Introduction

Cancer is a group of diseases characterized by unregulated cell growth and the invasion and spread of cells from the site of origin (primary site) to other sites in the body. Tumor is not synonymous with cancer. A tumor can be benign, pre-malignant, or malignant, whereas cancer is by definition malignant.

Proto-oncogenes and Oncogenes

Proto-oncogenes are the normal genes involved in the regulation of controlled cell growth. These genes encode proteins that function as (growth factors, growth factor receptors, signal transducing proteins, and nuclear transcription factors). When the proto-oncogene is mutated or overregulated, it is called an oncogene and results in unregulated cell growth and transformation.

Growth factors

Aberrant production of these proteins or response to the signals they elicit (in cells normally in the G₀ phase) can result in aberrant transition from G₀ → G₁, with subsequent uncontrolled growth.

- **Fibroblast growth factors (FGFs)** are a family of proteins that are normally expressed during the proliferation of cells required for normal wound healing, but overexpression can lead to tumor formation.

- **Platelet-derived growth factor (PDGF)** is a polypeptide that is normally important for extracellular matrix production, but overexpression may result in proliferation as a result of autocrine stimulation.

Growth factor receptors

What are Cell Surface Receptors?

Are receptors that are embedded in the membranes of cells. They are specialized integral membrane proteins that allow communication between the cell and the extracellular space. The extracellular molecules may be hormones, neurotransmitters, cytokines, growth factors, cell adhesion molecules, or nutrients; they react with the receptor to induce changes in the metabolism and activity of a cell.
Structurally, receptors have three main domains:
1. Extracellular domain (recognize and response to the extracellular molecules)
2. Transmembrane domain
3. Intracellular (or cytoplasmic) domain (interacts with the interior of the cell, most often through the tyrosine kinase enzyme activity).

Several growth factor receptors have been identified that are capable of activation, even in the absence of specific ligand. Many of these receptors have intracellular domains that function as tyrosine kinases.

- **Epidermal growth factor receptors (EGFRs):** There are at least three members of this family of tyrosine kinase receptors, erb b-1, erb b-2, and erb b-3, which, when mutated, lead to aberrant signaling and growth in the absence of the cognate ligand (epidermal growth factor [EGF]).

- **Rearranged during transfection (RET):** Although this tyrosine kinase receptor does not directly bind growth factors, it is important in the transduction of a signal upon binding of glial cell line–derived neurotrophic factor (GDNF), with mutations resulting in autonomous growth-promoting signals in the absence of ligand binding.

**Overexpression of the growth factor receptor erb b-1, also known as HER2/neu, is associated with the development of breast cancers.**

*Mutations in RET are commonly associated with multiple endocrine neoplasia (MEN) syndromes, including MEN I, and MEN II*

**Signal transducing proteins**

The next level at which defects in cell growth and development can occur is at the level of downstream signal transduction proteins. Two such examples are given: the **ras gene** and **nonreceptor tyrosine kinase proteins**.

- **The ras gene (ras protein)**
  Is a GTP-binding protein anchored to the inner cell membrane.
  In the inactive state, Ras binds GDP.
  In stimulation of the cell by growth factor, Ras exchanges GTP for GDP, leading to activation of downstream signaling events.
  The ras protein has intrinsic GTPase activity, terminating the signal transduction events when GTP is hydrolyzed back to GDP, returning ras to its inactive state.

*The ras gene is the most commonly mutated oncogene in cancer, with 10% to 20% of tumors harboring mutations in ras. Mutations in ras are found in a large number of tumors of the colon, pancreas, and thyroid.*
Nuclear transcription proteins
These transcription proteins, such as the *proto-oncogene myc*, integrate divergent growth-promoting pathways and ultimately lead to the production of proteins that allow the cell to advance through the cell cycle.
Such genes are rapidly induced when quiescent cells receive signals to divide and are rapidly translocated to the nucleus to mediate gene transcription.

Cell cycle regulators
Alterations in the normal function of cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors, often result in unchecked cell growth.
- **Cyclin D**: Increased expression of this regulator of the G\(_1\) to S transition is commonly found in tumors.
- **CDK4**: This regulator is among the most commonly altered genes in this class of genes.

