Mechanisms of Apoptosis and Molecular Basis of Carcinogenesis in Humans

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Abstract: Cancers is a group of diseases involving abnormal cell growth and proliferation with a potential to invade other adjunct and distant organs. According to World Health organization (WHO) 2021 report, mortality attributable to cancers was about 10 million of people. In Africa, according to GLOBOCAN 2018, there are about 36 cancer types, there were 811, 200 new case (4.5% of the world population) and 534, 000 cancer deaths (7.3% of the total world). The annual incidence of cancers in Kenya is about 47, 887 new cases with an annual mortality of 32, 987 cases, that is, 68.8% (National cancer screening guidelines, 2nd Ed). Malignancy is the 3rd most important cause of mortality in Kenya, Infections and cardiovascular taking first and second positions respectively. To combat this big public health problem, molecular understanding of cancers is key among medical practitioners in Kenya and global at large.

Keywords: Malignancy, Tumors, Caspase, Genes, Proto-oncogenes, P53 Suppressor genes, Gene Mutations

1. Apoptosis

Apoptosis is defined as genetically programmed cell death. This is a complex mechanism that requires energy in form of Adenosine triphosphate (ATP), Apoptosis gene regulation and does not results into inflammation.

For apoptosis to take place, there is an activation of Pro-caspase (Pro-C) to caspase which in turn it will activate Endonuclease’s enzyme. This will result into enzymatic nuclear DNA damage leading to Chromatid condensation. It is important to understand that enzymatic nuclear DNA damage involves highly genetically controlled specific fragment removal and the size of the fragments is at the multiples of 180 - 200 Base pairs. The end product is Apoptic bodies with intact membranes, which are phagocytosed by cells of immune system and because the plasma membrane is intact, there is no stimulation of inflammation.

Tissues that have cells that have undergone through the process of Apoptosis can be analyzed in a special examination called Agarose gel Electrophoresis which will give a characteristic Step ladder appearance. This finding is not diagnostic but highly suggestive. This is because Step ladder appearance can also be seen in necrosis. Smearing pattern on Agarose gel electrophoresis is diagnostic to necrosis with high specificity and sensitivity.

2. Mechanisms of Apoptosis

The mechanisms of apoptosis are divided into 2 major phases i.e., Initiation Phase and Execution Phase.

2.1 Initiation Phase

This phase generally involved with stimulus initiating apoptosis and it involves 2 pathways;
2.1.1 Intrinsic pathway

This first part of the complex pathway involves activation of Pro-C9 to C9 as per the illustration bellow.

2.1.2 Extrinsic pathway

This pathway involves the activation of Pro-C8 to C8 and also Pro-C10 to C10. Not that, in human only C10 is present and C8 is present in lower animals. C9 from intrinsic pathway and C8 and 10 from the extrinsic pathway will activate execution phase which will in turn activate endonuclease enzymes to damage Nu-DNA.

2.2 Execution Phase

This phase is activated by caspases secreted in initiation phase which includes C9, C8 and C10. These caspases will activate Pro-C3, Pro-C6 and Pro-C7 in execution phase to their active forms that is, C3, C6 and C7 which will activate Endonuclease enzymes.

3. Concept of Proto-Oncogen

These are normal genes in human cells that control cell growth and proliferation. It is an important concept to understand in order to comprehend detailed mechanisms of apoptosis and molecular basis of carcinogenesis in humans.
Proto - oncogenes are divided into 2 groups as shown below;

3.1 Pro - Apoptotic Proto - oncogenes

These genes tend to promote apoptosis mechanisms. They include BAK, BAX, BCL - Xs and BH3 only protein family. BH3 only proteins family is the stress sensors on the surface of the cells. BH3’s includes Bid, Bim, Bad, PUMA and NOXA only proteins.

3.2 Anti - Apoptotic Proto - oncogenes

These genes tend to inhibit apoptotic mechanisms. They include BCL - 2, MCL - 1 and BCL - XI

4. Detailed Explained Initiation Phase Using Proto - Oncogene Concept

4.1 Intrinsic Pathway

This is the major pathway of the initiation phase. It is also called Mitochondrion pathway because it is the major organelle involved in this biological pathway. On the surface of cells, there is a stress sensor protein called BH3 which detect stress from different agents such as Viruses (Human papilloma virus), radiation such as x rays, toxic chemical substances etc. Once BH3 detect stress, it deactivates BCL - 2 and MCL - 1 on the surface of the outer mitochondrion membrane and activated BAX and BAK on the surface of the inner mitochondrion membrane. Activation of BAX and BAK will lead to the expression on the surface of outer mitochondrion membrane from the inter mitochondrion membrane and also increase mitochondrion permeability. Increased mitochondrion is a characteristic of apoptosis. This permeability will make 2 channels leaky, one for Cytochrome c and the second one for SMAC DIABLO molecules.

