NEOPLASIA

Dr. Raghad Hanoon **LEC.4**

Molecular basis of Cancer (Carcinogenesis):

Fundamental principles of carcinogenesis:

Nonlethal genetic damage lies at the heart of carcinogenesis.

*This genetic damage (or mutation) may be acquired by the action of environmental agents like (chemicals, radiation, viruses), or may be inherited in the germ line.

*Tumors are monoclonal: A tumor is formed by the clonal expansion of a single precursor cell that has acquired genetic damage (founding cell).

***Four classes of genes are the principal targets of genetic damage:**

I. *Growth promoting* ***protooncogenes*** (dominant genes, can transform cells into malignant cells with single allele is damaged)**.**

II. *Growth inhibiting* ***cancer suppressor genes*** (recessive genes, can transform cells into malignant cells only if both alleles of gene are damaged).

III. ***Genes that regulate the apoptosis.***

IV.DNA repair gene, disability of DNA repair genes can predispose to widespread mutation & neoplastic transformation.

Carcinogenesis: is a multistep process resulting from the accumulation of multiple mutations. These mutations accumulate independently in different clonal cells, generating **subclones** with varying abilities to grow, invade, metastasize, and resist (or respond to) therapy. Over a period of time tumors not only increase in size but become more aggressive and acquire greater malignant potential (**tumor progression)**.

***Eight changes in cell physiology are considered the hallmarks of cancer.**

These changes are including: -

1. Self-sufficiency in growth signals

*In a normal cell, Proto-oncogenes have multiple roles, participating in cellular functions related to growth and proliferation.

*Self-sufficiency for growth to a cancerous cell is provided by ***oncogenes,*** which are the mutant proto-oncogenes.

*Oncogenes encode proteins called *oncoproteins* that promote cell growth, even in the absence of normal growth-promoting signals.

*To aid in the understanding of the nature and functions of oncoproteins, it is necessary to review briefly the sequence of events that characterize normal cell proliferation;

1. The binding of a growth factor to its specific receptor on the cell membrane

2. Transient and limited activation of the growth factor receptor, which in turn activates several signal-transducing proteins on the inner leaflet of the plasma membrane
3.Transmission of the transduced signal across the cytosol to the nucleus via second messengers or a cascade of signal transduction molecules
4. Induction and activation of nuclear regulatory factors that initiate DNA transcription
5. Entry and progression of the cell into the cell cycle, resulting ultimately in cell division

Mechanisms of action of Oncoproteins:

A. Growth factors:

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

*Many glioblastomas secrete platelet-derived growth factor (PDGF) and express the PDGF receptor.

*Many sarcomas make both transforming growth factor-α (TGF-α) and its receptor.

B. Growth factor receptors: Mutant genes lead to one of two consequences:

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

**EGFR (ERBB1);* is overexpressed in 80% of squamous cell carcinomas of the lung, 50% or more of glioblastomas, and 80% to 100% of epithelial tumors of the head and neck.

**HER2/NEU (ERBB2);* is amplified in approximately 20% of breast cancers.

C. Signal transduction proteins:

*Cancer cells acquire autonomous growth is by ***mutation in these genes that control the signaling pathway*** (transfer of signal from inner side of cell membrane to nucleus).

*Two important genes that control this pathway (**Ras**…. increase cells proliferation & **ABL…**……inhibit cell proliferation).

1. RAS:

***RAS is the most commonly mutated oncogene in human tumors. Approximately 30% of all human tumors contain mutated *RAS* genes, and the frequency is even higher in some specific cancers (e.g. colon and pancreatic adenocarcinoma).

***Activated RAS stimulates down-stream regulators of proliferation, which floods the nucleus with signals for cell proliferation.

***The RAS gene mutations (mostly point mutations) interfere with the inactivation of RAS, thus persists in its activated form, and the cell is forced into a continuously proliferating state.

2. ABL:

******Is a proto-oncogene with tyrosine kinase activity, that is reduced by internal negative regulatory mechanism. In chronic myeloid leukemia and certain acute leukemias, this activity is set free because the ABL gene is translocated from its normal abode on chromosome 9 to chromosome 22, where it fuses with part of the breakpoint cluster region *(BCR)* gene. The BCR-ABL hybrid protein has potent, unregulated tyrosine kinase activity, leading to growth autonomy.

D. Nuclear Transcription Factors:

Mutations affect the genes that regulate transcription of DNA may result in autonomous growth of cancers.

e.g. ***Myc gene;***

-Dysregulation of MYC expression is seen in Burkitt's lymphoma

-MYC is amplified in some cases of breast, colon, lung, and many other carcinomas.

