THE ETIOLOGY OF CANCER

The cause of cancer has been a focus of scientific researches for over half a century.

Evidences now indicate that for a large number of cancer types, there exist not only environmental influences but also hereditary predisposition.

Hereditary forms of cancer can be divided into 3 categories:

a. **Inherited cancer syndromes**: whereby, inheritance of a single mutagen (i.e. an agent that causes mutation) greatly increases the risk of developing a tumor. The predisposition to these tumors shows an **autosomal dominant type of inheritance**. Examples include
   - Retinoblastoma.
   - Familial adenomatous polyps.

b. **Familial cancers**. Many known types of cancer are included here whereby tumors
   - Occur in an early age.
   - Arise in two or more relatives.
   - Are sometimes multiple or bilateral.

The predisposition is either dominant or multifactorial (*multifactorial: multiple factors*). Sometimes tumors are linked to certain genes such as the linkage of BRCA-1 & BRCA-2 to familial breast & ovarian cancers. *(BRCA stands for breast carcinoma)*

c. **Autosomal recessive syndromes of defective DNA repair** e.g. xeroderma pigmentosa(um).
THE MOLECULAR BASIS OF CANCER

1. **Non-lethal genetic damage** lies at the heart of carcinogenesis. **This damage (mutation) may be**
   A. **Acquired** by environmental factors such as
      - Radiation,
      - Chemical substances
      - Viruses
   B. **Inherited** in the germ line cells.

2. The tumor mass is the result of **clonal expansion of a single progenitor (precursor) cell** that incurred the genetic damage. When such tumors are analyzed in women who are heterozygous for x-linked markers, they are made up of cells that contain the active maternal $X_A$ or the paternal $X_B$ chromosomes but not both.

   *Progenitor cell is that from which another cell or a family of cells is descended, (an ancestor; a parent).*

   The most commonly used method to determine tumor clonality involves the analysis of methylation patterns adjacent to the highly polymorphic locus of the human androgen receptor gene, $AR$. The frequency of such polymorphisms in the general population is more than 90%, so it is easy to establish clonality by showing that all the cells in a tumor express the same allele. For tumors with acquired cytogenetic aberrations of any type (e.g., a translocation) their presence can be taken as evidence that the proliferation is clonal. Immunoglobulin receptor and T-cell receptor gene rearrangements serve as markers of clonality in B- and T-cell lymphomas, respectively.

3. **Involvement of normal regulatory genes.** Four classes of normal regulatory genes are involved in carcinogenesis;
   a. The growth promoting proto-oncogenes.
   b. The growth inhibiting cancer suppressor genes.
   c. Genes that control programmed cell death. *The programmed cell death is termed apoptosis.*

These three types of genes are the principal targets of genetic damage. Mutant alleles of the first group are considered dominant because they transform cells despite the presence of a normal counterpart. However, loss of a single allele of a tumor suppressor gene sometimes reduces levels or activity of the protein enough that the brakes on
cell proliferation and survival are released. Loss of gene function caused by
damage to a single allele is called haploinsufficiency. Such a finding
indicates that dosage of the gene is important, and that two copies are
required for normal function. The third group may act in both ways, i.e. may
behave as proto-oncogenes or tumor suppressor genes.

d. Genes regulating repair of DNA damage. A forth category of genes
are those that regulate repair of damaged DNA. These affect cell
proliferation or survival indirectly by influencing the ability of the
organism to repair non-lethal damage in other genes. Both alleles must
be inactivated to induce genomic instability. In this respect, they can be
considered as tumor suppressor genes. A disability in the DNA-repair
genes can predispose cells to widespread mutations in the genome and
thus to neoplastic transformation. Cells with mutations in DNA repair
genes are said to have developed a mutator phenotype. Interestingly, a
new class of regulatory molecules, called microRNAs (miRNAs), has
recently been discovered. Even though they do not encode proteins,
different families of miRNAs have been shown to act as either
oncogenes or tumor suppressors. They do so by affecting the translation
of other genes.

4. Carcinogenesis is a multi-step process at both the phenotypic and the
molecular (genetic) levels.

At the phenotypic level, excessive growth, local invasiveness & distant
metastasis are acquired in a stepwise fashion so that over a period of
time many tumors become more aggressive and acquire greater
malignant potential, a phenomenon called tumor progression.
(Phenotypic: pertaining to the observable features of organisms)

At the genetic (molecular) level, these features are due to accumulation
of genetic lesions that are favored or facilitated by defects in the DNA
repair.

Every cancer reveals multiple genetic alterations involving activation of
several oncogenes & loss of tumor suppressor genes. Tumor
progression and associated heterogeneity result from multiple mutations
that accumulate independently in different cells, generating subclones
with varying abilities to grow, invade, metastasize, and resist (or
respond to) therapy. Some of the mutations may be lethal; others may spur cell growth by affecting additional proto-oncogenes or tumor suppressor genes. *Even though most malignant tumors are monoclonal in origin, by the time they become clinically evident their constituent cells are extremely heterogeneous.* During progression, tumor cells are subjected to immune and nonimmune selection pressures. For example, cells that are highly antigenic are destroyed by host defenses, whereas those with reduced growth factor requirements are positively selected. A growing tumor therefore tends to be enriched for subclones that “beat the odds” and are adept at survival, growth, invasion, and metastasis. Genes that seem to regulate the entry into the multistep carcinogenesis pathway are called *gatekeeper genes*. Those that affect genomic stability are called *caretaker genes*. Their loss leads to increase mutations of all genes including the gatekeepers.