Tumor suppressor genes
Tumor suppressor genes are genes, under normal circumstances, suppress cell growth. Some do so by encoding transcription factors for other genes needed to slow growth. When their activity is reduced, result in uncontrolled cell growth.
✓ Tumor suppressor genes act by three mechanisms

I. Cell surface molecules
There are numerous cell surface molecules that antagonize normal cell growth and development
- Transforming growth factor-b (TGF-b) receptor mediates its inhibitory effects by stimulating the production of CDK inhibitors.
- The protein product of the DCC (deleted in colon carcinoma) gene regulates cell growth through the integration of signals from the cellular environment.

II. Molecules that regulate signal transduction
These molecules possess an antagonistic role to the actions of intracellular proto-oncogenes.
- NF-1 (Neurofibromatosis-1) gene product and GAP (GTPase-activating protein) activate the GTPase function of ras, converting GTP to GDP and suppressing the growth-promoting function of ras (Figure -1).
- The adenomatous polyposis coli (APC) gene product promotes the degradation of b-catenin, which otherwise normally translocates to the nucleus to induce cellular proliferation.

III. Molecules that regulate nuclear transcription
Several tumor-suppressor genes residing in the nucleus encode proteins that play an important role in the integration of growth-promoting and growth-inhibiting signals.
- The retinoblastoma (Rb) gene is an important negative regulator of cell growth.
- The p53 gene has an important gate-keeper role in cellular proliferation.
Molecular Biology

The p53 gene (the gate keeper)
The p53 gene is induced when DNA is damaged by irradiation, ultraviolet (UV) light or chemical mutagenesis.
The p53 gene then exerts its growth-inhibitory function in one of two ways to ensure adequate repair of the damaged DNA before proceeding through the cell cycle.
1. The p53 gene induces the transcription of the CDK inhibitor p21, which inhibits the CDK- and cyclin-mediated phosphorylation of Rb required for the cell to transition to the S phase (see figure above).
2. If DNA damage inflicted on the cell cannot be successfully repaired, p53 mediates the transcription of genes implicated in the process of programmed cell death, or apoptosis (see below).

Patients with Rb loss of function are prone to the development of tumors of the retina early in life and osteosarcomas of the bone later in life.

Mutations in the tumor-suppressor gene, p53, are the most common molecular alterations in cancer, with more than 50% of human tumors harboring mutations in p53.

Germ line mutations in p53 result in Li-Fraumeni syndrome.
Patients in families with a history of Li-Fraumeni syndrome inherit one mutant copy of p53 in their cells, thereby requiring only one sporadic mutation in their other allele to develop cancer.

APOPTOSIS

Apoptosis is defined as the programmed destruction of the cell. It is characterized by a decrease in cell volume, mitochondrial destabilization, chromatin condensation with nuclear fragmentation, and cellular dispersion into fragmented apoptotic bodies without the release of cellular material.

Apoptosis is the endpoint of a cascade of converging events that results in cell death.
- Growth factor withdrawal occurs with the activation of the proto-oncogene myc in conditions of sparse nutrients in the cellular milieu.
- Signals are provided by the proapoptotic cytokines, tumor necrosis factor (TNF), and Fas ligand, whose receptors stimulate the activation of proapoptotic enzymes, called caspases.
- Activation of the proapoptotic gene Bax by the tumor-suppressor gene p53 occurs if DNA mutations detected during the G1/S checkpoint cannot be repaired with adequate fidelity.

Agents such as infliximab and etanercept are biologic agents that are used in the treatment of autoimmune diseases. These drugs trigger TNF receptors on autoreactive immune cells, inducing these cells to undergo apoptosis.
Terminal events in the process of apoptosis

The release of the electron transport chain protein cytochrome c located on the outer mitochondrial membrane is a critical regulator in the process of apoptosis.

