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Review article

Apoptosis : Understanding of the signaling pathways

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ABSTRACT

Apoptosis (programmed cell death) or cell annihilation imparts important role during various normal physiological systems of human body. Moreover, the inappropriate rate of apoptosis has been linked with pathophysiology of various diseases like preeclampsia, alzheimer's disease, huntington's, disease autoimmune diseases and various cancers. Therefore, understanding the mechanisms of apoptosis becomes the utmost priority. This review focuses on the morphological changes occurring during the process of apoptosis and the major apoptotic signaling pathways involved during this physiological phenomenon.

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1.Introduction:

During evolution of cellular life, the mechanisms of cell death has been found equally important as that of cell proliferation and maturation. Throughout the eukaryotes, the cell renewal is brought about by the production of reactive oxygen species. "Apoptosis" (in Greek meaning "falling off") is now considered to be an important and unique process occurring in most of human systems like normal cell turnover, fetal development, immune systems etc.

Apoptosis is a highly organised and well orchestrated process having three phases of morphological changes [1].

In phase 1, chromatin gets condensed at the periphery of nuclei which are reducing in size along with nucleolar disintegration. There is shrinkage of total cell volume, dilation of endoplasmic reticulum and rest all cell organelles gets compactly arranged.

In phase 2, there is formation of membrane bound apoptotic bodies having cytoplasmic and nuclear contents.

In phase 3, the remaining nuclei and cytoplasmic contents get further degenerated. The other mode of cell death is necrosis but apoptosis differs from necrosis by the following [2]: (Fig:1)

a). Apoptosis is the programmed cell death dependent on the internal machinery of the cell. Whereas, necrosis is an unplanned mode of cell death caused by factors external to the cell.

b). Apoptosis has a characteristic morphologic pattern of chromatin condensation and formation of membrane bound apoptotic bodies whereas in necrosis, cell organelles undergo swelling and there is mottled chromatin condensation. The cell membrane ruptures keeping all the cellular contents together.

c). Apoptotic cells undergo rapid recognition and degradation by phagocytes without release of inflammatory contents. Whereas necrosis results in inflammatory tissue injury.

Recent advancements has been made in understanding the pathophysiology of various diseases in which the role of altered apoptosis has been proposed. So, the understanding of apoptosis pathway becomes the initial step which can provide us the various ways to design the future preventive and therapeutic measures for the diseases associated with altered apoptotic phenomenon.

To unlock the mechanism of apoptosis, it is important to understand the key factors taking part in this phenomenon. Caspases (cysteine dependent aspartate specific proteases) play the most important role in processing, propagation and amplification of apoptotic signals that result in destruction of the cells. Caspases are present in the cytoplasm, mitochondrial intermembranous space and nuclear matrix of all the cells. Apoptosis can be initiated by the factors which can be the intrinsic stimuli like mitochondrial factors and extrinsic stimuli like cytokines and other death receptors. The caspases bring about the cleavage of various vital cellular proteins after an aspartic acid molecule that ultimately leads to cellular death [3,4]. There are at least 14 members of this family which have been identified in mammals [5] and can be divided into 3 subtypes depending on the presumed role in apoptosis [6]. These include initiators caspases (caspase 2, 8, 9,

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executioner caspases (caspase 3, 6, 7) and participants in cytokine activation (caspase 1, 4, 5, 11, 12, 13, 14). Once the intrinsic or extrinsic stimulus is given to the cell, the initiator caspases gets activated which then activate the executioner caspases. The activated executioners bring about the cleavage of cellular key proteins required for the maintenance of homeostasis leading to the collapse and demise of the cell.

Various studies have indicated that caspases cleave a set of key proteins like membrane associated microfilaments that play a role in the accomplishment of apoptotic morphology. But the exact mechanism of how the degradation of these proteins results in apoptotic morphology remains unknown.