**FEATURES (PROPERTIES) OF TRANSFORMED CELLS:**

On cell culture, one can differentiate normal cells from those undergoing transformation as follows:

1. **Density independent growth.** The tumor cells are usually density independent in their growth. Instead of producing confluent monolayer on tissue culture (as is the case with normal cells), they continue to pile up on top of each other.

2. **Anchorage independent growth** i.e. there is no attachment to solid support such as plastic surface. Transformed cells grow in suspension in a semisolid medium like soft agar.

3. **Immortality.** Fetal fibroblasts undergo 50 divisions before they senesce (*grow old*). Transformed cells continue on dividing.

4. **Decreased dependence on exogenous growth factors.** They produce their own growth factors leading to autocrine stimulation of growth i.e. respond to growth factors that they secrete.

5. **In vivo tumorigenicity.** When used in suspension, they produce tumors in nude mice.

6. **Formation of blood vessels (capillaries).**

7. **Metastasis.**

Upon applying those features on malignant cells we can summarize the essential alterations for malignant transformation as follows:
• **Self-sufficiency in growth signals:** Signalling in between cell could be one of the folliwings:
  1. **Autocrine:** cells respond to signaling substances that they secrete (growth factors). The cell produces a growth factor & its receptor leading to an autocrine loop.
  2. **Paracrine:** cells produce molecules that affect target cells in vicinity, such as what happens between macrophages & fibroblasts.
  3. **Endocrine:** substances are produced in endocrine glands & affect distant organs.

Tumors have the capacity to proliferate without external stimuli (autocrine), usually as a consequence of oncogene activation.

• **Insensitivity to growth-inhibitory signals:** Tumors may not respond to molecules that are inhibitory to the proliferation of normal cells such as transforming growth factor-β (TGF-β), and direct inhibitors of cyclin-dependent kinases.

• **Evasion of apoptosis:** Tumors may be resistant to programmed cell death, as a consequence of inactivation of p53 or other changes.

• **Defects in DNA repair:** Tumors may fail to repair DNA damage caused by carcinogens or unregulated cellular proliferation.

• **Limitless replicative potential:** Tumor cells have unrestricted proliferative capacity, associated with maintenance of telomere length and function.

• **Sustained angiogenesis:** Tumors are not able to grow without formation of a vascular supply, which is induced by various factors, the most important being vascular endothelial growth factor (VEGF).

• **Ability to invade and metastasize:** Tumor metastases are the cause of the vast majority of cancer deaths and depend on processes that are intrinsic to the cell or are initiated by signals from the tissue environment.

• **Defects in DNA repair:** Tumors may fail to repair DNA damage caused by carcinogens or incurred during unregulated cellular proliferation, leading to genomic instability and mutations in proto-oncogenes and tumor suppressor genes.
In order to understand what happens in transformation of cells to malignant ones, we must think of the normal micro physiology of cells.

The sequence of events that take place during normal cell growth include:

1. The binding of growth factor to its specific receptor on the cell membrane.
2. Transient & limited activation of the growth factor receptor which in turn activates several signal transducing proteins on the inner leaflet of the plasma membranes.
3. Transmission of the transduced signal across the cytosol to the nucleus via second messengers.
4. Induction & activation of nuclear regulatory factors that initiate DNA transcription.
5. Entry & progression of the cell into the cell cycle resulting ultimately in cell division.

**ONCOGENES**

**Oncogenes** are derived from proto-oncogenes; these are cellular genes that promote normal growth & differentiation. They were first discovered in retroviruses (RNA viruses containing reverse transcriptase enzyme), which can produce DNA molecules on an RNA template.

During evolution, these genes are transduced (captured) by the virus through a chance of recombination of DNA of normal host, infected by the virus. The designation v-onc indicates viral oncogene & c-onc indicates cellular oncogene.

**Transformation of proto-oncogenes to oncogenes:**

Transformation of proto-oncogenes to oncogenes takes place through the following ways:
1. **Proto-oncogenes may be damaged** & thus activated by extracellular events such as radiation or contact with a chemical carcinogen that lead to point mutations in the gene sequence.

2. They may be **damaged as they are translocated from one DNA position to another** when they are carried or transduced by viruses (v-onc).

3. They may be excessively **activated by unfamiliar genes lying adjacent to them** following translocation e.g. translocation of myc proto-oncogene from the long arm of chromosome 8 to the long arm of chromosome 14. This translocation if takes place in a B lymphocyte renders the myc gene becoming near the gene of immunoglobulin heavy chain and under its promoter. The immunoglobulin heavy chain gene is active all the time in a B cell to produce antibodies. There will be excessive activation of c-myc rendering it becoming oncogenic. This genetic abnormality is found in certain B cell lymphoma (Burkitt’s lymphoma).