The role of cytochrome c is carrying out electron transfer chain for oxidative phosphorylation. Once it leaks into cytoplasm, it changes from mitochondrion Cytochrome c to Cytosolic cytochrome c whose role is to promote apoptosis. In the cytoplasm, Cytochrome c combines with Apoptosis activating factor - 1 (ApaF - 1) to form Apoptosome which then activate Pro - C9 to active C9. This activation maybe inhibited by a molecule called Inhibitor of Apoptosis (IAP) in the cytoplasm. SMAC DIABLO from mitochondrion has to deactivate IAP in order for Pro - C9 to take place.

4.2 Extrinsic Pathway

This pathway is also known as Death - R - Mediated pathway. Death receptors include Tumor Necrosis Factor - R (TNF - R) and FAS. FAS is also known as CD95. When death ligand binds to death receptor e.g., TNF binds on TNF - R this mechanism activates adaptor receptor in the cytoplasm and this will further activate Pro - C8 and Pro - C10 to their active form of C8 and C9 respectively.
5. Molecular Basis of Carcinogenesis

This complex molecular mechanism requires good understanding of medical biochemistry and genetics. These mechanisms will be discussed under the following areas; non-lethal mutations, monoclonality and 4 targets of malignant pathogenesis.

5.1 Non-Lethal Mutations

Mutation is defined as an alteration in the nucleotide sequence of the genome of an organism. Mutation may result into cell death, which is a good thing in malignant pathogenesis, cell may survive but remains neutral or cell may survive and continues to grow. This is what is known as non-lethal mutation and this is the core of malignant pathogenesis. These cells that have mutated genome and continue to grow are at increased risk of abnormal growth and proliferation and hence malignancy.

5.2 Monoclonal

This means that, tumors are derived from a single ancestral cell that underwent conversion from a normal cell to a cancerous state. This state can also be inherited and it forms the basis of malignant pathogenesis.

6. 4 Targets of Malignant Pathogenesis Proto-oncogene

As discussed under apoptosis, these are normal genes that control cell growth and proliferation. Mutation of these genes (Oncogenes) down regulates apoptosis and increases chances of carcinogenesis.

**Tumor Suppressor genes**

They are a normal gene that regulates cell division, repair of DNA mistakes and activate apoptosis. Mutation of these genes will also down regulate apoptosis which is opposite to the normal physiology of these genes. TP53 gene code for the P53. Mutation of this gene has been identified in more than 50% of human malignancies.

**Mechanism**

In normal circumstances, P53 proteins are unstable with a very short half-life (t1/2) of 5 to 20 minutes but in an event that, there is a DNA damage, the concentration and t1/2 increase rapidly. P53 proteins interact with WAF - 1 genes resulting into WAF - 1 m RNA transcription and finally P21 synthesis. During cell cycle, there is a check point between G1 and S phase which is controlled by CDk and Cyclin.

CDk is independent to Cyclin so these two molecules for a complex known as CDk - Cyclin complex and binds with P21 to stop cell cycle progression in case of DNA damage. Another pathway involves signaling proapoptotic proto-oncogenes such as BAX to initiate apoptosis or sending another signal to Genes responsible to regulate DNA repair to avoid mutation.

**Genes Regulating Apoptosis**

As discussed previously, these are genes that control
apoptosis and are divided into 2 groups;

a) Pro - Apoptotic genes
b) Anti - Apoptotic genes Genes Regulating DNA Repair

Mild DNA damage can be repaired by these genes and cell resumes its physiological function. These genes include Chromatin assembly factor - 1 (CAF - 1) major subunits CHAF1A and CHAF1B, Chromatin modifying protein and DNA double strand break repair proteins. Mutation of these molecules put at risk DNA for poor repair or no repair and this can be the nidus for carcinogenesis.

7. Essential Alteration for Malignant Transformation

1) Self - sufficiency to growth.

This is mainly done by Oncogenes that allows cells to proliferate uncolorable. Cells are able to grow even at the absence of the stimulus that initiated it.

2) Insensitivity to growth inhibitory signals.

Tumor cells are insensitive to growth inhibitory signals that cause inhibition of cell proliferation in normal cells. Transforming growth factor - Beta (TGF - B) is an inhibitory signal molecule to cells, however, malignant cells are insensitive to these molecules and continue to grow.

3) Evading Apoptosis

As discussed previously, malignant cells growth is associated with mutations of genes regulation apoptosis and down regulation is lost, allowing these cells to grow and proliferate without apoptosis.

4) Impaired DNA repair

Genes regulating DNA repair mutate and repair is no longer performed.

5) Unlimited Replicative capability

When cells get unlimited replicative capability, it has a chance to transform into malignancy. This happens by maintaining length and function of Telemere. Telemere is repetitive sequence of nucleotide and they are found at the end of chromosome.

6) Sustained Angiogenesis

Angiogenesis is the formation of new blood vessels. Malignant cells secrete vascular growth factors such as Vascular Endothelial growth factor (VEGF) in order to get nutrients and oxygen. This supports their growth due to availability of requirements of metabolism and also removal of end products of metabolism.

7) Ability of invasion and metastasis

Once a malignant tumor has grown now, it invades the adjacent tissues and metastasize via lymphatics, blood vessels or directly to distant tissues and organs.

References