- Cytochrome c is normally prevented from translocating out of the mitochondria by the antiapoptotic gene, bcl-2.
- The exiting of cytochrome c occurs via the mitochondrial channel protein Bax.
- The relative abundance of bcl-2 and Bax determines the ultimate fate of the cell:
  - If Bax predominates, cytochrome c is liberated through the channel and associates with the cytoplasmic molecule, proapoptotic protease activating factor (Apaf-1).
  - Apaf-1 then activates a cascade of proteolytic events via the activation of caspases.

Caspases contain both “C” (cysteine) and “aspase” (aspartic acid) protease activity, and once activated, they begin the apoptotic cascade.

- Caspases normally exist in the cytoplasm as inactive zymogens until stimulated through any of the major pathways triggering apoptosis.
- Caspases degrade intracellular proteins and activate DNases, with resultant DNA fragmentation, or DNA laddering, which is characteristic of apoptosis.

The normal programmed cell death pathway, when perturbed, can also result in the accumulation of cells and uncontrolled cell growth.
Mechanism of Oncogenesis

Numerous mechanisms exist to create the genetic changes that result in uncontrolled cell growth.

Point mutations

Point mutations are changes in the individual nucleotides in the gene encoding either proto-oncogenes or tumor-suppressor genes that can result in cancer.

Chromosomal translocations

Such translocations occur during chromosomal replication, with whole segments of different chromosomes becoming aberrantly attached and fused, resulting in two distinct mechanisms of aberrant growth factor production.

<table>
<thead>
<tr>
<th>Chromosomal Translocation</th>
<th>Malignancy</th>
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<tbody>
<tr>
<td>t(8;14)</td>
<td>Burkitt lymphoma</td>
</tr>
<tr>
<td>t(14;18)</td>
<td>Follicular lymphoma</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>Mantle cell lymphoma</td>
</tr>
<tr>
<td>t(9;22)</td>
<td>Chronic myelogenous leukemia</td>
</tr>
<tr>
<td>t(15;17)</td>
<td>Acute myelogenous leukemia</td>
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Molecular Carcinogenesis

As cells proceed through the multiple rounds of division during the growth and maintenance of the organism, mistakes in the replication of the genome are inevitable. And as the cell is subjected to insults, such as chemicals, radiant energy, or viruses, the normal DNA repair mechanisms may become overwhelmed, leading to the chemical changes that result in mutations.

Chemical carcinogenesis

Both natural and synthetic compounds are capable of damaging cells either directly, after being acted on by the cell, or in synergy with other chemicals.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline dyes</td>
<td>Bladder cancer</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Mesotheliomas</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Skin cancer</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>Angiosarcoma of the liver</td>
</tr>
<tr>
<td>Nitrosamines (food preservatives)</td>
<td>Stomach cancer</td>
</tr>
</tbody>
</table>
o **Initiators**

These compounds cause direct damage to cellular macromolecules, but their effects are not sufficient, in and of themselves, for tumor formation.

- Direct acting compounds are usually highly reactive electrophiles, such as alkylating agents that form adducts with various cellular components (DNA, RNA, proteins, or lipids).
- Indirect acting compounds (procarcinogens): This larger group requires enzymatic activation to produce an ultimate carcinogen.

o **Promoters**

These compounds are noncarcinogenic but facilitate the abnormal growth of cells that have been exposed to initiators. These compounds facilitate tumor formation by stimulating proliferation of the cell mutated by the initiator.

➤ **Radiation carcinogenesis**

DNA and other macromolecules are capable of being damaged by different wavelengths of electromagnetic radiation including both UV and ionizing radiation.

- **UV radiation** from the sun is responsible for causing mutations in DNA by the formation of dimers between two adjacent pyrimidines (thymine dimers), which must be removed for normal replication to proceed.
- **Ionizing radiation**: High-energy radiation (x-rays and gamma rays) causes direct damage to DNA and creates highly reactive hydroxyl and hydrogen radicals that further interact with various cellular macromolecules.

➤ **Viral carcinogenesis**

Viruses have evolved numerous strategies for promoting the aberrant growth of their host cell types.