4. There may be an **amplification of the oncogene** itself i.e. the neoplastic cell DNA may contain multiple copies of the same oncogene either in the form of homogenously stained region (HSR) or double minutes (DM), as what happens in N-myc gene in neuroblastoma. Both HSRs and DMs can be transcribed and expressed into the encoded protein N-myc.

5. Combination of more than one process could occur even in a single gene.

**ONCOPROTEINS**

These are the protein products of oncogenes. They are required for self-sufficiency in growth signals.

They include:

1. **Growth factors**: mutations of genes that encode growth factors render them oncogenic e.g. c-sis encodes the Beta- chain of platelet-derived growth factor (PDGF). Cancer cells acquire the ability to synthesize the same growth factors to which they are responsive, generating an autocrine loop. Many glioblastomas secrete platelet-
derived growth factor (PDGF) and express the PDGF receptor, and many sarcomas make both transforming growth factor α (TGF-α) and its receptor. Growth factor driven proliferation contributes to the malignant phenotype by increasing the risk of spontaneous or induced mutations in the proliferating cell population but is not sufficient for neoplastic transformation. Cells could be forced to secrete large amounts of growth factors by products of other oncogenes that lie along many signal transduction pathways, such as RAS which causes overexpression of growth factor genes.

2. **Growth factor receptors**: The oncogenic versions of these receptors are associated with persistent activation without binding to growth factor, delivering continuous mitogenic signals to the cell. They become oncogenic due to mutation, gene rearrangement and overexpression. e.g. receptors for EGF. Two members of the epidermal growth factor (EGF) receptor family are the best described. The normal form of ERBB1, the EGF receptor gene, is overexpressed in up to 80% of squamous cell carcinomas of the lung, in 50% or more of glioblastomas and in 80% to 100% of head and neck tumors. The ERBB2 gene (also called HER-2/NEU), the second member of the EGF receptor family, is amplified in approximately 25% of breast cancers and in human adenocarcinomas arising within the ovary, lung, stomach, and salivary glands. Because the molecular alteration in ERBB2 is specific for the cancer cells, new therapeutic agents consisting of monoclonal antibodies specific to ERBB2 have been developed and are currently in use clinically, providing an example of targeted therapy.

Greater than 90% of gastrointestinal stromal tumors have a constitutively activating mutation in the receptor tyrosine kinase c-KIT or PDGFR, which are the receptors for stem cell factor and PDGF, respectively. These mutations are amenable to specific inhibition by the tyrosine kinase inhibitor imatinib mesylate.

3. **Signal transducing proteins**: most of these are located in the inner leaflet of the plasma membrane where they receive signals from outside the cell & transmit them to the nucleus. Normal RAS proteins are tethered to the cytoplasmic aspect of the plasma membrane and can be activated by growth factor binding to receptors at the plasma membrane. (Fig-8) It is a member of a family of small G proteins that bind guanosine nucleotides (guanosine triphosphate, GTP and guanosine diphosphate, GDP). RAS proteins flip back and forth between an excited signal-transmitting state and a quiescent state. In the inactive state, RAS proteins bind GDP. Stimulation of cells by growth factors leads to exchange of GDP for GTP and
subsequent conformational changes that generates active RAS. The activated RAS stimulates downstream regulators of proliferation, such as the *mitogen-activated protein (MAP) kinase cascade*, which floods the nucleus with signals for cell proliferation. Several distinct point mutations of RAS have been identified in cancer cells. The affected residues lie within either the GTP-binding pocket or the enzymatic region essential for GTP hydrolysis, and thus markedly reduce the GTPase activity of the RAS protein. Mutated RAS is trapped in its activated GTP-bound form, and the cell is forced into a continuously proliferating state.

**Alterations in non-receptor-associated tyrosine kinases** which normally function in signal transduction pathways that regulate cell growth could be due to chromosomal translocations or rearrangements that create fusion genes encoding constitutively active tyrosine kinases. An important example of this oncogenic mechanism involves the c-ABL tyrosine kinase. In CML and some acute lymphoblastic leukemias, the *ABL* gene is translocated from its normal abode on chromosome 9 to chromosome 22 where it fuses with the *BCR* gene. The resultant chimeric gene encodes a constitutively active, oncogenic *BCR-ABL tyrosine kinase*.

4. **Nuclear transcription proteins**: DNA replication & cell division are regulated by genes whose products are localized to the nucleus where they control the transcription of growth related genes. The transcription factors contain specific amino acid sequences that allow them to bind DNA e.g. *c-myc*. In contrast to the regulated expression of *MYC* during normal cell proliferation, persistent expression, and in some cases overexpression, of the MYC protein are commonly found in tumors. Dysregulation of *MYC* expression resulting from translocation of the gene occurs in Burkitt lymphoma, a B-cell tumor. *MYC* is amplified in some cases of breast, colon, lung, and many other carcinomas. The related N-*MYC* and L-*MYC* genes are amplified in neuroblastomas and small-cell cancers of the lung, respectively.

