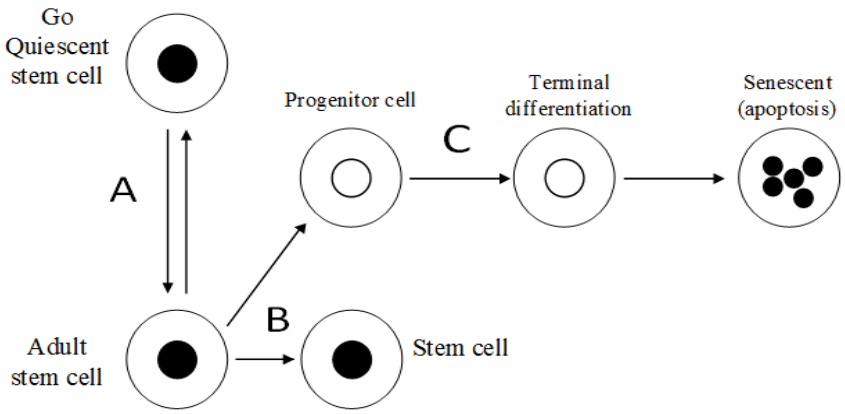

Fig 6-6 *Cell renewal by stem cell. A) An adult stem cell is activated from its quiescent Go state. (B) Stem cell divides into a progenitor and another stem cell by asymmetrical division. (C) The progenitor cell undergoes terminal differentiation into an adult cell which becomes senescent and ultimately dies by apoptosis.*

(subventricular zone and hippocampus), *(2) Slow renewal tissues*, where mature cells are long-lived and renewal is slow (examples: glial cells, connective tissue, endocrine glands), and (3) *Rapid renewal tissues* with high turnover rate including: hematopoietic tissue, gastrointestinal epithelium, skin, testis and urothelium.

Genomic Stability

DNA is regularly subjected to damage by *endogenous factors* (such as: oxygen radicals, replication and recombination errors), as well as, *exogenous factors* (such as chemicals, ultraviolet light and radiation). As a result of exposure to these harmful agents, DNA undergoes serious changes such as: depurination, deamination, hydrolysis, cross links, DNA breaks, and nonenzymatic methylation (alkylation) which attach chemical groups called adducts to DNA (Fig 6-7 and Fig 6-8).

Cellular repair of a macromolecule is known to occur only for DNA, a vital molecule for cell survival. DNA damage repair occurs during cell-cycle checkpoints with tumor suppressor gene *TP53 (the guardian of the genome)* playing a key role. TP53 detects DNA damage through the mediation of ataxia telangiectasia protein (pAT), then TP53 causes arrest of all cycle to give time for DNA repair enzymes. If the DNA lesions are severe and irrepairable, the mutant cells are eliminated by apoptosis (Fig 6-9).

Typically, DNA repair involves 3 main steps, namely: damage recognition, excision of the damaged DNA, and finally filling of the resultant gap

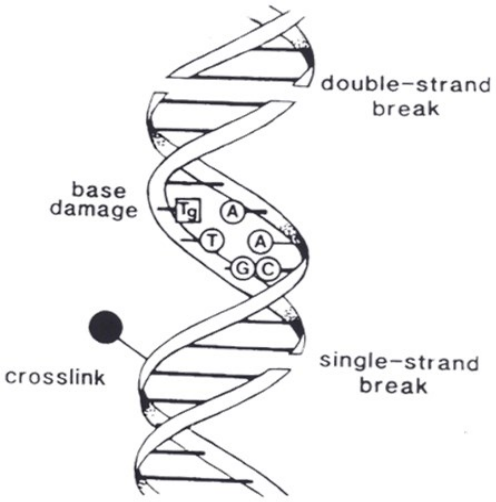

Fig 6-7 *Types of DNA damage caused by chemical or radiation injury.*

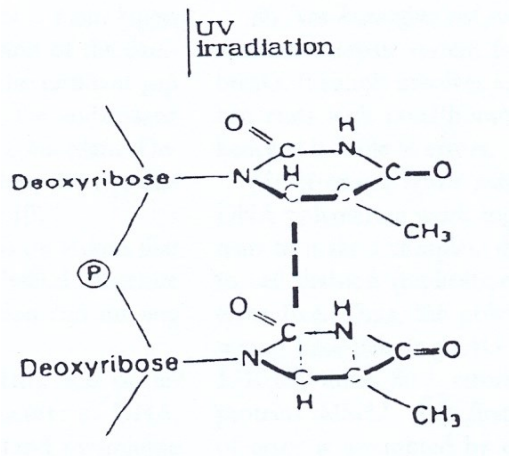

Fig 6-8 *Pyrimidine-dimer mutation resulting from ultraviolet (UV) irradiation. Neighbouring thymine molecules in the same DNA strand are attached together by a covalent bond (dashed line).*

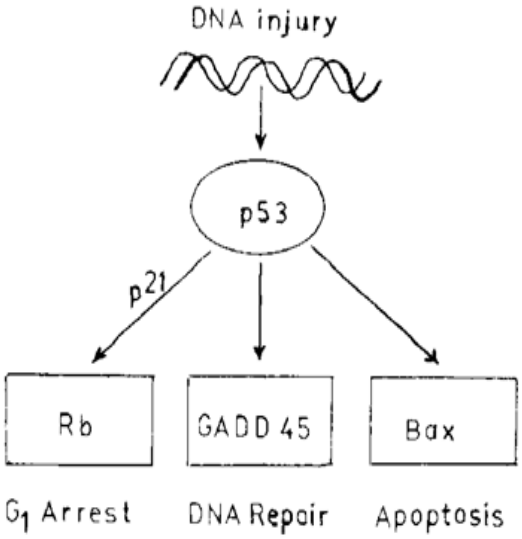

Fig 6-9 *TP53 gene, the guardian of the genome. In response to DNA damage, wild TP53 keeps genomic stability through transcriptional control of other genes responsible for cell cycle arrest, DNA repair and apoptosis.*

by newly synthesized DNA using the undamaged complementary DNA strand as a template. Depending on the type of damage, there are five distinct DNA repair pathways (Fig 6-10).

1. Base excision repair (BER): acts on lesions that do not disrupt the overall double helical structure of DNA, e.g. oxidation, methylation and missing bases.

2. Nucleotide excision repair (NER): acts on lesions that disrupt the helical structure of DNA, e.g. cross-linked bases as intrastrand pyrimidine dimmers, interstrand cross-links and bulky chemical adducts (Fig 6-11). Xeroderma pigmentosum (XP) protein plays an important role in recognition of helical distortion.

3. Homologous recombination repair (HRR): this mechanism acts on double-strand breaks. It uses a homologous DNA strand to act as a template, hence, it is error free. This system is a recombination repair, involving exchange of DNA between the two strands (Fig 6-12). Many gene products are involved to locate and bind broken ends of DNA, including: breast cancer protein 2 (BRCA-2) and mutated in ataxia telangiectasia (ATM).

4. Non homologous end joining (NHEJ): this is an alternative repair system for double-strand DNA breaks. It simply involves joining together the broken ends with non-homologous chromosomes, hence, it is liable to errors.

5. Mismatch repair genes (MMR): normally, DNA polymerase works together with MMR proteins to make a complete duplicate of DNA prior to cell division (replication). This process is not error free. Thus, the polymerase incorporates a wrong base-pair (e.g. AG instead of CG) every 1/10,000 times. Such errors are detected by MMR proteins (MSH2). As a first repair step, correction of error is attempted by exonulease (Fig 6-13), but if it fails, correction is made by MMR through the action of MLH1 (Fig 6-14). MMR genes mutation is linked to a specific colonic cancer (hereditary non-polyposis colorectal cancer, HNPCC).

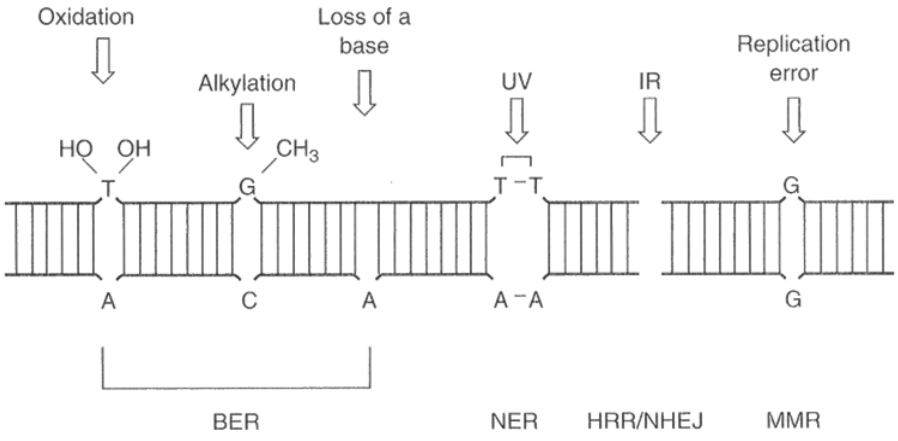

Fig 6-10 *Types of DNA injury and their corresponding repair pathways. Abbreviations: UV=ultraviolet, IR= irradiation, BER=base excision repair, NER=nucleotide excision repair, HRR=homologous recombination repair, NHEJ=nonhomologous end joining, and MMR=mismatch repair.*

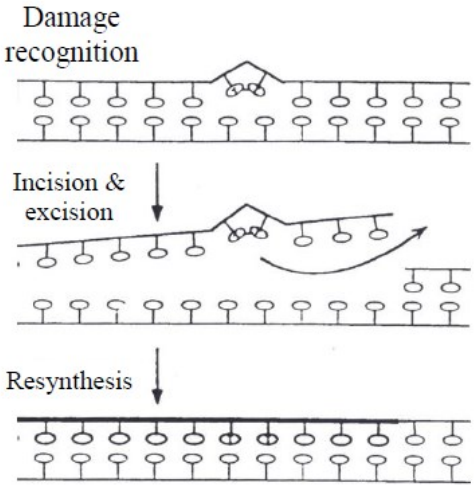

Fig 6-11 *Steps of nucleotide excision repair. The base damage is recognized and excised by a glycosylase, removal of the sugar residue by endo and exonuclease, followed by resynthesis and ligation by polymerase and ligase enzymes respectively.*

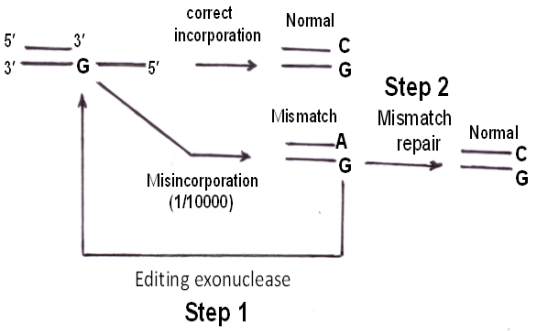

Fig 6-13 *The two step model for mismatch repair. The repair of mismatched bases (A-G) is done in two steps. The first step is an attempt to repair by exonuclease, but if it fails a second step is done by the mismatch repair system.*

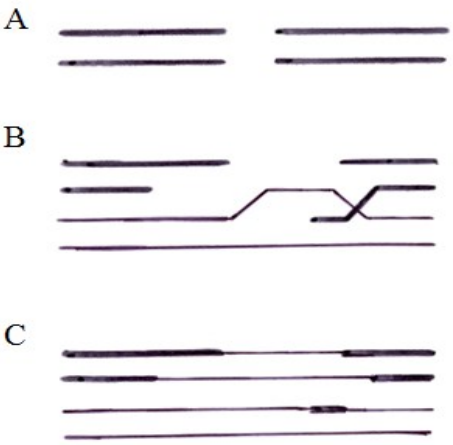

Fig 6-12 *Homologous recombination repair (HRR). (A) Double-strand breaks (B) Recruitment of homologous DNA strand to act as a template (C) Gap filling by repair synthesis.*

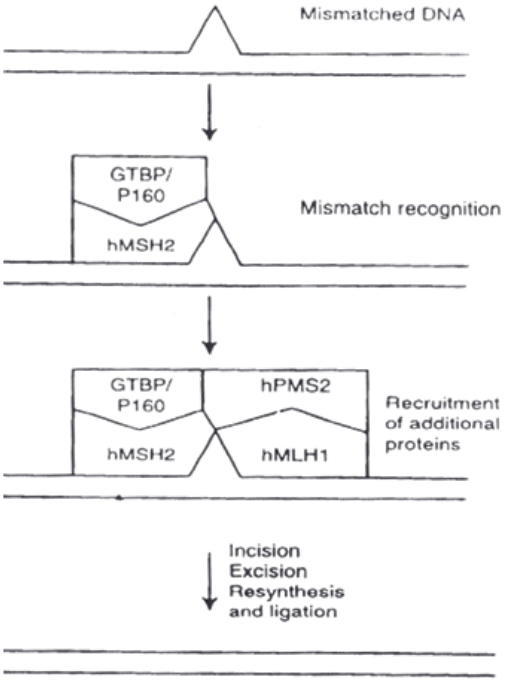

Fig 6-14 *Mismatch repair (step 2). Mismatch is recognized by MSH2 and then repaired by MLH1.*

BASIC CONCEPTS OF TUMOR BIOLOGY

Monoclonality

According to this concept a neoplasm arises from a single cell (usually an adult stem cell which divides symmetrically by clonal expansion). The monoclonal origin of cancer is supported by the following 3 observations in malignant tumors:

1. G6PD isoenzymes: normal cells contain two isoenzymes (A and B) of the enzyme glucose 6-phosphate dehydrogenase (G6PD), whereas, malignant tumors of that tissue contain only one isoenzyme, either A or B, hence confirming the monoclonal origin of tumor (Fig 6-15).