E. Cyclins and Cyclin-Dependent Kinases

-Normal cell cycle is consisting of five phases (G_0, G_1, S, G_2, M).

-All these phases are under control of proteins (Cyclins & Cyclins dependent Kinase).

-Mutations that dysregulate the activity of cyclins and CDKs would favor cell proliferation.

-Cyclin D overexpression is seen in many cancers (breast, esophagus & liver).

2. Insensitivity to Growth-Inhibitory Signals (Tumor Suppressor Gene):

*All tumor suppressor genes cause inhibition of cell growth by two pathways: -

1. *Stimulate antigrowth signal, causing cells to enter G_0 phase.*
2. *Prevent the cell to pass from phase G_1 to S phase.*

*Disruptions of **tumor suppressor genes** make the *cells resistant to inhibition of growth & increase their proliferation.*

***Examples of Tumor suppressor genes:**

1. RB gene:

This is the *first discovered suppressor gene, loss of normal RB gene was discovered initially in Retinoblastoma, but recently proved it lost in many tumors (breast cancer, bladder & lung cancer, osteosarcoma).*

Both alleles of RB gene must be mutant in order to regard this gene is mutant.

2. Adenomatous Polyposis coli (APC) gene:

Loss of this gene can be seen in 70- 85% of sporadic carcinoma of colon.

Individuals born with Loss or mutant of one of alleles of APC gene, they develop hundreds to thousands of adenomatous polyps in the colon which on second decade of life one or more of these polyps will undergo malignant transformation.

3. P53:

-It is one of most commonly mutated gene in human cancers.

-Normal *p53* prevents neoplastic transformation ***by***: -

I. activation of temporary cell cycle arrest (quiescence),
II. induction of permanent cell cycle arrest (senescence),

III. triggering of programmed cell death (apoptosis).

-*In view of these activities,* p53 *has been rightfully called a "**guardian of the genome.**"*

-More than 70% of malignant human tumor show defect in functions of TP53.

-Most of mutations in TP53 are acquired & less commonly they are inherited mutations like ***Li- fraumeni syndrome*** (patient have many cancers like sarcomas, breast cancer, leukemia, brain tumors, adrenal tumors).

-With homozygous loss of *p53,* DNA damage goes unrepaired, mutations become fixed in dividing cells, and the cell turns onto a one-way street leading to malignant transformation.

3. Altered cellular metabolism.

*Even in the presence of sufficient oxygen, cancer cells demonstrate a distinctive form of cellular metabolism characterized by high levels of glucose uptake and increased conversion of glucose to lactose (fermentation) via the glycolytic pathway. This phenomenon, called the Warburg effect and also known as aerobic glycolysis.

*Aerobic glycolysis provides rapidly dividing tumor cells with metabolic intermediates that are needed for the synthesis of cellular components, whereas mitochondrial oxidative phosphorylation does not.

4. Evasion of apoptosis:

Normally, there are two pathways that lead to apoptosis: **the extrinsic pathway,** which is mediated by *TNF- Fas receptor* & inhibited by FLICE protein (FLIP). and **the intrinsic pathway** (also known as the mitochondrial pathway), *is mediated by* BAX, BAK genes & inhibited by bcl2 gene.

Changes in mechanism of apoptosis in malignant tumors are:

1. Decrease level of Fas protein (CD95).
2. Increase level of FLICE protein (FLIP); which inhibits apoptosis by inhibit activation of caspases.
3. Reduced levels of pro-apoptotic BAX resulting from loss of p53.
4. Increase level of Bcl2 gene which inhibits apoptosis (as in 85% of B-cell lymphoma).

5. Limitless replicative potential (immortality)

Most of normal human cells have capacity of 50 to 70 doublings, after this, the cells lose the capacity to divide (due to loss of Telomerase enzymes which is important in DNA replication). In malignant tumors, there is increased level of Telomerase.

6. Sustained Angiogenesis:

*Most of cancers cannot grow more than 1 to 2 mm in diameter or thickness unless they are vascularized.

**Angiogenesis has many effects on tumor growth:*

1. Supplies nutrients & oxygen.
2. Newly formed endothelial cells during angiogenesis, will stimulate the growth of adjacent tumor cells by secretion of polypeptides (like platelets growth factor).
3. Angiogenesis is also important for development of metastasis.