The *MYC* proto-oncogene is expressed in virtually all eukaryotic cells and belongs to the immediate early response genes, which are rapidly induced when quiescent cells receive a signal to divide. After a transient increase of *MYC* messenger RNA, the expression declines to a basal level. The molecular basis of MYC function in cell replication is not entirely clear. As with many transcription factors, it is thought that MYC is involved in carcinogenesis by activating genes that are involved in proliferation. MYC interacts with components of the DNA-replication machinery, and plays a role in the selection of origins of replication. Thus, overexpression of MYC may drive activation of more origins than needed for normal cell division, or bypass checkpoints involved in replication, leading to genomic damage and accumulation of mutations. It is regarded as one of a handful
of transcription factors that can act in concert to reprogram somatic cells into pluripotent stem cells. MYC may also enhance self-renewal, block differentiation, or both.

5. **Cyclines & cycline-depending kinases**: the orderly progression of cell through the various phases of cell cycle is orchestrated by cycline-dependent kinase (CDKs), which are activated by binding to cyclins, so called because of the cyclic nature of their production and degradation. The CDK-cyclin complexes phosphorylate crucial target proteins that drive the cell through the cell cycle. On completion of this task, cyclin levels decline rapidly. The cyclin D genes are overexpressed in many cancers, including those affecting the breast, esophagus, liver, and a subset of lymphomas. Amplification of the CDK4 gene occurs in melanomas, sarcomas, and glioblastomas. More than 15 cyclins have been identified; cyclins D, E, A, and B appear sequentially during the cell cycle and bind to one or more CDK. The cell cycle may thus be seen as a relay race in which each lap is regulated by a distinct set of cyclins, and as one set of cyclins leaves the track, the next set takes over.

Inhibitors of the cycline-CDK complexes are also important in the cell cycle (e.g. p21). They are regarded as cancer suppressor genes.

**Cell cycle checkpoints:**

There are two main cell cycle checkpoints, one at the G₁/S transition and the other at G₂/M. The S phase is the point of no return in the cell cycle. Before a cell makes the final commitment to replicate, the G₁/S checkpoint checks for DNA damage; if damage is present, the DNA-repair machinery and mechanisms that arrest the cell cycle are put in motion. The delay in cell cycle progression provides the time needed for DNA repair; if the damage is not repairable, apoptotic pathways are activated to kill the cell. Thus, the G₁/S checkpoint prevents the replication of cells that have defects in DNA, which would be perpetuated as mutations or chromosomal breaks in the progeny of the cell. DNA damaged after its replication can still be repaired as long as the chromatids have not separated. The G₂/M checkpoint monitors the completion of DNA replication and checks whether the cell can safely initiate mitosis and separate sister chromatids. This checkpoint is particularly important in cells exposed to ionizing radiation. Cells damaged by ionizing radiation activate the G₂/M checkpoint and arrest in G₂; defects in this checkpoint give rise to chromosomal abnormalities. To function properly, cell cycle checkpoints require sensors of DNA damage, signal transducers, and effector molecules. The sensors and transducers of DNA damage seem to be similar for the G₁/S and G₂/M checkpoints. They include, as sensors, proteins of the RAD family and ataxia telangiectasia mutated (ATM) and as transducers, the CHK kinase.
families. The checkpoint effector molecules differ, depending on the cell cycle stage at which they act. In the G1/S checkpoint, cell cycle arrest is mostly mediated through p53, which induces the cell cycle inhibitor p21. Arrest of the cell cycle by the G2/M checkpoint involves both p53-dependent and p53-independent mechanisms. Defects in cell cycle checkpoint components are a major cause of genetic instability in cancer cells.

VIRAL CARCINOGENESIS

Most of the viruses that participate in cancer production in human are DNA viruses. In order for the cell to become transformed by a virus, it must sustain the infection healthily. (Of course, when it undergoes a lytic cycle of virus infection the cell dies i.e. there is no cancer).

DNA oncogenic viruses: Some of these viruses contain oncogenic sequences like human papilloma virus. Others like hepatitis B virus & Epstein Bar virus (EBV) do not contain oncogenic sequences so they act indirectly. The DNA virus must be integrated in the DNA of the host cell. Early genes (containing the promoters & core protein genes) must be integrated. Late genes (coat protein genes) are excluded. When these genes are integrated they code for the production of transforming proteins, which bind to cellular proteins that regulate growth.

RNA oncogenic viruses: All RNA viruses involved in carcinogenesis are retroviruses i.e. they contain the enzyme reverse transcriptase. The latter helps in DNA synthesis by these viruses (using their genomic RNAs as templates).

Retroviral genome contain:

1. The gag region coding for virion core proteins.
2. The pol region coding for reverse transcriptase enzyme.
3. The env region coding for the envelope glycoprotein.

These are bound at each side by long terminal repeats (LTRs) which are untranslated sequences that contain promoters & enhancer sequences for the synthesis of adjacent viral RNA.