2. Light chain restriction: normal B lymphocytes carry on their surface immunoglobulin with two types of light chains (kappa and lambda). But non-Hodgkin lymphoma or myeloma cells which arise from B lymphocytes contain only one type of light chain, either kappa or lambda.

3. Immunoglobulin receptor and T-cell receptor gene rearrangement serve as markers of monoclonality in B and T-cell lymphomas respectively.

Field carcinogenesis

In carcinomas, and because of high carcinogenic exposure, the oncogenic process usually involves large area of mucosa, with an increased risk of developing multiple tumors (Slaughter 1953). This phenomenon is particularly observed in tumors of upper aerodigestive tract, oral cavity, urothelium, as well as, syndromic cancers of hereditary origin.

Two hypotheses were introduced to explain multiple tumors (Fig 6-16): *(1) Migrating clone theory (multifocal origin)*, satellite tumors are considered intraepithelial or stromal spread from the original tumor, (2) *Independent events theory (multicentric)*, considers the two tumors as different primaries. It seems likely that both hypotheses are true and explain the concept of field carcinogenesis. Grossly, if the tumors are close to each other, they are considered multifocal, but if they are separated by a long distance they are more likely to be multicentric (Oijen, 2000).

Field carcingogenesis is expressed clinically as multicentricity of tumors in the same organ, bilaterality of tumors in paired organs, and multiple primary cancers in different organs. The incidence of *multicentricity* varies according to tumor site being high (>50%) in xeroderma pigmentosa, thyroid, prostate and ulcerative colitis; moderate (about 25%) in breast, polyposis coli and bladder; and low (<10%) in oral cavity and stomach. The incidence of *metachronous bilateral* cancers (occurring at different times) is highest in inherited cancer (e.g. 90% in familial retinoblastoma); moderate in abdominal undescended testis (30%)

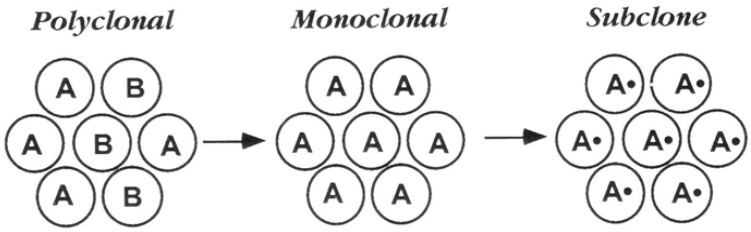

Fig 6-15 *Monoclonality. Cancer is monoclonal in origin since it arises from a single cell. The subsequent development of subclones will lead to tumor cell heterogeneity.*

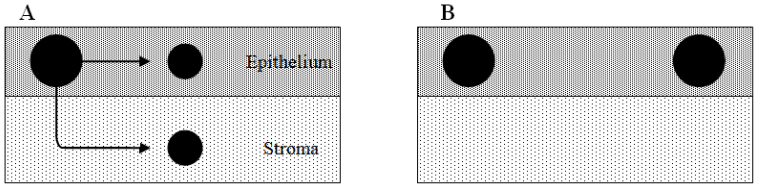

Fig 6-16 *Field carcinogenesis in carcinoma. (A) multifocal origin, tumors arise from one clone followed by intraepithelial or stromal spread, and (B) multicentric origin represent two distinct widely separated, different tumor clones.*

and lobular carcinoma of breast (25%) and; low incidence in Wilms' tumor (10%) and invasive duct carcinoma of breast (4%). The incidence of *multiple primary* cancers varies between 3 to 5% of total cancers. They are classified on etiologic basis into: spontaneous (inherited or environmental factors), or iatrogenic (therapy induced).

The Multistep Model

According to this concept, malignancy develops after passing through three successive stages, namely: initiation, promotion and progression in that order (Fig 6-17). This was the first model proposed for carcinogenesis. Thus, Brenblum and Rous (1941) first recognized the initial two stages of carcinogenesis through their classic experiments on mice, using tar as an initiating mutagenic agent and croton oil as a promoting mitogenic agent. The initiating agent alone was incapable of producing a tumor. Thus, the two driving forces of neoplasia are mutagenesis and mitogenesis. The concept of tumor cell progression (the third step) was developed by Foulds (1954) and defined as the stepwise evolution of malignant characteristics. The three stages of cancer development have the following distinctive features:

1. Initiation: It is caused by mutation following the application of an initiating agent (chemical carcinogen or radiation). It is irreversible and cummulative. Histologically, the cells appear normal inspite of the genetic damage. The initiated cells are also diploid, with normal DNA content.

2. Promotion: It is characterized by cell proliferation (symmetrical division of stem cells) and is reversible after cessation of the promoting agent. Human promoting agents include: bile acids (increased by fat diet), hormones and oncogenic viruses. Promoting agents produce cell hyperplasia by activating protein kinase and inducing growth factors through epigenetic mechanisms. Histologically, there is always a precancerous lesion in the form of dysplasia or benign tumor. Promoted cells are usually diploid.

3. Progression: It results from multiple mutations in the proliferating cellular subclones, and is an irreversible process. Histologically, there is cellular anaplasia and the tumor acquires malignant biologic features such as: angiogenesis, local invasion, metastatic potential and drug resistance. The tumor cells are usually aneuploid with abnormal DNA content.

Clonal Evolution Model

This model was first proposed in 1976 by Nowell, and confirmed in 1990 by Fearon and Vogelstein studies on the adenoma carcinoma sequence in the colon (chapter 13). For cancer to develop, multiple mutations are required (usually 6 to 10) involving oncogenes and tumor suppressor genes. Accumulation of mutations in different subclones will ultimately lead to *molecular intratumor heterogeneity* (Fig 6-17). The tumor cell subclones

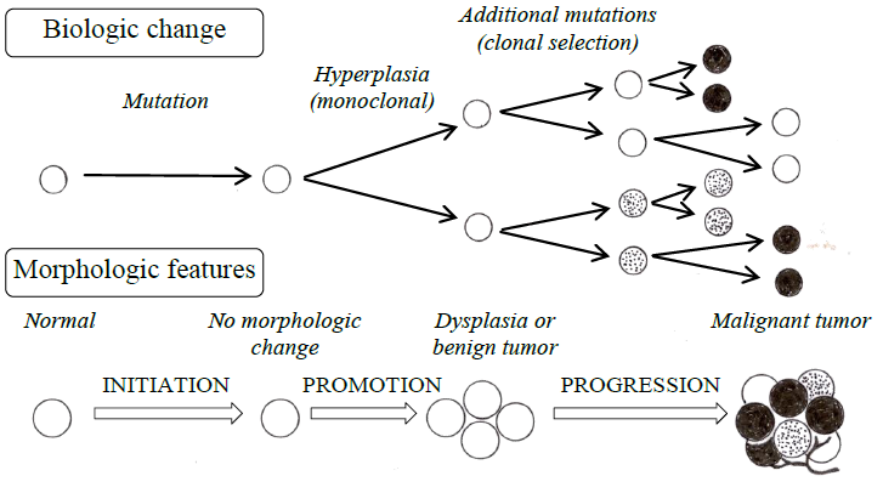

Fig 6-17 *The multistep model of carcinogenesis and its associated cytomorphological and biological changes. Initiation results from mutational change of adult stem cells into malignant stem cells. Promotion involves clonal expansion of malignant stem cells by symmetrical cell division, whereas, progression involves clonal selection of the proliferating stem cells (Malignant Darwinism) due to multiple mutations.*

compete with each other on the basis of growth rate, with ultimate survival and predominance of the more aggressive subclones (*clonal evolution* or *malignant Darwinism*). Thus, in this model it is the total accumulation of genetic lesions, rather than their order of sequence, which is most critical in cancer development (a multihit rather than a multistep process).

Reversibility of Malignancy

The growth of cancer is usually progressive, but in some occasions the malignant process may be reversible as outlined in the following four examples:

1. Most *dysplasias* of the uterine cervix, and even some carcinoma in situ, are reversible.

2. *Spontaneous regression* of cancer, without therapy, is a well documented but rare phenomenon (one case per 100,000). It is commonly observed in neuroblastoma, renal cell carcinoma and malignant melanoma. The possible mechanisms of this phenomenon are: tumor cell differentiation, immune rejection or vascular insufficiency.

3. Regression is possible in some tumors by chemical induction of *differentiation*. Examples include: the differentiation of acute premyelocytic leukemia (APL) by retinoids, and neuroblastoma by cyclic adenosine mono-phosphate (C-AMP).

4. The mouse *blastocyst/teratoma experimental model* is another example of reversibility of cancer. In this experiment, malignant teratoma cells from a black mouse are inoculated into a normal white mouse. A mosaic normal offspring is born with normal skin showing black areas (derived from the reverted teratoma cells) and white areas (from normal embryonic cells).

THE GENETIC BASIS OF CANCER

The genetic basis of cancer is supported by three facts, namely: (1) carcinogenic agents are also mutagenic, (2) gene mutations and chromosomal abnormalities are inherited, transmitted from parent to daughter cells, and (3) genes determine cell phenotype in normal cells, hence, malignant phenotype could only be explained on genetic basis.

Genes which may cause cancer are already present in the normal genome as precursors (protooncogenes and tumor suppressor genes), constituting about 1% of genome. Normally these genes encode proteins that control cell proliferation, differentiation, apoptosis and genomic stability. Alteration of these genes will give rise to cancer genes responsible for the malignant phenotype. Thus, the activation of *protooncogenes* will result in *oncogenes* which have a growth stimulating effect on cells. Conversely, *tumor suppressor genes* normally serve to restrain cell proliferation (cell brakes), hence, their inactivation will lead to cell overgrowth.

With advances made in molecular cell biology, several other genes were found to participate in oncogenesis. At present, *survival genes* (*antiapoptosis* and *telomerase genes*), *angiogenesis* and *cell migration genes* are classified under oncogenes, whereas, *proapoptosis genes*, *DNA repair genes* and *cell adhesion genes* (*E-cadherin*) are considered tumor suppressor genes.