*Cells of malignant tumor are the main inducer of angiogenesis by their production of growth factors (**Angiogenetic factors);** the most two important ones are:
1-Vascular endothelial growth factor.
2- Fibroblast growth factor.

7. Invasion and metastasis

The Metastatic pathway of cancer can be divided into two phases:
1. Invasion of Extracellular matrix: Include the following steps
I. Detachment of tumor cells from each other (by losing of E- cadherin & B- catenin molecules).
II. Degradation of Extracellular Matrix (by Metalloproteinase).
III. Attachment of tumor cells to new Extracellular Matrix components.
IV. Migration of tumor cells to the vessels (mediated by Cytokines).
2. Vascular dissemination & Homing of tumor cells:
-***In circulation***, Tumor cells are liable to destruction by host immune cells. To avoid such destruction, tumor cells are arranged themselves into small emboli (by adhesion to circulating leukocytes & platelets).
-***Then*** tumor cells leave circulation by adhesion to the endothelial cells & destruction of basement membrane of vessels, to enter the extracellular matrix of metastatic site.
-Distribution of metastasis can be predicted by the location of primary tumor & its vascular & lymphatic drainage e.g. cancer of breast is expected to involve the lung & bones of thorax.
-Some cancers have *unexpected metastatic pathway e.g. caner of lung metastasizes to the adrenal glands.*

8. Evasion of immune surveillance

*Cancer cells express a variety of antigens that stimulate the host immune system, which appears to have an important role in preventing the development of cancers.
*Despite the antigenicity of cancer cells, the immune response to established tumors is ineffective, due to acquired changes that allow cancer cells to avoid anti-tumor responses.

Genomic Instability due to defects in DNA repair

*Although humans swim in environmental agents that are mutagenic (e.g., chemicals, radiation, sunlight), cancers are relatively rare outcomes because the cell is able to repair DNA damage and the death of cells with unrepairable damage.
****Defects in three types of DNA-repair systems contribute to different types of cancers;***
1. Defect in DNA mismatch repair gene:
*When a strand of DNA is being repaired, these genes act as ―spell checkers. For example, if there is an erroneous pairing of G with T rather than the normal A with T, the mismatch-repair genes correct the defect. Without these ―checkers, errors accumulate at an increased rate.

Ex. HNPCC (Hereditary nonpolyposis colon carcinoma (Lynch syndrome)

*****This disorder, characterized by familial carcinomas of the colon affecting predominantly the cecum and proximal colon

*A characteristic finding in the genome of patients with mismatch repair defects is ***microsatellite instability (MSI).*** Microsatellites are repeats of one to six nucleotides found throughout the genome. In normal people, the length of these microsatellites remains constant. By contrast, in patients with HNPCC, these satellites are unstable and increase or decrease in length. Although HNPCC accounts for only 2% to 4% of all colonic cancers, MSI can be detected in about 15% of sporadic cancers due to acquired mutations that disrupt the function of mismatch repair genes.

2. Defect in nucleotide excision repair gene: Ex. Xeroderma Pigmentosum

*Xeroderma pigmentosum is an autosomal recessive disorder caused by a defect in DNA repair that is associated with a greatly increased risk for cancers arising in sun exposed skin.

*****UV radiation causes cross-linking of pyrimidine residues, preventing normal DNA replication. Such DNA damage is repaired by the nucleotide excision repair system.

*Several proteins are involved in nucleotide excision repair, and an inherited loss of any one can give rise to xeroderma pigmentosum.

3. Defects in DNA Repair by Homologous Recombination:

*Germ line mutations in two genes, ***BRCA1*** **and** ***BRCA2***, account for 50% of cases of familial breast cancer. In addition to breast cancer, women with ***BRCA1*** mutations have a higher risk for developing epithelial ovarian cancers, and men have a slightly higher risk for developing prostate cancer. Likewise, germ line mutations in the ***BRCA2*** gene increase the risk for developing breast cancer in both men and women, as well as cancers originating from the ovary, prostate, pancreas, etc. Cells that lack these genes develop chromosomal breaks. Both genes seem to function in the homologous recombination DNA repair pathway. *Similar to other tumor suppressor genes,* both copies of *BRCA1* and *BRCA2* must be inactivated for cancer to develop. Although linkage of *BRCA1* and *BRCA2* to familial breast cancers is well established, these genes are rarely inactivated in sporadic cases of breast cancer. In this regard, *BRCA1* and *BRCA2* are different from other tumor suppressor genes, such as *APC* and *TP53*, which are frequently inactivated in sporadic cancers.