There are three types of retroviruses depending on their transforming activities & genomic structure:
1. **Acute transforming viruses**: These produce tumors in animals & transform cells in vitro. All members (except Rous sarcoma virus) have lost genetic information coding for replicative genes i.e. they are replication defective. The new genetic set sequences inserted instead of the deleted material are responsible for their transforming ability. V-oncs are named according to the virus and the type of malignancy e.g. simian sarcoma = sis. Where did the v-oncs sequences come from? There are proto-oncogenes that become introduced into the viral genome during the process of viral replication within the normal cell. Replication defective viruses usually act in association with replication-competent ones (helper viruses) that provide genes for completing the viral life cycle (pol sequence).

**How do v-oncs cause malignancy?**

a. During transduction, mutation in v-oncs sequences leads to formation of an altered gene product, which causes unregulated growth.

b. Transduction brings proto-oncogenes into proximity of retroviral promoters, which causes excessive expression. Rous sarcoma virus genome contains both reverse transcriptase genes & oncogenic sequence (src).

2. **Slow transforming viruses**: These have the typical retroviral genome; they are replication competent but do not possess oncogenes. They are called chronic leukemia viruses because they are responsible for causation of leukemia. Their neoplastic transforming mechanism of action is through insertional mutagenesis i.e. the newly synthesized viral DNA is integrated near the proto-oncogene. The presence of retroviral promoter in the vicinity (upstream) of proto-oncogene leads to its increased transcription & conversion to cellular oncogenes (c-oncs). They also cause structural changes in the cellular genes.

**Human T-cell leukemia virus-I (HTLV- I)**: this is the only oncogenic retrovirus that is implicated in human cancer causation. It is associated with a form of T-cell leukemia/lymphoma. Like the AIDS virus, HTLV-I has strong tropism to CD4 cells (which are the target for neoplastic transformation). It does not contain v-onc but acts by insertional mutagenesis. Its genomic structure reveals gag, pol, env & LTR regions. In addition, it contains the (tat region) between env & 3’LTR. This is responsible for the production of transforming proteins. The latter products induce activation of IL-2 & its receptor genes in the infected T-cell that lead
to the formation of an autocrine system for growth & proliferation. The outcome is clonal T-cell proliferation.

CANCER SUPPRESSOR GENES

Like oncoproteins, protein products of the members of this category of genes function in different localities in the cells. Similar to mitogenic signals, growth-inhibitory, pro-differentiation signals originate outside the cell and use receptors, signal transducers, and nuclear transcription regulators to accomplish their effects; tumor suppressors form a portion of these networks.

1. Genes acting on the cell cycle:
The most important of these are the Rb (retinoblastoma) & P53 genes. Loss or malfunction of key regulatory proteins that these genes encode can cause malignancy. Malignancy occurs when the cell becomes homozygous for the mutant allele or loses its heterozygosity (LOH) for the normal allele.

The retinoblastoma gene (Rb)
The product of this gene, Rb protein (pRb) in its active form serves as a brake to DNA replication in cell cycle. Mutation renders the protein inactive & thus the cell divides non-stop.

The deletion or mutations of the Rb locus on chromosome 13q14 lead to neoplastic proliferation of the retinal cells. Functioning in the two-hit theory model, two types of retinoblastoma are found:

In the familial form of retinoblastoma, all somatic cells inherit one mutant Rb gene from a carrier parent (1\textsuperscript{st} hit). The second mutation affects the Rb locus in one of the retinal cells after birth (2\textsuperscript{nd} hit).

In the sporadic form of retinoblastoma, on the other hand, both mutations at the Rb locus are acquired by the retinal cells after birth.

The pRb polices the normal cell cycle. Quiescent cells (in the G0 or early G1) contain the active hypophosphorylated form of the pRb. In this state, pRb prevents
cell replication by binding & possibly sequestering the E2F family of transcription factors. The hyperphosphorylated form of the pRb releases the E2F transcription factors. The released E2F proteins then activate the transcription of several target genes such as cyclin E.

P53
Another very important gene; it is the guardian of the genome or the molecular policeman.

P53 prevents replication of damaged or faulty DNA. P53 prevents neoplastic transformation by three interlocking mechanisms: activation of temporary cell cycle arrest (quiescence), induction of permanent cell cycle arrest (senescence), or triggering of programmed cell death (apoptosis).

Normal (wild type) p53 is called into action in emergency breaks after exposure to irradiation, UV light or mutagenic chemicals. The accumulated wild type p53 binds to DNA & stimulates transcription of several genes that mediate the two major effects of p53:

a. Cell cycle arrest: Transcription up regulation of the p21gene is mediated by the p53 protein. P21 arrests the cell cycle at the G1 phase whereby promotion of DNA repair is mediated by the group of genes which are collectively called GAAD45 (growth arrest DNA damage 45).

b. Apoptosis: When the repair is efficient or the damage was beyond the capacity of the repair system, the p53 causes transcriptional upregulation of an apoptosis gene, the bax gene.

c. Another p53 function is to mediate gene repression by activating transcription of miRNAs. P53 activates transcription of the mir34 family of miRNAs. mir34s repress translation of both proliferative genes, such as cyclins, and anti-apoptotic genes, such as BCL2. Repression of these genes can promote either quiescence or senescence as well as apoptosis.

In such cases there is no cancer.