Cancer is essentially a multigenic disease. Thus, apart from retinoblastoma which results from inactivation of a single suppressor gene (Rb), most cancers develop after dysfunction of multiple cancer genes (usually 5 to 10) of different classes in order to complement each other. The number and types of cancer genes involved varies tremendously among different organ sites. For this reason, the molecular oncogenic profiles are presented in the chapters covering different systems. However, we will review in the following section only the general aspects of mutations, oncogenes and, tumor suppressor genes. A detailed account will be made, however, on RAS oncogene, Rb and TP53 tumor suppressor genes, since they represent a prototype (model) for other cancer genes of their classes, beside their common participation in many cancers.

Mutations
Causes and Types

A mutation is defined as any permanent and heritable change in DNA base sequence. Mutations are classified in many ways according to: etiology, type of mutation, type of cell affected and extent of genes involved.

Spontaneous or Induced

Spontaneous mutation is due to either chemical instability of DNA bases or replication errors. The following are four examples: (1) *deamination* of cytosine ultimately leading to a mutant daughter cell in which the normal GC pair is replaced by AT, (2) *depurination* with loss of purine bases, (3) *misincorportion* of bases during replication leading to mismatching of bases, and (4) *loop formation* during replication leading to the creation of a long DNA strand with extra nucleotides (Fig 6-18). Induced

mutations will result from DNA damage by chemicals, reactive oxygen species or radiation.

Dominant or Recessive

A gene occurs in duplicate, called *alleles*, one on each of the homologous chromosomes. The normal allele is called *wild type* and the altered allele *mutant*. If the alleles are similar, the gene pair is called *homozygous*, but, if alleles are different, the gene is considered *heterozygous*. In dominant mutation, affection of only one allele is sufficient to induce the phenotypic change (gain of function). Conversely, in a recessive mutation, a change of one allele (heterozygous) is insufficient to induce the phenotypic change, and affection of both alleles is required (loss of heterozygosity LOH) leading to loss of function of the gene.

Germline or Somatic

Germline mutation is hereditary or induced in the embryo (gamete cells), hence the mutation will be passed to all cells of the body. Conversely, *somatic mutation* occurs postnatally, affects somatic cells and passes to only the descendents of that cell lineage with a restricted distribution of mutation in a particular organ. Cancer genes are affected by somatic mutation in 90% of cases, 20% by germline mutation and 10% by both. Contrary to recessive genes, germline mutation of dominant genes (proto-oncogenes) are usually incompatible with embryonic life.

Point Mutation

A mutation involving a change in a single base pair is called *point mutation*. Three types are recognized (Fig 6-19); namely : (1) *missense mutation* is a single neocleotide change which results in a protein in which one amino acid is substituted for another, (2) *nonsense mutation*, in which a stop codon (TAA) replaces an amino acid codon, leading to premature termination of translation, thereby, generating a short or truncated protein, and (3) *frameshift mutation* involves the addition or deletion of any number of nucleotides (not a multiple of three) causing a change in the reading frame and leading to the introduction of unrelated amino acids into the protein.

Chromosomal abnormalities

Chromosomal alterations may be *numerical* (a change in number) or *structural*. The former is usually the result of *nondysjunction* (Fig 6-20) and results in loss or gain of one or more chromosome (*aneuploidy*) and may be fatal to the cell in case of loss of vital chromosomes. Structural chromosomal abnormalities involve gain or loss of only a segment of chromosome as a result of breaks (Fig

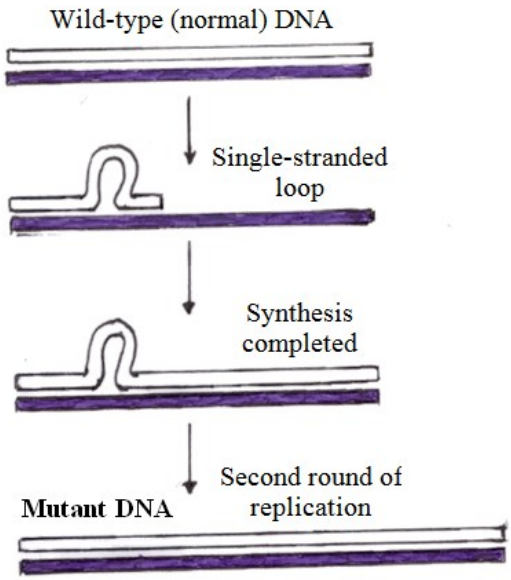

Fig 6-18 *DNA loop formation. This will lead to a mutant DNA long strand with excessive number of nucleotides.*

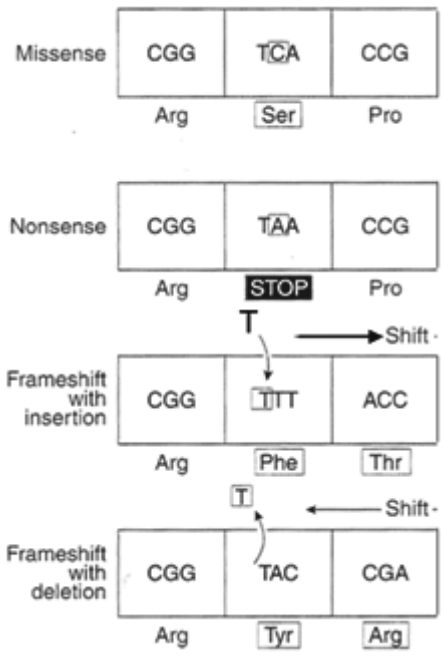

Fig 6-19 *Point mutation and the resulting changes in amino acids in the protein product. A nonsense mutation will result in a short (truncated) protein.*

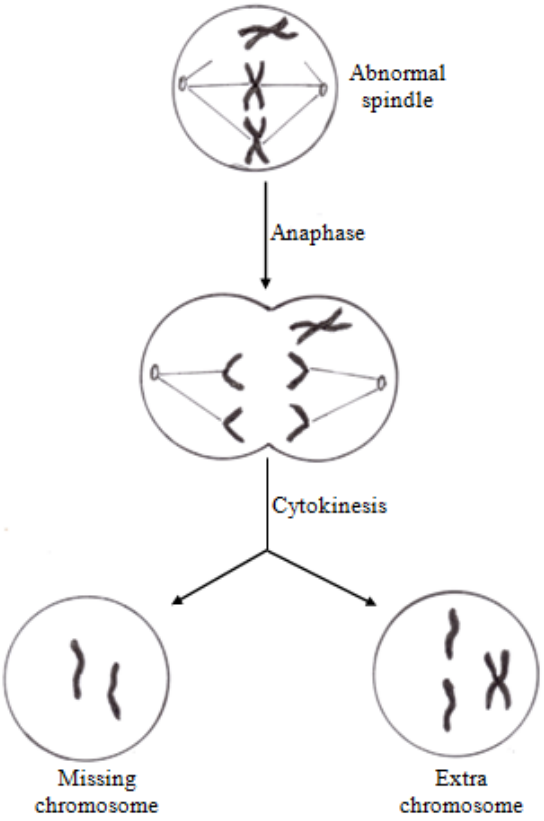

Fig 6-20 *Nondysjunction. Abnormal nonattachment of spindle to chromosomes during mitosis results in a cell with missing chromosome and a cell with extrachromosomes. Chromosomal loss is a mechanism operable in tumor suppressor gene inactivation.*

6-21), or focal increase of copy numbers of genes resulting in amplification (Fig 6-22). Chromosomal abnormalities may be *spontaneous* (defects in cell cycle) or *induced* by exogenous factors. These abnormalities are studied by conventional *G-band Karyotyping*, and recently by other molecular cytogenetic techniques such as: *fluorescent in situ hybridization (FISH)* and *comparative genomic hybridization (CGH)* (for numerical changes). Chromosomal abnormalities may be simple or *complex*, the latter shows 3 or more abnormalities in the karyotype. Structural chromosomal abnormalities ultimately result in gene rearrangement mutation, of which nine types are recognized (Fig 6-21):

1. Translocation (t) is the most common chromosomal abnormality, especially in hematopoietic and soft tissue malignancies (Table 6-4). Reciprocal translocation refers to the exchange of a chromosomal segment between different (non-homologous) chromosomes. The translocation is balanced if there is no loss of genetic material in this process, or unbalanced if there is loss of such genetic material. Translocation can activate protooncogenes in two ways, namely: (a) *fusion* with other genes with the creation of chimeric gene with oncogenic protein product, such as t (9; 22) in chronic myeloid leukemia (Fig 6-23), or (b) *transactivation* of the translocated gene by a promoter enhancer sequence in the new location, such as t (8;14) in Burkitt lymphoma (Fig 6-24).

2. Deletion (del) is the second most common chromosomal abnormality especially in solid tumors and is the main mechanism of inactivating tumor suppressor genes (Table 6-5). Deletions occur as a result of breaks in chromosomes during cell division and the liberated segment is not incorporated into other chromosomes and hence is

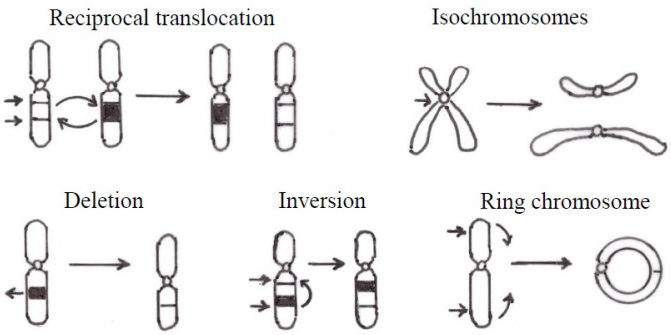

Fig 6-21 *Structural chromosomal abnormalities resulting in gene rearrangement.*

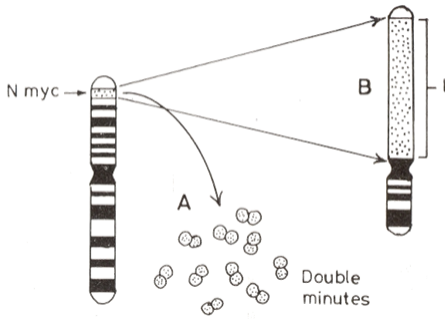

Fig 6-22 *Karyotypic features of gene amplification in neuroblastoma. (A) small paired fragments of chromatin (double minutes) and (B) homogeneous staining regions on chromosome.*

Table 6-4 Some Diagnostic Chromosomal Translocations in Human Cancers

Translocation	Affected Genes	Malignancy
Leukemias		
t (9; 22) (q34;1;q11)	ABL1-BCR	CML, ALL, AML
t (8;21) (q22;q22)	RUNX1-RUNXIT1	AML-MZ
t (15;17) (q22;q12)	PLM-PARA	APML
t (11;14) (q13;q32)	BCL-1 (PRAD1)	CLL
Lymphomas		
t (8;14) (q24;q32)	MYC-IGH	BL, FL, DLBCL
t (14;18) (q32;q21)	BCL2-IGH, MALT1-IGH	FL, DLBCL, MALT lymphoma
t (11;14) (q13;q32)	CCND1-IGH	MCL,MM
t (2;5) (p23;q25)	ALK	ALCL
Soft Tissue Sarcomas		
t (2;13) (q35;q14)	PAX3-FKHR	Alveolar RMS
t (12;16) (q13;q11)	FUS-CHOP	Liposarcoma
t (X;18) (p11.2;q12)	SYT-SSX1	Synovial sarcoma
t (11;22) (q24;q12)	EWS-FLI1	Ewing / PNET
t (X;17) (p11;q25)	ASPL-TFE3	Alveolar soft part sarcoma
t (17;22) (q21;q13)	COL1A1-PDGFB	Dermatofibrosarcoma
t (11;22) (p13;q12)	EWS-WT1	Desmoplastic small round cell tumor
t (12;15) (p13;q26)	ETV6-NTRK3	Infantile fibrosarcoma
Others		
t (10;17) (q11.2;q23)	RET-ptc2	Papillary thyroid carcinoma
t (21;22) (q21;q22.3)	TMPRSS2-ERG	Prostatic carcinoma

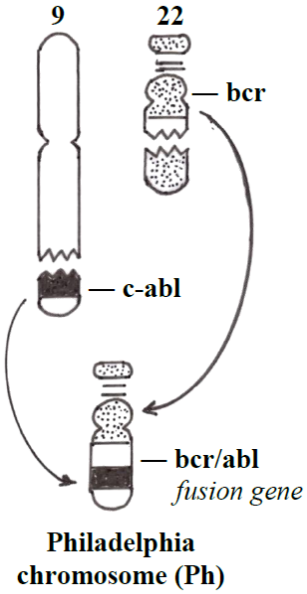

Fig 6-23 *Translocation t (9; 22) in chronic myeloid leukemia resulting in Philadelphia chromosome. The expressed chimeric protein is oncogenic due to its high tyrosine kinase activity.*

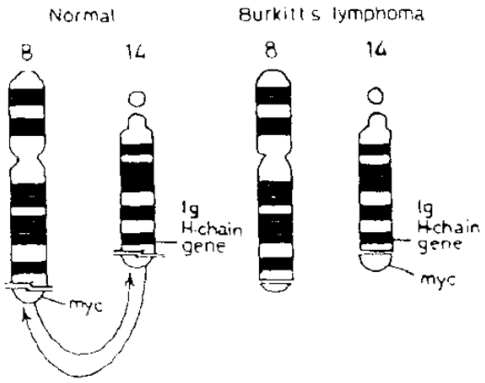

Fig 6-24 *Chromosomal translocation t(8;14) in Burkitt's lymphoma leading to the activation of c-myc proto-oncogene by the promoter enhancer of immunoglobulin (Ig) gene in the new location in chromosome 14.*

lost.