- **Human papilloma virus (HPV)**: Members of this family are capable of causing abnormal cell cycle progression; some facilitates the degradation of cellular p53, whereas others perturb the normal function of the protein Rb.
- **Epstein-Barr virus (EBV)** causes abnormal accumulation of cells through the production of the protein LMP-1 (latent membrane protein-1), which promotes the expression of bcl-2, leading to protection from the normal apoptotic pathways that trigger cell death.
- **Hepatitis B virus (HBV)**: Although this virus does not encode any known oncoproteins, its association with human liver cancer has been clearly demonstrated as most likely multifactorial.

> *HPVs, particularly are closely associated with the development of cervical cancers.*
> *EBV is associated with several malignant conditions including Burkitt lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma.*
> *HBV is the leading cause of hepatocellular carcinoma worldwide.*
DNA Repair and Carcinogenesis

The cell has evolved several mechanisms for the repair of DNA damaged by the multitudes of insults encountered in the environment. Multiple proteins exist to correct such errors in two major DNA repair pathways.

1. **Nucleotide excision repair**
   This repair mechanism is responsible for surveying the topology of the DNA double helix and removing such local distortions. This occurs through endonuclease cleavage of the damaged bases and restoration of the original segment through the concerted actions of a DNA polymerase using the intact strand as a template, to correct the complementary strand.

2. **Mismatch repair**
   Sometimes during replication, DNA polymerases insert nucleotides that defy the normal Watson-Crick base pairing (i.e., a G may pair with a T instead of the normal A to T pairing.) Such “misspellings” need to be recognized and corrected before perpetuation of the error to the next round of division.

The genes BRCA-1 and BRCA-2, located on chromosomes 17 and 13, respectively, are DNA repair genes implicated in human cancers. Mutations in these genes underlie 5% to 10% of familial cases of breast cancer. Both genes also are implicated in the development of ovarian cancer.

Molecular Progression of Cancer

Many genetic alterations are required for the about $10^9$ tumor cells required to form 1 g of tissue mass, which corresponds to the smallest clinically detectable mass. Every human cancer reveals the activation of several oncogenes and the loss of two or more tumor-suppressor genes. This is evidenced in the molecular model of colon carcinogenesis known as the adenoma-carcinoma sequence.
Tumor growth

Numerous variables contribute to the growth of transformed cells.

1. **Growth factors:** Many tumors require the presence of various hormones or other growth factors to fuel the growth of the tumor mass. Lack of such factors retards the growth of the developing mass.

2. **Angiogenesis:** Tumor cell growth requires the presence of nutrients and oxygen, and because of normal diffusion limits, tumors can only grow to a thickness of about 2 mm without a nutrient supply. As such, hypoxic conditions elicit the production of angiogenic molecules, such as vascular endothelial growth factor (VEGF), which promotes vascularization of the growing tumor mass.

Invasion

To disseminate throughout the body, the cancer must gain access to the circulation. This requires breaking through the basement membrane, the thick extracellular matrix that separates tissue layers. Such steps are facilitated by the rendering of various proteases, such as matrix metalloproteinases (MMPs), cathepsin D (a cysteine protease), and urokinase-type plasminogen activator (a serine protease).

Metastasis.

Once in the circulation, tumor cells alter the expression of adhesion molecules, allowing them to deposit as “seeds” to distant sites, which serve as the “soil” for continued growth of the transformed cells.

Molecular Markers in Cancer Biology

There are numerous proteins that are overexpressed in cancer cells. Some are actually capable of being detected in the serum of patients with specific cancers. Many of these proteins are simple markers and have no special significance with respect to the pathology of the disease.

Many of these proteins lack either the specificity or sensitivity needed for their use as screening tests. Their true utility is in monitoring the progression of the disease once confirmed or in monitoring therapy or recurrence.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cancer</th>
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<tbody>
<tr>
<td>Carcinoembryonic antigen (CEA)</td>
<td>Colon cancer</td>
</tr>
<tr>
<td>Human chorionic gonadotropin (hCG)</td>
<td>Trophoblastic tumors; testicular tumors</td>
</tr>
<tr>
<td>Prostate-specific antigen (PSA)</td>
<td>Prostate cancer</td>
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</tbody>
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