Faulty p53 molecules allow cell with damaged DNA to survive & replicate. The existing mutation will pass to the progeny cells, which will have the chance to accumulate additional mutations to pass to neoplasia.
**P53 gene is the single most common target of genetic alteration in human tumors.** 50% or more of human tumors have either loss of p53 gene in both alleles or have what is called “a negative dominant mutation”. Mutated p53 protein has got a longer half life and can be visualized by immunohistochemistry methods.

NOTE: The discovery of p53 family members p63 and p73 has revealed that p53 has collaborators. Indeed, p53, p63, and p73 are players in a complex network with significant cross-talk. p53 is ubiquitously expressed, while p63 and p73 show more tissue specificity. For example, p63 is essential for the differentiation of stratified squamous epithelia, while p73 has strong pro-apoptotic effects after DNA damage induced by chemotherapeutic agents. Furthermore, both p63 and p73, and probably p53 as well, are expressed as different isoforms, some of which act as transcriptional activators and others that function as dominant negatives.

2. **Down regulation of growth promoting signals** is another area where products of cancer suppressor genes can operate. Germ line mutations at certain loci are associated with benign tumors that are precursors of carcinoma later on. Patients with mutant allele of adenomatous polyposis coli (APC), which is genetically transmitted, develop hundreds to thousands of adenomatous polyps in the colon at ages 10-20 years; the condition is also called familial adenomatous polyposis (FAP). One or two of these polyps undergo malignant transformation i.e. cancer of the colon. Both loci for the APC gene must be lost for the tumor development (adenoma). Several additional mutations are needed for carcinoma to occur.

APC protein is located in the cytoplasm. It interacts with other intracellular proteins such as B-catenin (a transcription activator of growth promoting genes). APC causes degradation of B-catenin, thus maintaining its low level in the cytoplasm (negative regulator of B-catenin signaling). Sometimes, Certain mutations in B-catenin render it refractory to APC protein regulation, although the latter is normal.

Inheritance of one mutant allele of NF-1 gene (*NF stands for neurofibromatosis*) causes the appearance of numerous benign neurofibromas as a result of inactivation of the second copy of the gene. The condition is called neurofibromatosis type-1. Later on neurofibrosarcoma may develop. The protein product of the gene is neurofibromin. Its function is to regulate signal transduction via the ras protein. Neurofibromin is a GTPase activating protein that facilitates conversion of active GTP-ras into inactive GDP-ras. With loss of neurofibromin, ras is continuously active.
3. The third group of cancer suppressor genes involves cell surface receptors. The binding of TGF-B to its receptor up-regulates transcription of growth inhibitory genes. This is done partly by stimulating the synthesis of CDK inhibitors, which regulate the cell cycle. Cadherins are glycoproteins that act as glues between epithelial surfaces. Their loss leads to easy disaggregation with local invasion & later on metastasis. The cytoplasmic aspect of cadherin is bound to β-catenin whereby the latter stabilizes cadherin.

GENES THAT REGULATE APOPTOSIS
These genes either prevent programmed cell death (apoptosis) e.g. bcl-2, or induce programmed cell death e.g. bax & bad genes. Juxtaposition of immunoglobulin heavy chain gene located on chromosome 14q with bcl-2 located on chromosome 18q causes over-expression of bcl-2. By a not well-understood mechanism this over expression protects lymphocytes from apoptosis; they survive causing lymphadenopathy in a malignant process called small cell lymphoma. The location of the bcl-2 protein is on the outer leaflet of mitochondrial membrane, endoplasmic reticulum & nuclear membrane. This protein regulates the exit of cytochrome-c from mitochondria to the cytoplasm. In turn, cytochrome-c assists in activation of a proteolytic enzyme caspase responsible for cell death. The tumor suppressor gene P53 mediates up-regulation of the bax gene promoting apoptosis.

CELLULAR SENESCENCE
With each cell division there is shortening of specialized structures at the end of chromosomes called telomers. This reaches to a point whereby loss of telomers function leads to an end to end fusion of chromosomes & cell death. In germ cells telomers are prevented from shortening by the action of the enzyme telomerase. This enzyme is absent from most somatic cells. Tumor cells have the ability to reactivate this enzyme.

GENES THAT REGULATE DNA REPAIR
There are several inherited disorders in which genes that encode proteins involved in DNA repair are defective. Those are at great risk of developing cancer. In **hereditary nonpolyposis colon cancer** (HNPCC), familial carcinoma of the colon (affecting its right side) does not arise from adenoma. Defects in genes involved in DNA mismatch repair lead to HNPCC (genes as spell checker). DNA repair genes are not oncogenic but allow mutations in other genes in normal cell cycle. Cells with such defect are said to be replication error (RER) phenotype. **Xeroderma pigmentosum** is another example whereby genes encoding enzymes like nucleases, polymerases & ligases, which cut DNA, add nucleotides & join them in the DNA strand respectively.

**CHEMICAL CARCINOGENESIS**

Chemical carcinogenesis involves the generation of malignant cells by going through multiple steps. These steps can be grouped into two stages, initiation & promotion.