3. Amplification (amp), gene amplification denotes an increase of DNA copies (amplicons) in a chromosomal region. Such a change is probably the result of defects in the cell cycle check point (R2) resulting in excessive unscheduled DNA synthesis. There are two karyotypic manifestations of gene amplification, namely: homogeneous staining regions and double minutes (Fig 6-22). Neuroblastoma and breast cancers are the best examples of gene amplification involving N-MYC and HER-2 genes respectively.

4. Inversion (inv) refers to a process in which a chromosome is broken at two points and the resulting segment rotates then reattaches (Fig 6-21).

5. Ring chromosome (r) is formed by two breaks involving both telomeric ends of a chromosome with subsequent end-to-end fusion (Fig 6-21).

6. Isochromosomes (I) are formed by faulty centromere division in a transverse manner (Fig 6-21). An increase of isochromosome I (12p) is commonly observed in germ cell tumors.

7. Telomere dysfunction (BFB cycle), this results from telomere shortening with loss of the protective protein caps and creation of sticky ends. This leads to end-to-end fusion of different chromosomes with subsequent breakage at anaphase. A subsequent breakage-fusion-bridge (BFB) cycle is repeated leading to multiple translocations, deletions and amplifications.

8. Recombination errors: genes are normally exchanged between similar chromosomes during meiosis. A process of crossing over occurs followed by recombination, resulting in an equal or reciprocal exchange of genetic information between homologous chromosomes (Fig 6-25). However, if chromosomal alignment is not exact during metaphase, unequal crossing over will result, with unequal exchange of genes. Thus, one chromosome will receive less genes (a deletion), whereas, the other chromosome will receive more genes (insertion).

9. Gene transposition: small DNA sequences are capable of moving around within the genome of the same cell. These mobile DNA elements are called *transposones* or jumping DNA. This results is a change of normal gene sequence (rearrangement mutation).

Clinical Relevance

Gene rearrangement analysis is most useful in every aspect of cancer management, including: (1) diagnosis of distinct disease entities (Tables 6-4

Table 6-5 Diagnostic Deletions in Some Human Cancers and Their Related Tumor Suppressor Genes

Chromosome	Gene	Malignancy
5q 21-22	APC, MCC	Colon
17p 13.1	TP53	Several Cancers Li-Fraumeni
13q 14	RB	Retinoblastoma
17q	HICI, REN	Medulloblastoma
1p, 19q	CDKN2C	Oligodendroglioma
22q	NF2	Ependymoma
3p 25-26	VHL	Renal, pheochromocytoma
11p 13	WT1	Nephroblastoma (Wilm)
11p 15.5	WT2	Nephroblastoma(Wilm)
9q	TSC1, PTCH DBC1, PI3KCA	Bladder papillary Carcinoma
3 p 14.2	FHIT	Lung (SCLC)
22q	INI 1	Rhabdoid tumor

and 6-5), (2) guide therapy, e.g. tyrosine kinase inhibitors in t (9;22) positive CML and AML, as well as, all-transretinoic acid in t (15;17) positive acute promyelocytic leukemia, (3) to monitor response to therapy, as well as, detect minimal residual disease, and (4) predict prognosis, especially in hematologic malignancies.

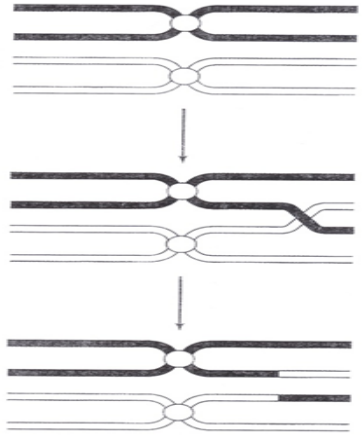

Fig 6-25 *Recombination error. It may result from unequal crossing over of chromosomes during metaphase*

ONCOGENES

Oncogenes are genes which when activated will cause cancer, due to their growth stimulating effect on cells. Oncogenes arise from activation of normal precursors *(protooncogenes)* resulting in a quantitative or qualitative change of the gene product. Historically, the oncogenes Myc and RAS were first discovered in birds and rodents (Stehelin, 1976) and found to be related to retrovirus infection. *DNA transfection assay* (Weinberg, 1981) is the experimental evidence of presence of oncogenes. Thus, DNA isolated from tumors induces malignant transformation of benign fibroblasts grown in culture. RAS was the first human oncogene discovered in bladder cancer (Cooper and Weinberg, 1982) and found to be activated by point mutation, rather than viral infection. At present, about 50 human oncogenes are recognized.

Oncogenes are *dominant genes*, since a mutation in a single allele is sufficient to cause cancer. Most germline mutations of protooncogenes are lethal to the embryo. For this reason, oncogenes are not involved in hereditary cancer syndromes, with the exception of RET protooncogene in multiple endocrine neoplasia (MEN) syndromes. Several mechanisms are operable in producing oncogenes from protooncogenes (Fig 6-26), almost all of them are somatic and postnatal.

Oncogenes are classified according to the site of action of their oncogenic protein products at

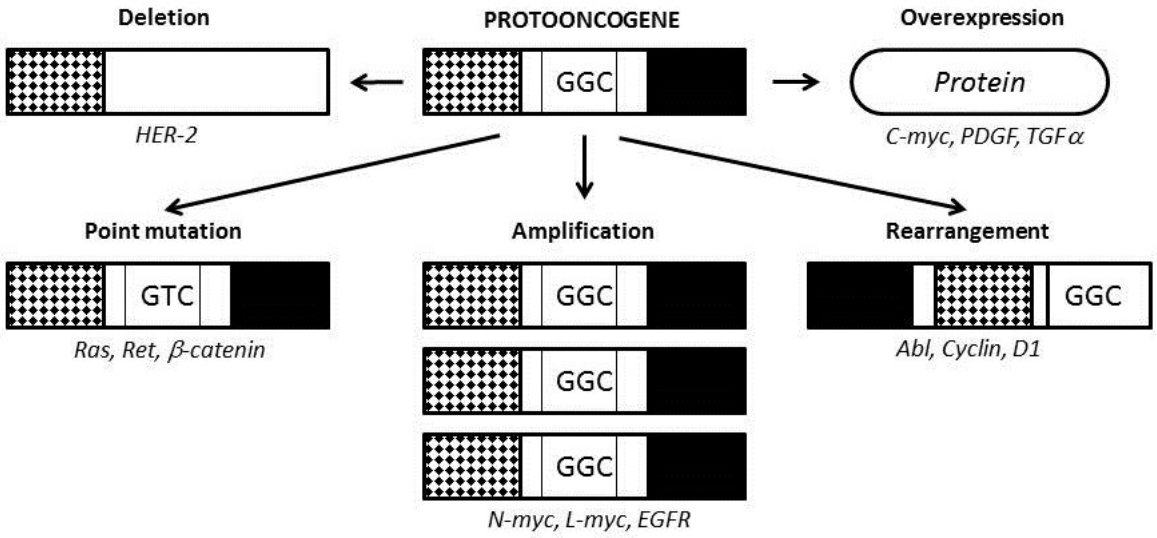

Fig 6-26 *Genetic mechanisms of proto-oncogene activation leading to oncogenes. Oncoprotein overexpression may also result from epigenetic mechanisms.*

subcellular level (Fig 6-27 and Table 6-6). All these sites are important components of signal transduction leading to mitogenic signal and/or survival advantage. In addition, the following two groups are also classified as oncogenes, namely: (1) Inhibitors of tumor suppressor genes (e.g. MDM2 inhibiting p53, and WNT signal inhibiting APC mediated degradation of β-catenin), and (2) genes involved in tumor progression by either promoting local invasion (e.g. matrix degrading metalloproteinases enzymes) or enhancing metastatic spread (e.g. VEGF and loss of nm23).

RAS Oncogene

RAS oncogene (resulting from activation mutation of RAS protooncogene) is the most common oncogene encountered in malignancy, observed in about 30% of all human cancers. However, the frequency of such mutations varies considerably among different tumors, being 90% in pancreatic cancer, 50% in colonic and 30% in lung carcinomas.

There are 3 members of RAS family in human genome, namely: HRAS, KRAS and NRAS). Normally, RAS protooncogene codes for a guanosine-nucleotide binding protein (p21). For signal transduction, there is an orderly cycling of RAS protein between guanosine-diphosphate GDP (inactive) and RAS-guanosine triphosphate GTP (active from). The enzyme GAP (GTPase activating protein) causes hydrolysis of RAS- GTP converting it to the inactive RAS-GDP form (Fig 6-28). RAS receives upstream signals from nearby protein kinase receptors (e.g. FGFR, EGFR, PDGFR and C -kit receptor) through the mediation of bridging proteins. When RAS is activated, it conveys two important down stream signals, namely (1) a mito-

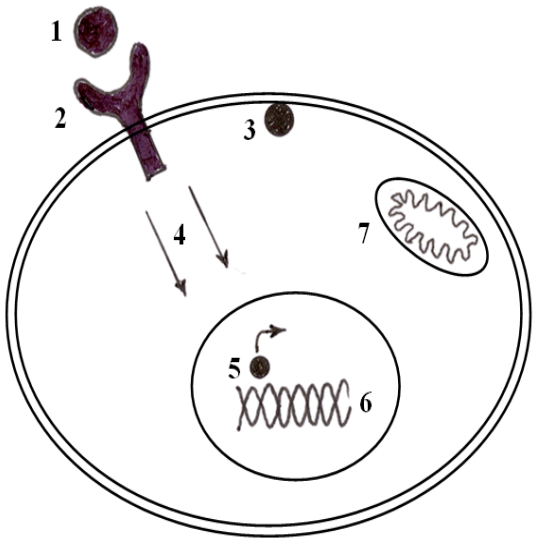

Fig 6-27 *Classification of oncogenes according to their site of action in the cell, (1) Growth Factor, (2) Growth factor surface receptor, (3) Membrane associated receptor, (4) Cytoplasmic receptor, (5) Transcription factor, (6) Telomerase and (7) Antiapoptotic protein acting on mitochondrial membrane (for details refer to Table 6-6).*

Table 6-6 Selected Oncogenes and their Associated Human Malignancies

Class (oncogene product)	Associated Cancer
1. Growth Factors (Ligands)	
FGF	Gastric, Kaposi
TGF-α	Astrocytoma, HCC
HGF	Thyroid, Renal
HSS	Kaposi Sarcoma
SIS	Glioma, Fibrosarcoma
2. Growth Factor Receptors	
EGFR	Lung, Gliomas
RET	MEN-2
PDGFR	Gliomas, Leukemias
C-kit	GIST, Seminoma, Leukemias
HER-2	Breast, Ovary
3. Membrane Associated	
H- RAS	Bladder
K- RAS	Pancreas, Colon
N- RAS	Leukemias
BRAF	Melanoma
PI-3K / PTEN	Breast, Gliomas
4. Cytoplasmic	
BCR / ABL	CML
JAK-2	PV, ET, PMF
SRC	Colon
5. Transcription Factors	
C- MYC	Burkitt (BL)
N- MYC	Neuroblastoma, SCLC
L- MYC	SCLC
FOS, JUN, MDMZ	Sarcomas
6. Telomerase	Many Cancers
7. Mitochondrial (antiapoptotic)	
BCL-2	FL, CLL

N.B. Oncogenes are written in capital letters, whereas their expressed proteins are written in small letters.