**INITIATION**

This signifies exposure of cells to an appropriate dose of a carcinogenic agent (initiator) so that it becomes transformed (altered). However, *initiation alone is not sufficient to cause tumor*. Initiation is a rapid process & has memory. Multiple applications of an initiator have the same effect because initiating carcinogens produce permanent damage in the DNA of target cells. Tumor arises in an initiated cell only after the application of another substance (promoter).

**PROMOTION**

Promoters can cause tumor in an initiated cell even if their application was delayed for several months but *they cannot cause tumors by themselves*. Multiple applications of a promoter are also associated with tumor formation in an initiated cell. Tumor does not occur when the promoter is applied before an initiator. This indicates that cellular changes by promoters are reversible (they do not affect the DNA). If the time between multiple promoter applications is prolonged, no tumor occurs.

**Chemicals are either**

- **Complete carcinogens** having both initiation & promotion effects or
- **Incomplete carcinogens** capable only of initiation.
**INITIATORS** are of two types either **natural or synthetic; they are either**

1. **Direct acting**, which do not require chemical transformation for their carcinogenicity. They include alkylating & acylating agents.
2. **Indirect acting** (pro-carcinogens), which require metabolic conversion in vivo by the cytochrome p-450 system dependent activation & formation of “ultimate carcinogen”.

The carcinogenic potency of such chemicals is determined by the inherent reactivity of their electrophilic derivatives & by the balance between its metabolic activation & detoxification.

The susceptibility to carcinogenesis is regulated in part by polymorphism in genes encoding metabolizing enzymes. For example, CYP1A1 is a product of P-450 gene, which metabolizes polycyclic aromatic hydrocarbons such as benzopyrenes. Ten percent of the white population has highly inducible form of the enzyme with an increase risk of cancers in smokers.

All directly acting initiators are highly reactive electrophiles (have electron deficient atoms) that can react with nucleophilic (electron rich) sites in the cell such as DNA, RNA & proteins in non-enzymatic reaction resulting in the formation of covalent adducts (addition products) between the chemical carcinogen & nucleotides in the DNA. Interaction with DNA, RNA or proteins is sometimes lethal.

In the initiated cell the target is usually DNA (proto-oncogenes) & the interaction is non-lethal.

**Chemical carcinogens are mutagens** (cause mutation). However carcinogen induced DNA damage do not always lead to initiation since several forms of DNA damages can be repaired by cellular enzymes.

For the changes to be heritable in the initiated cell, the damaged DNA template must be replicated & for initiation to occur, carcinogen altered cell must undergo at least one cycle of proliferation so that the changes in the DNA become fixed.

No single unique DNA alteration can be associated with initiation, however the interaction with DNA is not completely random & each carcinogen produces its “molecular finger print” that can link specific chemicals with their mutational effects.
PROMOTERS
These are not electrophilic & they do not damage DNA. Their action involves altered expression of genetic information in the cell; this is mainly by binding or activation of protein kinases that catalyze phosphorylation of protein causing change in the intracellular pH & increase growth & activity. By this, promoters induce clonal proliferation of initiated cells & alter their differentiation program by activating enzymes that are part of the physiologic signal transduction pathways (kinases).

The process of promotion involves
1. Proliferation of pre-neoplastic cells.
2. Malignant conversion.
3. Tumor progression.

Examples of carcinogenic chemicals
Initiators
1. Direct acting alkylating agents. These are activation independent, weak carcinogens that are important for being therapeutic agents used in anticancer therapy like cyclophosphamide, chlorambucil & busulfan. Some are immuno-suppressive and used in the treatment of immunological disorders such as rheumatoid arthritis. They cause interaction with the damaged DNA & can cause leukemia, lymphoid neoplasms & other cancers.

2. Polycyclic aromatic hydrocarbons. These are the most potent carcinogens requiring metabolic activation. They can cause skin cancers, sarcomas & local cancers in specific organs. They are produced by combustion of tobacco particularly with cigarette smoking & are present in smoked fish & meat. They lead to causation of lung cancer & bladder cancers.

3. Aromatic amines & azodyes. Their carcinogenicity is exerted in the liver mainly where the ultimate carcinogen is formed e.g. acetylaminofluorene which is implicated in hepatocellular carcinoma & B-naphthylamine in bladder cancer in workers in aniline dye & rubber industries.
4. **Naturally occurring carcinogens.** The potent hepatic carcinogen Aflatoxin B1 is produced by some strains of *Aspergillus flavus* that thrive on improperly stored grains.

5. **Nitroseamines & amides.** These may form in the GIT of human & contribute to GIT cancers. They are formed from the reaction of nitrostable amines & nitrates by bacteria.

6. **Miscellaneous agents.**
   - **Occupational exposure to asbestos** leads to bronchogenic carcinoma, mesothelioma & GIT cancers.
   - **Cigarette smoking** is associated with bronchogenic carcinoma.
   - **Vinyl chloride** (the monomer of polyvinyl chloride) exposure is associated with in hemangiosarcoma of the liver.
   - Chromium, Nickel & other metals (vapors) exposure is associated with lung cancers.
   - Arsenic exposure is associated with skin cancers.