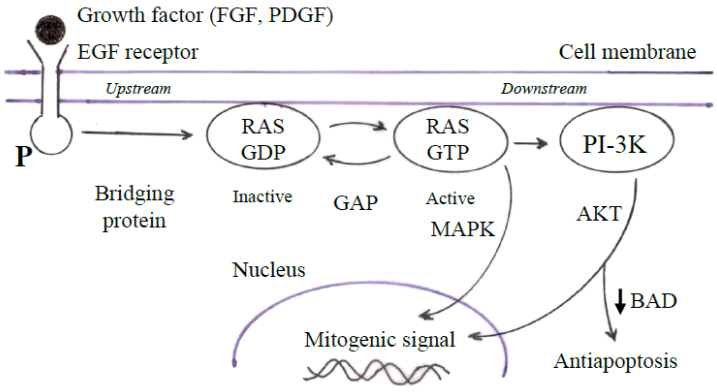

Fig. 6-28 *The RAS signal transduction pathways. RAS is activated from upstream membrane receptors, RAS/GTP activates two downstream signal pathways (RAF/MAPK and PI-3K/AKT), the latter has mitogenic and antiapoptotic effects.*

genic signal through RAF, MAPK pathway (Fig 6-1), and (2) a mitogenic and antiapoptotic signals through PI-3K/AKT pathway (Fig 6-28).

RAS protooncogene is activated by point mutation, changing the base sequence of codon from GGC to CTC, resulting in substitution of valine for glyceine amino acid. This mutation causes a change in the three dimensional structure of RAS protein rendering it resistant to hydrolysis by GAP, hence RAS remains in a continuous active state of RAS-GTP (a constitutively active oncoprotein) stimulating the downstream pathways.

There are two other mechanisms of activating RAS, namely: (1) the tumor suppressor gene NF-1 encodes a protein with RAS-GAP enzyme activity, hence, mutation of NF-1 will arrest RAS-ATP hydrolysis, keeping RAS in the active RAS-ATP from, (2) RAS signal transduction may be stimulated through a mutational activation of one of its downstream signaling kinases (e.g. mutation of BRAF in melanoma, a member of the RAF family).

TUMOR SUPPRESSOR GENES (TSG)

Tumor suppressor genes (TSG) are genes which when inactivated will lead to malignancy. The development of malignant phenotype is due to loss of normal protective functions of TSGs (cell growth suppression, DNA repair and proapoptotic functions).

Historically, *the retinoblastoma gene (RB)* was the first human tumor suppressor gene discovered, first postulated by Knudson (1971), and later cloned by Lee (1987). The experimental evidence of presence of TSG is *somatic cell fusion* (Harris, 1988). Thus, when normal and malignant cells are fused in culture, nonmalignant hybrid cells result. At present, about 35 tumor suppressor genes are discovered (Table 6-7).

Tumor suppressor genes are *recessive genes*. Accordingly, mutations of both alleles are required to achieve inactivation and develop malignancy. In other words, a change of the heterozygous to homozygos state, or, *loss of heterozygosity (LOH)*. Moreover, since TSGs are recessive, germline mutations are compatible with embryonic life, and the *mutation* is transmitted to all cells of the body. Such *germline* mutation is the basic oncogenic mechanism of all *hereditary cancers* (which constitute 1% of human malignancies) and explain their distinctive clinical features, namely: early age of onset and multiplicity. According to the *"two-hit" model* of hereditary oncogenesis (Knudson, 1971), the first hit is a germline mutation of gamete cells inherited from an affected parent, and the second hit is a somatic mutation acquired postnatally, leading to loss of heterozygosity (LOH) and development of malignancy (Fig 6-29). In addition, tumor suppressor genes are also involved in the development of sporadic cancers. In such cases, both hits (mutations) occur in somatic cells after birth. For this reason, somatic cancers (contrary to hereditary cancers) are usually solitary and affect older age groups.

Tumor suppressor genes are inactivated by several mechanisms (Fig 6-29), both genetic (e.g. mutation on deletion) or epigenetic (e.g. promotor hypermethylation, histone deacetylation or RNA interference with silencing of mRNA translation). Moreover, the protein product of a wild TSG may be inhibited by binding with other proteins, such as viral proteins or a protein product of a mutant TSG (a phenomenon known as *dominant –negative effect*). Finally, loss of function of wild TSG may also result from increase of its degradation through the proteosome.

Tumor suppressor genes are classified into two main classes according to their normal functions, namely: (1) *gatekeeper genes* which keep cell population stability by acting as an antiproliferative molecular brakes of cell cycle, as well as, an proapoptotic function to eliminate unwanted cells, and (2) *caretaker genes* which keep genomic integrity through DNA repair mechanisms (Table 6-8). Generally, gatekeeper genes are more important as initiating agents of cancer, whereas, caretakers contribute to additional mutations needed for tumor progression. Some genes (e.g. TP53) combines the characteristic features of both gatekeepers and caretakers. It is also noteworthy, that genes whose products inhibit oncogenes are considered TSGs. The following are illustrative examples: (1) P14/ARF inhibiting MDM-2, (2) PTEN phosphatase inhibiting PI-3K/AKT progowth prosurvival signaling pathway, (3) inhibitors of cell cycle of families KIP (p21, p27, p57) and INK-4 (p15, p16, p18, p19), (4) transforming growth factor beta (TGF-β) which inhibits C-myc, CDK 2 and 4, as well as, cyclins A and E, (5) E-cadherin binding to β-catenin (6) NF-1 protein product inhibiting active RAS-GTP and (7) NOXA inhibition of the anti-apoptotic bcl-2.

Table 6-7 Tumor Suppressor Genes (TSG) Classified According to Their Mechanism of Action (Kinzler and Vogelstein)

TSG	Mechanism	Tumor type/Syndrome
GATEKEEPER GENES (Antiproliferative and proapoptotic)		
RB	Cell cycle regulation	Retinoblastoma, osteosarcoma
TP53	Transcription factor cell cycle regulator and proapoptosis	Several cancers Li- Fraumeni syndrome
PTEN	Inactivates kinases	Prostate, Gliomas, Breast, thyroid, Endometrium, Cowden syndrome
WT-1	Inhibits PDGF and IGF-1	Wilms tumor
APC	Degradation of β-catenin	Colon (polyposis)
NF-1	Neurofibromin, inhibits RAS	MPNST, Gliomas
NF-2	Merlin, Contact inhibition	Gliomas, meningioma
P15 & p16	Inhibits cell cycle	
DPC4	Promotes TGF-β action	Pancreas
BAX	Proapoptosis	Colorectal
CARETAKER GENES (Genomic stability and DNA repair)		
XP	Nucleotide excision repair	Xeroderma pigmentosum skin cancer
MSH2, MLH1	DNA mismatch repair	HNPCC, ovary
ATM	Detection of DNA damage	Ataxia telangiectasia
BLM	Helicase enzyme	Bloom syndrome leukemia, gastrointestinal
FANC	Hypersensitivity of DNA to radiation	Lymphoma, Leukemia
BRCA-1 and BRCA-2	Repair of DNA double strand breaks	Hereditary breast cancer
VHL	Inhibits RNA polymerase by inhibiting elongin	Renal cell carcinoma, Hemangioblastoma, Pheochromocytoma
WRN	Genomic stability	Werner syndrome, Osteosarcoma

N.B. TSGs are written in capital letters, whereas their protein products are written in small letters proceeded by "p".

The Retinoblastoma Gene (RB-1)

The retinoblastoma gene is a model (paradigm) of tumor suppressor genes. It is located in the long arm of chromosome 13 (13q14). Loss of function of RB gene is involved, not only in retinoblastoma, but also in several other cancers.

Most retinoblastomas (90%) are sporadic, but about 10% are familial. The latter is characterized by early age of onset and frequent bilaterally. To explain these features, Knudson in 1971 proposed his *"two-hit" hypothesis*. In familial cases, one genetic change (first hit) is inherited from an affected parent (germ line mutation) and therefore present in all somatic cells. The second mutation (second hit) occurs after birth in one of the retinal cells leading to loss of heterozygosity (LOH) and tumor formation (Fig 6-30), conversely, in sporadic type of retinoblastoma, both mutations are acquired after birth in somatic cells.

The main function of retinoblastoma gene product is the control of cell cycle at G1-S interphase (Fig 6-31). Retinoblastoma protein (pRb) is a phosphoprotein, and in the hypophosphorylated state it binds with the transcription factor (E2F) leading to arrest of cell cycle at G1 phase (a molecular brake mechanism). But, with phosphorylation of pRb (as a result of mitogenic signals) the E2F transcription factor is released and induce DNA polymerase and cyclin E formation and the cell enters the S phase. The molecular brake system of Rb is lost under several pathologic conditions such as p53 mutation, RAS activation, human papilloma virus oncoprotein (E7) and mutation of Rb gene.

Patients with the inherited form of retinoblastoma are at increased risk for the development of other types of cancers later in life, particularly osteosarcoma, breast carcinoma and melanoma. These tumors arise after additional somatic mutations in their already predisposed cells.

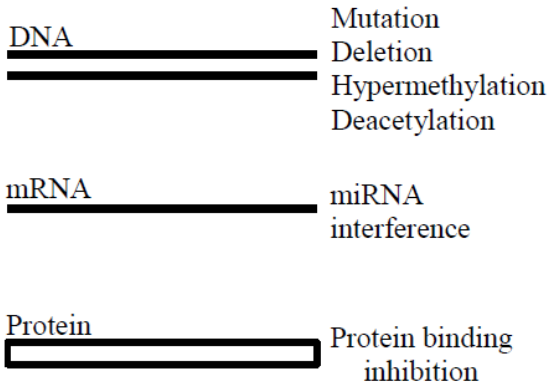

Fig 6-29 *Mechanisms of inactivation of tumor suppressor genes. It occur at three different levels, namely: DNA, mRNA and protein.*

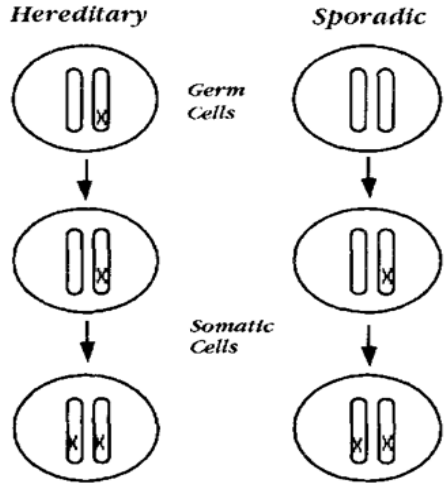

Fig 6-30 *Knudson's double-hit model of retinoblastoma. In the hereditary type of the disease mutations are both germline and somatic, but in the non-hereditary sporadic type both*

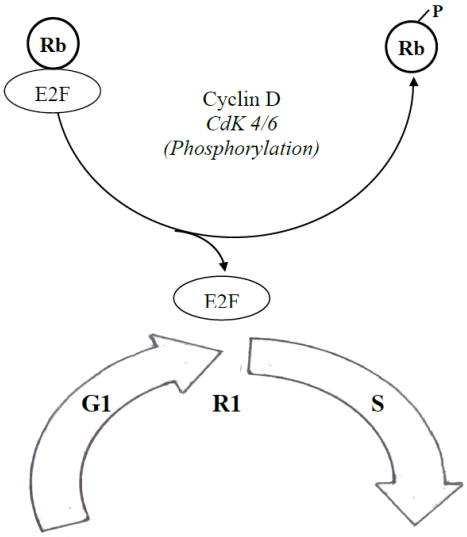

Fig 6-31 *Role of Rb protein in the control of cell cycle. Phosphorylation of RB release the transcription factor E2F protein which plays key role in regulation of cell cycle progression at G1-S interphase. Three cyclin-CDK complexes target and activate Rb protein, namely cyclin D/cdk 4/6 phosphorylates Rb during early G1, cyclin E/cdk2 during late G1 and cylin A/cdk2 during S phase.*

TP53 Tumor Suppressor Gene The Guardian of the Genome

The TP53 gene is the most frequently altered gene in malignant tumors, being inactivated in more than 50% of human cancers. Under normal conditions, wild type TP53 is involved in a number of protective reactions to DNA damage such as: cell cycle arrest, DNA repair and apoptosis (if DNA damage is irreparable), hence the gene has been called the guardian of the genome.

The term *TP53* refers to the gene, whereas, p53 refers to its protein product. The *p53* is a phosphoprotein of 53 kilodaltons (KD), hence its name. It is composed of 393 amino acids. The protein is divided into three main functional regions (Fig 6-32): an acidic amino terminus which contains the transcriptional activity of the molecule (activates transcription factors), a carboxyl terminus which controls the three dimensional structure of the molecule (p53 is only active in tetramer form), and a central region which is responsible for DNA binding. The wild-type p53 has a short half-life of only 20 to 30 minutes, hence, it is not detected by immunohistochemistry. However, the mutated p53 has a longer half life, hence it accumulates in the nucleus and could be detected by immunohistochemistry.

Wild-type TP53 is activated in response to chemicals or anoxia. Such damage is sensed by ataxia telangiectasia gene product PAT (Fig 6-33). TP53 gene is a transcriptional regulatory protein which combines both gatekeeper and caretaker functions of tumor suppressor genes. Important *target genes* controlled by p53 are the following: (1) activation of *WAF-1* which induces (p21/WAF1) protein, a strong inhibitor of cyclin dependent kinases (CDK 4, 6) leading to hypophosphorylation of pRb and arrest of cell cycle in G1-S transition, (2) transcription activation of *miRNA* resulting in an epigenetic antiproliferative effect through mRNA interference, (3) activates transcription of *GADD-45* (growth arrest DNA damage) gene responsible for DNA repair, (4) promotes *apoptosis* either through activation of proapoptosis genes (BAX, BAD, PUMA) or through genetic or epigenetic mechanisms (miRNA), (5) inhibits telomerase gene h-TERT (human telomerase reverse transcriptase), thus favoring cell mortalization, (6) activates the expression of *MDM-2* (murine double minute) gene which inactivates p53 in two ways: (a) by binding to the transcription terminus of p53 (Fig 6-33) and (b) by enhancing p53 degradation through the upiquitin proteosome pathway. This is considered a negative feed-back regulatory mechanism. Expression of p14/ARF will inhibit MDM-2, hence restoring wild p53 function (Fig 6-34), and (7) activation of expression of *thrombospondin* and inhibition of b-FGF resulting in antiangiogenic effect.

Inactivation of wild p53 is accomplished at one of three levels, namely: gene, m-RNA or protein level (Fig 6-29).

1. *Gene mutation*: missense mutation is the most common (74%), and loss of heterozygosity (loss of both gene alleles) is required for TP53 inactivation. Mutations commonly affect the central region of the gene (axons 1 to 9) leading to loss of DNA binding property of p53 (Fig 6-32). Etiolog-

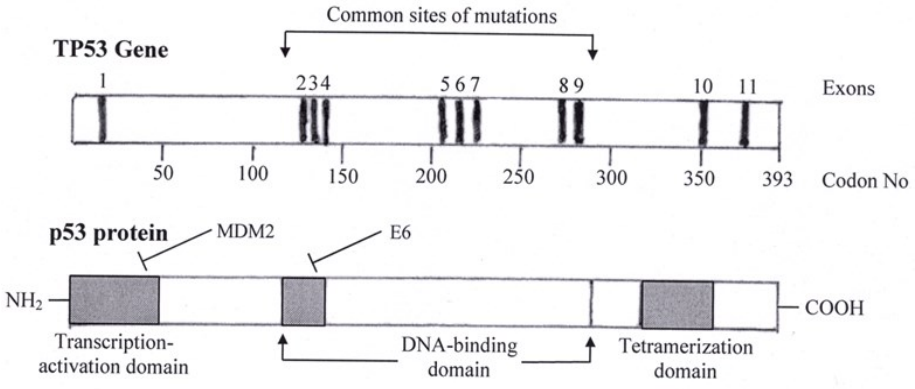

Fig 6-32 *The structure of TP53 gene and p53 protein. Shown are common sites of gene mutations, and functional zones of protein molecule, as well as, sites of its inactivation by MDM2 and E6.*

ically, the mutation may be spontaneous (deamination of cytosine), or induced by ultraviolet irradiation (pyrimidine dimers) or carcinogens (chemical adducts). *Germ line mutation* of TP53 gene may occur resulting in *Li-Fraumeni syndrome* (LFS) characterized by the development of multiple tumors in children, affecting different systems of the patient, including: soft tissue sarcomas, osteosarcomas, brain, breast and leukemias.

2. RNA level (epigenetic mechanisms): These play a significant role in p53 inactivation leading to arrest of transcription or translation of RNA. This is accomplished by one of three mechanisms: (1) hypermethylation of cytosine in gene promoter regions, which contributes to gene silencing, (2) hypoacetylation of histone leading to histone remodeling and (3) miRNA binding to mRNA leading to arrest of translation process, thus inhibiting protein formation.

3. Protein-binding inactivation: p53 inactivation at the protein level may result by one of three ways: (1) binding with *MDM-2* protein expressed by TP53, a negative autoregulation mechanism (Fig 6-32), (2) binding with E6, a HPV viral protein product (Fig 6-32), or (3) binding with a mutated p53 protein leading to loss of function of the wild p53 molecule *(dominant negative effect)*.

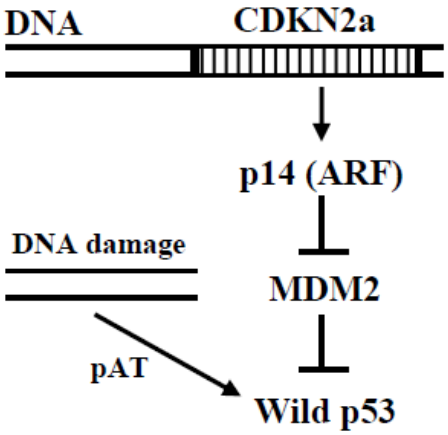

Fig 6-33 *Genetic regulation of wild (normal) TP53. DNA damage is sensed by TP 53 gene through ataxia telangiectasia (pAT) protein leading to its activation. MDM2 protein, induced by TP53, is a negative autoregulator. P14 is an inhibitor of MDM2, hence, an activator of TP53 (since, inhibition of an inhibitor is activation).*

Cell Immortalization

Telomere Dysfunction and Cancer

Telomere shortening and dysfunction promotes carcinogenesis through the generation of chromosomal rearrangement. Thus, the loss of the protective tolemere protein caps, renders these ends sticky. Chromosomes subsequently join each other by end-to-end fusion, and are subjected to breaks during mitosis. The process is repeated resulting in *breakage-fusion-bridge (BFB)* cycle with significant gene rearrangement in the form of translocations, deletions and amplification (Fig 6-34). The outcome is dependent on the integrity of TP53 and Rb genes. If these genes are intact, chromosomal breaks are sensed by TP53, mitosis is arrested, telomerase enzyme inhibited by inhibition of its gene hTERT (human telomerase reverse transcriptase) and the aberrant cells are eliminated through activation of the proapoptosis gene (BAX). Conversely, if these tumor suppressive genes (TP53 and Rb) are inactive, tumor cells will become immortal and progress to malignancy (Fig 6-34).

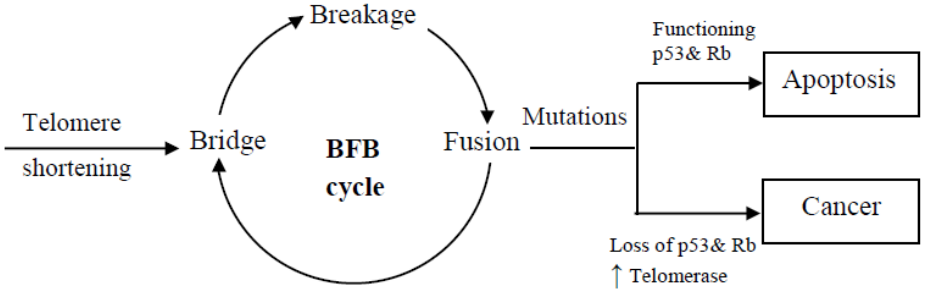

Fig 6-34 *The breakage-fusion-bridge cycle: It results from telomere shortening, leading to multiple gene rearrangement mutations and development of malignancy if repair by tumor suppressor genes (TP53 and Rb) is lacking.*

Genome Destabilization

DNA Repair Defects and Cancer

DNA repair genes (caretaker tumor suppressor genes) regularly survey DNA for any damage and repair it. The loss of these two genes functions (signaling and repair) will lead to the accumulation of mutations and development of malignancy. This is observed in five main cancer syndromes (Table 6-8), all are inherited as autosomal recessive, except HNPCC which is autosomal dominant.

1. Xeroderma pigmentosum (XP). Patients with this disease are sun-sensitive (UV) and have an extreme predisposition to different types of skin cancer, an increased incidence of perhaps 1000-fold. The disease is due to a defect in DNA excision-repair genes.

2. Hereditary nonpolyposis colon cancer (HNPCC). This is due to a defect in DNA mismatch repair genes (MMR) and is inherited as autosomal dominant. It is etiologically related to 5% of all colonic cancer (HNPCC), as well as, extracolonic cancer (Lynch syndrome). With errors in MMR genes, there is contraction or expansion of microsatellites of DNA (noncoding short repeats). Microsatellite instability is the result of misparing of nucleotides causing misalignment of DNA strands. Such microsatellite instability is a practical test of MMR defects.

3. Ataxia telangiectasia (AT). Also known as Louis-Bar syndrome, is a rare heredofamilial syndrome (autosomal recessive) with an incidence of 1/40,000 births. It is characterized by degeneration of cerebellar Purkinje neurons (ataxia), oculocutaneous telangiectasis, immunodeficiency, and mental deficiency. The etiology is related to a defect of AT protein which acts as p53 signal mechanism after DNA damage. Accordingly, these patients have increased sensitivity to radiotherapy and sun light, as well as, increased risk to develop hematolymphoid malignancies. Heterozygous carriers (1% of population) are also at risk to develop cancer, especially breast cancer, and are susceptible to ionizing irradiation.

4. Bloom syndrome: The patients show stunted growth, increased risk of infections and high risk to develop leukemia and gastrointestinal cancers.

5. Fanconi anemia; it is characterized by growth retardation, aplasia of thumbs, severe pancytopenia and high risk of leukemia and lymphoma.

6. Hereditary breast cancer. This contributes to about 5% of all breast cancers and is characterized by the early age of onset and frequent bilaterality. The molecular basis is a mutation of BRCA-1 or BRCA-2 genes, and this results in failure to repair DNA double strand breaks. The patients are also at risk of ovarian cancer.