**Promoters**
Tumor promotion may occur after exposure to an exogenous agent such as cigarette smoking or viral infections that cause tissue damage & reactive hyperplasia. Promoters may be endogenous such as hormones & bile salts. Estrogen can cause liver tumors in animals. Diethyl stilbesterol is associated with postmenopausal endometrial carcinoma. Increase dietary fat can lead to colon cancer due to increase synthesis of bile acids.

**RADIATION & CANCER:**
Radiation is the energy distributed across the electromagnetic spectrum as waves (having long wave length & low frequency) or particles (having short wave length & high frequency).

**TYPES**
- **Natural**
  - Cosmic rays of galactic & solar origin. These are not included in the electromagnetic spectrum they are high energy charged particles.
• Ultraviolet rays
• Radioactive elements in the earth crust.

**Human generated radiation**
• Medical diagnostic and therapeutic agents
• Industrial agents
• Nuclear weapons.

**Non-ionizing radiation**; these are electromagnetic radiation of long wave length & low frequency. They include electric power, radio waves, microwaves, infra red & ultraviolet light. They produce vibration & rotation of atoms in biological molecules.

**Ionizing radiations** are radiation energy of short wave length & high frequency. They can ionize biologic target molecules & eject electrons. They include x-rays produced in the roentgen tube & gamma rays emitted from natural sources. They also include particles released by natural decay of radio-isotopes or by artificial acceleration of subatomic particles. **All ionizing radiation produce their effect through transferring of energy to the molecules & atoms within the cell.** With sufficient energy transfer, orbiting electrons may be separated from the atomic nucleus producing ionization of the atom or the molecule. Transfer of less energy may move the electron into distant orbit with excitation of the molecule.

**Particulate radiation**
• **Alpha particles** constitute the nucleus of helium atom. It is generated when a heavy element is transformed to lighter one. They are positively charged particles.

• **Beta particles; negative beta particles** are negatively charged electrons which are produced when a neutron is converted into a proton & an electron in the nucleus. **Positive beta particles** are positively charged electrons (positrons) which are produced when a proton is converted into a neutron & a positron in the nucleus. A photon or more of gamma rays may be associated with these two reactions.

• **Neutrons** : are neutral particles emitted either in nuclear fission reaction in the nuclear reactors, or in fusion reactions
Alpha particles have a strong ionizing power but low penetration because of their large size (2 protons & 2 neutrons). Beta particles have weaker ionizing power & higher penetration than alpha.
MECHANISM OF ACTION

Target theory (direct hit)
Charged particles produce their effects by direct hits on target molecules within the cell, the DNA specifically the linkage & bonds leading to mutations having genetic or cancerous potentials or to inhibition of cell division & cell death having acute somatic effects, in addition to effects on enzymes & macromolecules within the membranes.

The indirect action theory
Radiation energy like x-ray & gamma rays exert their effects by producing free radicals. Absorbed radiant energy leads to radiolysis of cell water & the formation of the ionized water molecules H2O+ & H2O-, which dissociate to form free radicals H+ & OH-. These initiate reactions within themselves, their own reaction products & tissue water to form other free radicals like H2O2’ & HO2’. Free radicals interact with membranes, nucleic acids & enzymes leading ultimately to cell death or to inhibition of cell division.

Oxidant stress activates transcription factors that increase gene expression.

Cells are sensitive to radiant energy in direct proportion to their reproductive or mitotic activity & in an inverse proportion to their level of specialization.

In sufficient dose, radiation inhibits the cell capacity to divide & kill the cell. In the fetal life it causes teratogenicity.

Smaller doses induce mutations & heritable or non-heritable alteration in metabolism.

The effect on DNA include formation of pyrimidine dimmers, cross links single stranded or double stranded breaks & various rearrangements leading to a wide range chromosomal & chromatid alterations including deletions, breaks translocations, interadherence of chromosomes, fragmentation & all forms of abnormal chromosome morphology. Disordered mitotic spindle, polyploidy & aneuploidy; nuclear swelling, condensation of chromatin, nuclear membrane breaks, giant cells, nuclear pyknosis & lysis can all be seen.

A delayed carcinogenic effect of ionizing radiation is due to a phenomenon called induced genetic instability (mutations continue to be expressed, accumulation of these mutations is due to persistent DNA lesions that are not repaired).
UV LIGHT
UVB leads to generation of reactive oxygen species & damage chromophores such as melanin.

UV damages DNA with formation of pyrimidine dimmers between adjacent pyrimidines on the same DNA strand, formation of pyrimidine-pyrimidine (6-4) photo products, single stranded breaks & DNA protein cross links. Mutations involving adjacent pyrimidine bases in P53 C to T or CC to TT double base substitutions is an example.

Ultraviolet response pathway
This involves activation of the ras signal transduction with activation of mitogen activated protein kinases & induction of cellular proto-oncogenes & genes involved in cell proliferation.

Thymidine dinucleotides produced by UV activates the P53 pathway in a manner analogue to DNA breaks produced by ionizing radiation causing DNA repair. Nucotide excision repair (NER) includes:
   1. Recognition of the DNA lesion.
   2. Incision of the damaged strand on both sites of the lesion.
   3. Removal of the damaged oligonucleotide.
   5. Ligation.