Differentiation Arrest and Cancer

In this model, malignancy results from maturation arrest in the sequence of change from stem cell, to progenitor, to terminally differentiated cell which ultimately dies by apoptosis. According to this theory, cancer develops mainly as a result of *cell accumulation*, rather than cell proliferation. Two

Table 6-8 Hereditary Cancer Syndromes due to Defective DNA Repair

Syndrome	Repair defect	Associated malignancy
Xeroderma pigmentosum (XP)	Nucleotide excision repair	Basal cell carcinoma Squamous cell carcinoma Melanoma
Hereditary nonpolyposis colon cancer (HNPCC) Lynch syndrome	Mismatch repair	Colorectal, ovary , endometrium, gastrointestinal
Ataxia telangiectasia (ATM)	Detection of DNA damage to activate TP53 action	Lymphoma/Leukemia, stomach, breast
Bloom syndrome (BS)	Helicase enzyme	Leukemia, gastrointestinal
Fanconi anemia (FA)	Hypersensitivity of DNA to radiation and reactive oxygen	Lymphoma/Leukemia
Familial breast cancer (BRCA-1, BRCA-2)	Repair of DNA double strand breaks	Breast and ovary

cell receptors (*Notch* and *Wnt*) when activated represent a mechanism to prevent lineage specific cellular differentiation and maintains the cells in an undifferentiated stem-cell-like state. This model is probably operable in some cancers as: squamous cell carcinoma, lung carcinoma and malignant teratomas.

In some lymphomas and leukemias, cell *maturation arrest* may be accomplished through other genetic mechanisms. Thus, in *follicular* lymphoma, the translocation t(14; 18) transactivates the antiapoptotic gene *(Bcl-2)* resulting in cell accumulation. Also, in *acute promyelocytic leukemia (PML)*, translocation between chromosome 15 and 17 results in a chimeric fusion gene involving *retinoic acid receptor*. The chimeric protein product blocks myeloid differentiation at the promyelocyte stage, a process that is reversed by retinoic acid therapy.

EPIGENETIC MECHANISMS

Epigenetics (also known as epimutation) is defined as heritable modifications in gene expression that do not involve changes in DNA nucleotide sequence (contrary to mutations, epigenetic changes are reversible). It is likely that genes are more commonly affected by epigenetic than genetic mechanisms. Thus, epigenetics explains the normal differential gene expression in different tissues with silencing of unwanted genes (i.e. tissues have the same genotype but different phenotype). Epigenetics also explain the phenomenon of genomic imprinting, which refers to the relative silencing of one parental allele compared with the other parental allele. Finally, epigenetics is recognized to play an important role in activation or silencing of protooncogenes and tumor suppressor genes respectively. DNA methylation, histone acetylation and micro-RNA interference are, so far, considered the most important epigenetic mechanisms (protein phosphorylation and ubiquitin-mediated degradation are additional examples of epigenetic changes).

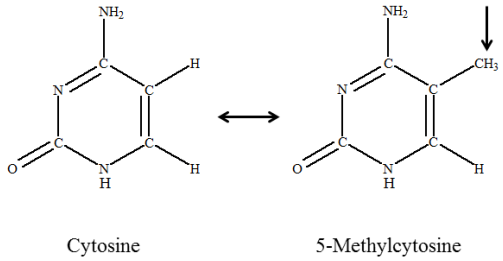

Fig 6-35 *Methylation of cytosine. It occurs at carbon atom number 5 of the ring (Arrow).*

DNA Methylation

In humans, DNA methylation occurs only in cytosines that precede guanines (called dinucleotide (CpGs). The CpG sites are not randomly distributed in the genome, instead, there are CpG-rich regions known as CpG islands. These islands are usually not methylated in normal cells. Hypermethylation of cytosine of CpG islands in gene promotor regions (Fig 6-35) will inhibit gene transcription by two mechanisms: (a) blocking the binding of transcription factors to DNA, and (b) causing histone deacetylation with subsequent chromatin remodeling, closing down the chromatin and hiding the promotor region of DNA from polymerase and transcription factors. Accordingly, DNA methylation offers a model for alteration of cancer genes activity. Thus hypermethylation inactivates tumor suppressor genes, whereas, hypomethylation activates proto-oncogenes (Fig 6-36).

Histone Acetylation Chromatin Remodeling

The promotor is the region of DNA where the enzyme DNA polymerase, with its associated transcription factor, will bind and initiate transcription of m-RNA. In the silent deacetylated state of chromatin, the nucleosomes are closely-packed or condensed, hiding DNA promoter from DNA polymerase and transcription factors, hence, the gene is inactive or silent (Fig 6-37A). These condensed areas of chromatin appear overstained and are known as heterochromatin.

Acetylation refers to the addition of acetyl group (CH_3CO). It affects lysine residues in histone molecules and is mediated by the enzyme histone acetyl transferase (HAT). This modification reduces the binding affinity of histone to DNA leading to nucleosomal dissociation with exposure of promotor regions of DNA to transcription factors (Fig 6 -37C). These acetylated areas of loose chromatin are understained and are referred to as *euchromatin*.

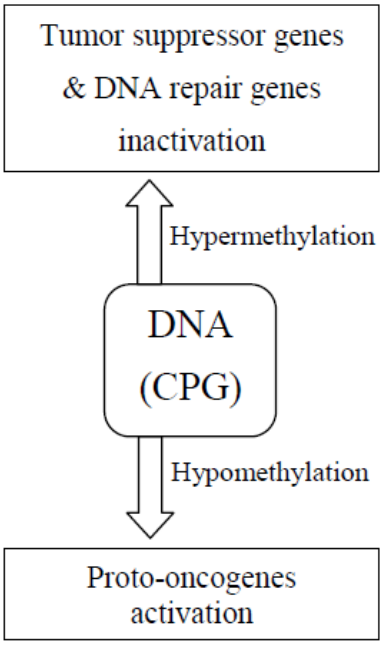

Fig 6-36 *Functional outcomes of DNA methylation on cancer genes. Hypermethylation inactivates tumor suppressor genes, whereas, hypomethylation activates protoncogenes.*

Chromatin remodeling is the epigenetic mechanism involved in *steroid hormonal action*. The classical estrogen signaling pathway is presented in (Fig 6-37B). Estradiol diffuses through the cell membrane and binds to cytoplasmic estrogen receptor (ER). The latter is an estrogen-dependent transcription factor that has two domains in its molecule, one for binding to an activator (with histone acetyl transferase HAT activity), and another for binding with DNA.

The binding of estrogen to ER results in its phosphorylation and conformational change in its shape rendering it more active in DNA binding. The activated complex is then translocated into the nucleus where it binds to the hormone receptor element (HRE) of DNA promotor (Fig 6-37C). The activated HAT will acetylate histone, resulting in chromatin remodeling and exposure of promotor to transcription factors. Genes that are regulated by the activated ER include: early gene responses (c-myc, c-fos, and c-jun), growth factors (IGF and EGF) and antiapoptotic factors (bcl- 2). The antagonistic ligand (e.g. antiestrogen tamoxifen) binds to ER, changing its active conformational shape, inhibiting its DNA binding and hence blocking estrogen effect.

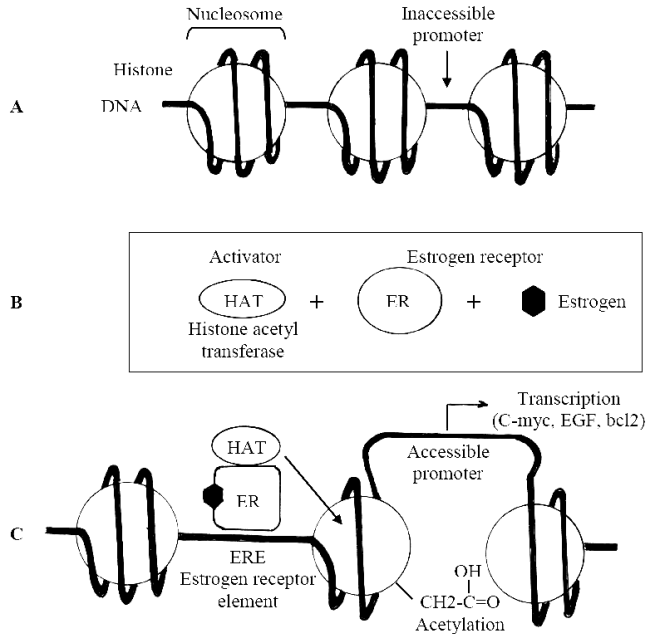

Fig 6-37 *Chromatin remodeling through histone acetylation and the epigenetic mechanism of estrogen action. (A) In the deacetylated state DNA promotor is inaccessible to transcription factors, hence, no transcription. (B) Estrogen receptor (ER) is a steroid-dependent transcription factor that also binds to the activator enzyme HAT. (C) ER-estrogen-HAT complex causes acetylation of histone with chromatin remodeling exposing DNA promotor to transcription factors and starting transcription.*

Micro RNA (miRNA)

Micro RNAs are short (22-nucleotides) noncoding RNAs that target mRNA. The result is mRNA degradation and inhibition of translation (arrest of protein synthesis). MiRNA is originally transcribed by the microgene as double strand (hairpin), then processed in the cytoplasm as single strands. One strand targets mRNA resulting in Blocking of translation (Fig 6-38). In this way, overexpression of miRNA inhibits tumor suppressor proteins, whereas, underexpression of miRNA will increase oncoprotein products. Micro RNA expression is controlled by the methylation status of the regulatory region of microgene.

AN ECLECTIC VIEW

This means to choose what seems to be the best from different doctrines or theories. Thus, different theories are not mutually exclusive, hence, they may complement rather than antagonize each other. Following this concept, Feinberg (2004) proposed that both genetic and epigenetic mechanisms complement each other in carcinogenesis. Thus, genetic changes leads to initiation, whereas, epigenetic changes lead to progression of cancer. This complementary role is supported by recent molecular studies, since in some tumor suppressor genes (e.g. MLH1 and p16) one allele is inactivated by mutation, and the other allele is silenced by hypermethylation.

MOLECULAR TARGETED THERAPY

The recent understanding of molecular cancer biology, combined with technological advances, has led to the development of agents that target proteins or RNA essential for cancer growth, differentiation, survival and spread. Tumor growth inhibition is achieved mainly through control of cell cycle in 3 ways: (a) inhibition of mitogenic growth factors, (b) inhibition of transcription factors or (c) accumulation of the inhibitors of cell cycle through inhibition of their proteosomal degradation. Moreover, survival of tumor cell population is shortened by stimulating terminal differentiation or apoptosis. The spread of malignant cells by invasion and metastases is reduced through inhibition of angiogenesis.

An ideal targeting agent should act selectively on malignant cells and spare normal cells (wide therapeutic index), and should preferably affect multiple targets. Also, an ideal target is the one involved in several aspects of carcinogenesis (e.g. NFκB and proteosome).

Targeted therapies are classified in two ways, namely: (a) according to their biochemical struc-

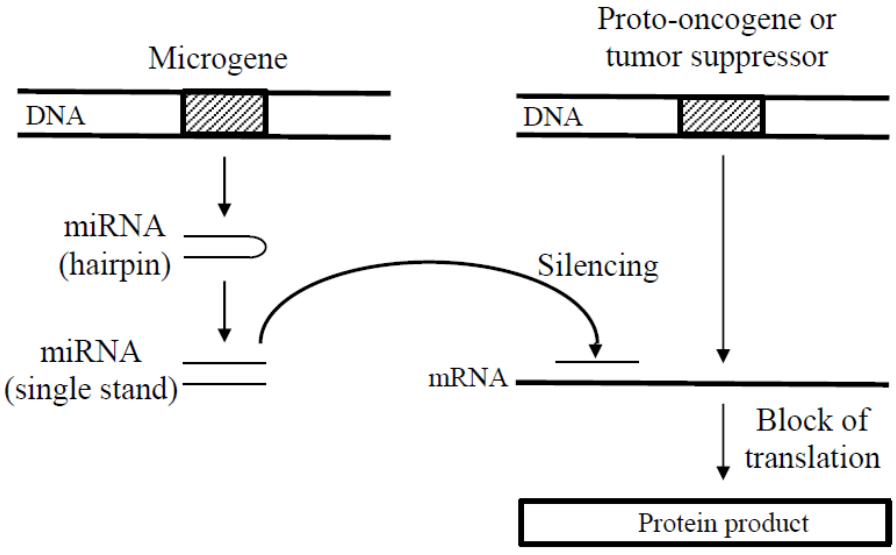

Fig 6-38 *Micro RNA (miRNA) interference (silencing) of gene expression. This is accomplished by binding and inactivating mRNA, hence arrest of protein synthesis. Overexpression of miRNA inhibits tumor suppressor genes, whereas, underexpression, will increase oncoprotein product.*

Table 6-9 Approved Targeted Therapy for Cancer Treatment

Generic (Trade) Name	Target	Malignancy
Small molecules		
Imatinib (Gleevec)	c-kit, PDGFR, JAK-2	CML, GIST, polycythemia
Gefitinib (Iressa)	EGFR	NSCLC lung
Bortezomib (Velcade)	Proteasome	Multiple myeloma
Denileukin difitox (ontak)	CD25	T-cell lymphoma
(Tamoxifen)	ER	Breast
Sorafenib, Sunitinib	BRAF, EGFR VEGFR, FLT-3	Renal cell carcinoma, Melanoma
Monoclonal antibodies		
Rituximab (Rituxan)	CD20	B-cell NHL
Tositumomab (Bexxar)	CD20	
^{131}I-conjugate		B-cell NHL
Trastuzumab (Herceptin)	HER-2	Breast
Bevacizumab (Avastin)	VEGF	Colorectal
Cetuximab (Erbitux)	EGFR	Colorectal
Gemtuzumab (Mylotarg)	CD33	AML

ture (Table 6-9) into small molecule inhibitors and monoclonal antibodies moAb (free or conjugated to a toxin, radioisotope or nanoparticle), or (b) according to the mechanism of action on specific sites of signal transduction pathways. The following are ten illustrative examples.

1. Ligand inactivation: A monclonal antibody will unite with the ligand, blocking its ATP binding site, rendering it inactive in binding with surface receptor (e.g. Bevacizumab targeting of VEGF in colorectal cancer).

2. Receptor inactivation: This could be accomplished by tyrosine kinase inhibitor (TKI) or monoclonal antibodies which will unite and inactivate the extracellular domain of the receptor (e.g. moAb cetuximab targeting EGFR in colorectal cancer) or a chimeric cytoplasmic oncoprotein (TKI imatinib targeting Bcr-Abl in chronic myeloid leukemia).

Another receptor tyrosine kinase is JAK-2 which acts through JAK-STAT signaling pathway. This is a potential target of TKI therapy in myelodysplastic syndromes and myeloproliferative neoplasms.

Her-2 is another receptor tyrosine kinase which does not have its own ligand, but instead, when mutated it prefers to dimerize with other HER family members with intact receptor, with subsequent activation of downstream MAPK and PI-3K signal pathways (Fig 6-39). HER-2 may be inactivated in two ways: (a) binding of monoclonal antibodies (e.g. Herceptin) to its surface receptor, or (b) small molecule thyrosine kinase inhibitor of downstream pathways (Fig 6-39).

RAS is a membrane-associated oncogene which uses guanosine nucleotides to transfer its signal. A critical post-translation of RAS protein activation (farnesylation) is required for its function. This is accomplished by the enzyme farnesyltransferase. Anti RAS agents include farnesyltransferase inhibitor (FTI) will abolish RAS function as an oncogene. Also, it was noted that the Rotavirus replicates selectively and eventually kills RAS-mutated cancer cells and spares normal cells. This led to the development of Reolysin as an anti -RAS agent. Finally, BRAF (a serine/threonine kinase) is a member of the RAF family, a component of RAS downstream signal pathway (Fig 6-1). BRAF mutation is frequent (70%) in melanoma, but the end results of its targeted therapy (Sorafenib) are so far limited.

3. Antitranscription factors: The estrogen receptor (ER) is considered a steroid dependent transcription factor (Fig 6-37). The antagonistic ligand (Tamoxifen), binds to ER, resulting in a change in its conformational structure, becomes unable to bind to estrogen or DNA, resulting in arrest of transcription, with loss of the mitogenic and antiapoptotic effect of estrogen.

Another example is NF-κB, which is a multi-

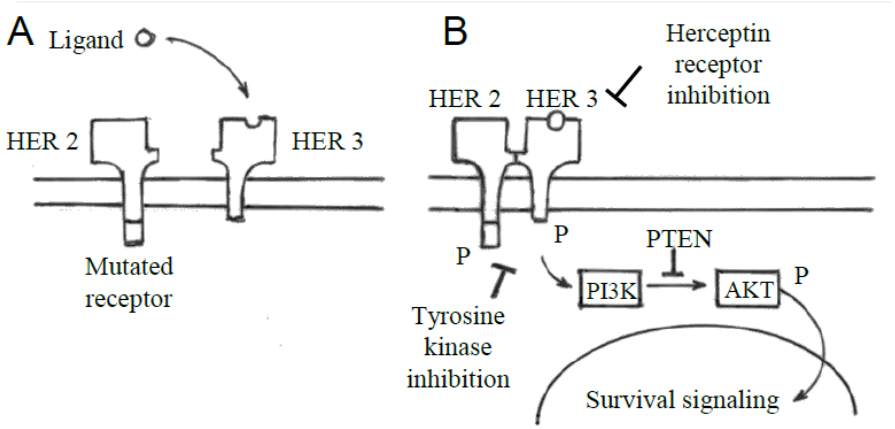

Fig 6-39 *HER-2 signaling and its targeted therapy. (A) Mutated HER-2 is incapable of binding to its ligand due to lack of a receptor function, (B) HER-2 dimerises with HER-3 which has intact receptor to bind the ligand and start signaling.Targeted therapy may be directed to the receptor (monoclonal antibodies, Herceptin) or to the signal transduction pathway (tyrosine kinase inhibitors, Sorafenib and Sunitnib).*

functional transcription factor (mitogenic antiapoptotic and angiogenic), hence, it is considered an ideal target for molecular therapy. NF-κb is inhibited by nonsteroidal anti-inflammatory drugs (NSAID), as well as, by a specific agent a repressor protein (IKB). Inhibition of IKB degradation by the proteosome is another potential approach to increase the cytoplasmic content of IKB and hence inhibits NF-κb.

4. Antitranscription mechanisms (epigenetic modifications): Epigenetics (cytosine methylation and histone acetylation) play a major role in gene transcription through their action in exposing DNA promotor to the transcription factor and influencing its binding capacity (Fig 6-37). Since epigenetic changes are reversible, they offer ideal targets for cancer therapy. For example, since DNA hypermethylation results in loss of tumor suppressor gene function, hypomethylation will have a therapeutic value. Demethylation is accomplished by the use of antisense oligonucleotides. Azacitidine and decitabine are two potent DNA demethylating agents approved for treatment of myelodysplastic syndromes and leukemias. Important target for this strategy is the tumor suppressor gene (CDKN2 A) which codes for p16, a major inhibitor of the cell cycle.

In a similar epigenetic way, proto-oncogenes are activated by histone acetylation which results in chromatin remodeling, exposing DNA promotors for transcription (Fig 6-37). Conversely, deacetylating agents which inhibit histone acetylase enzyme will inhibit gene expression. However, clinical application must await the development of safe agents in the future.

5. Antitranslation agents: Agents that can block the translation of mRNA to proteins use antisense oligonucleotides (ASON) to target either miRNA or mRNA. ASON is a synthetic DNA molecule with a nucleotide sequence complementary to the normal target mRNA (the sense molecule). The binding of ASON to RNA forms a hybrid RNA-DNA duplex that inhibits the translation process. Trials of this targeted therapy were made on CLL and melanoma with rather limited clinical success.

6. Proteasome inhibitors: The proteasome is the main mechanism of protein degradation, including proteins that promote apoptosis (BAX and BAD) or inhibit the cell cycle (p16, p21 and p27). Accordingly, proteosomal enzyme inhibition will result in accumulation of proapoptotic and cyclin-dependent kinase (CDK) inhibitors. The proteasome-inhibitor Bortezomib is an approved agent for the treatment of multiple myeloma or non-Hodgkin lymphoma.

7. Restoration of tumor suppressor gene (TSG) function: Regain of normal function (e.g. wild p53) is more difficult to accomplish than inhibiting an aberrant function (e.g. oncogene inactivation). Such restoration of TSG activity, if successful, will regain cell population and genomic stability. Two approaches were adopted to restore p53 function, namely: (a) to deliver a normal TP53 gene by gene therapy using adenovirus as a vector, and (b) selective destruction of tumor cells carrying TP53 mutation by introducing a genetically engineered ade-

novirus, which replicates and ultimately destroys only mutant cells and spares cells with normal TP53 gene.

8. Differentiation-inducing agents: This therapy will ultimately direct malignant cells to death by apoptosis (terminal differentiation). The main role of differentiation agents (mainly derivatives of vitamin A, retinoids) is in chemoprevention and treatment of minimal residual disease. Examples are: (a) the use of All trans-retinoid acid (ATRA) in acute premyelocytic leukemia (APML), (b) the use of bexarotene (retinoid x receptor agonist) in cutaneous T-cell lymphoma, and (c) the use of 13-cis retinoid acid in neuroblastoma, and chemoprevention of basal cell carcinoma in xeroderma pigmentosa patients.

9. Apoptosis-inducing agents: Apoptosis is an important mechanism of chemotherapy drug action to eliminate cells and reduce tumor population. Bcl-2 is an antiapoptotic oncogene, hence, its inhibition will promote apoptosis and chemotherapy effect on tumors. Antisense drugs (antisense-Bcl-2 drug ABT-737) is under clinical trials in follicular non-Hodgkin lymphoma and small cell lung cancer. Recently, the new retinoid fenretinide has a pro-apoptotic effect, used in neuroblastoma and Ewing sarcoma.

10. Antiangiogenic agents: Angiogenesis is an essential biologic phenomenon for tumor invasion and metastasis, to provide nutrition and oxygen supply for the growing cells. Thus, tissue hypoxia activates tumor and stromal cells to secrete a growth factor, vascular endothelial growth factor (VEGF), which binds with receptors (VEGFR) on endothelial cells leading to their proliferation (a process needing receptor tyrosine kinase activity). This signal pathway could be blocked by two therapeutic approaches, namely: (a) monoclonal antibodies against VEGF (Avastin), or (b) tyrosine kinase inhibitors (TKI) of the receptor VEGFR (Sunitinib and Sorafenib). The first approach is called antiligand, whereas the second approach is antiendothelial. Therapeutic outcome is, so far, transient without long-term survival benefit.

REFERENCES

Cagle PT and Allen TC (editors): Basic Concepts of Molecular Pathology, Springer, Heidelberg, New York, 2009.

Coleman WB and Tsongalis GJ: Molecular Pathology, the Molecular Basis of Human Disease. Elsevier, AP, Amsterdam, 2009.

Devita VT, Lawrence TS and Rosenberg SA (editors): Cancer, Principles and Practice of Oncology, 9th edition Wolters Kluwer / Lippincott Williams and Wilkins, Philadelphia, 2011.

Esteller M: Epigenetics in cancer (review article), N Engl J Med, 358: 1148-1159, 2011.

Hanahan D, and Weinberg RA (review article): The hallmarks of cancer, Cell, 100: 57-70, 2000.

Kumar V, Abbas AK, Fausto N and Aster JK: Pathologic Basis of Disease. 8th edition, Saunders Elsevier, Philadelphia, 2010.

Mokhtar N: Molecular Pathology of Cancer. NCI, Cairo, 1998.

Nussbaum R, Mclnnes R, and Willard H (editors): Genetics in Medicine. Saunders, Elsevier, Philadelphia, 2007.

Tannock IF, Hill RP, Bristow RG, and Harrington L (editors): The Basic Science of Oncology, 4th edition, Me