1.Pathway of apoptosis

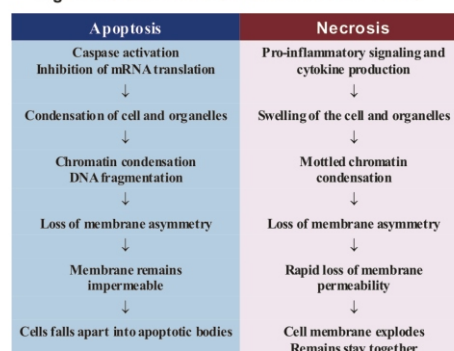
Two pathways of caspase activation have been characterized [7,8]

One (**Extrinsic or ligand receptor pathway**) is initiated outside the cell by the stimulation of proapoptotic signals or receptor ligands on the cell surface when the extracellular environment is not appropriate. These ligands include CD95L/FasL and binds to the corresponding receptors like CD95/Fas [7-10]. Once the ligand and receptor binding occurs, there is clustering and recruitment of adaptor protein Fas associated death domain (FADD) and the initiator caspases 8 or 10, thereby forming death inducing signaling complex (DISC)[11-14]. Formation of DISC leads to caspase 8 activation which then activates the executioner caspases [15]. DISC action is inhibited by the interaction of FADD with c-FLIP(Flice inhibitory protein) and therefore caspase activation is inhibited [16]. (Fig:II)

The second (**intrinsic or mitochondrial pathway**), gets started from within the cell in response to signals as a result of DNA damage, p53, hypoxia, any defect in cell cycle etc [17-20]. This pathway is also called as mitochondrial pathway as the main regulators of this pathway are the proteins of BCL-2 family which regulate the mitochondrial membrane permeability. This family includes two types of proteins- proapoptotic proteins (BAK,BAX,BAD,BIK) and antiapoptotic proteins(BCL-2,BCL-XL,MCL-1) [21]. The balance between proapoptotic and antiapoptotic proteins is an important checkpoint of apoptosis pathway. Any intrinsic injury within the cell leads to the apoptotic signals to the cell components and stimulation of proapoptotic proteins like BAK/BAX. This leads to the mitochondrial membrane permeabilisation and translocation of cytochrome c and SMAC/DIABLO proteins from the intermembrane space into the cytosol [22]. The released cytochrome c binds to the apoptotic protease activating factor (Apaf-1) and forms apoptosome. Then there occurs recruitment of procaspase 9 into the apoptosome leading to the activation of caspase9 [23]. This ultimately activates the executioner caspases which further cleaves cellular proteins. SMAC/DIABLO protein released from the mitochondria promotes apoptosis by directly interacting with inhibitors of apoptotic proteins(IAPs) and disrupting their ability to inactivate caspases. The IAPs are the important regulators of the apoptosis pathway and the IAP gene family constitutes around 7 members. These IAPs directly bind caspases particularly caspase 3,7 and 9 and inhibit them [24-26]. The balance between IAPs and their negative regulators (SMAC/DIABLO) is another checkpoint of apoptosis

pathway. The imbalance caused due to overexpression of IAPs or downregulation of their negative regulator proteins may suppress apoptosis. This is a very common event seen in a variety of carcinoma cell lines and primary tumor samples [27] and is the common baseline for the active investigations regarding the role of IAPs and other regulators in preventive and therapeutic measures. (Fig:III)

Fig I: PROGRAMMED CELL DEATH PATHWAYS



Differences between two modes of cell death: Apoptosis and Necrosis

Fig II: Extrinsic pathway (Death ligand pathway)

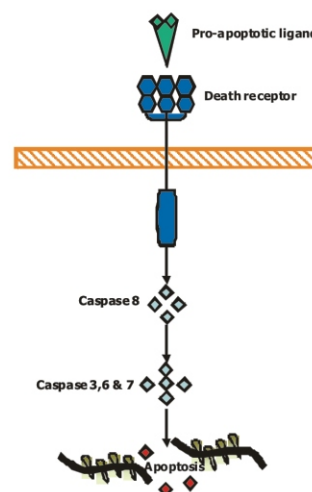
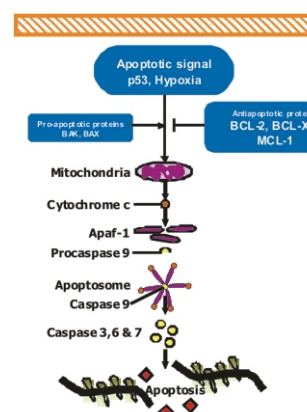


Fig III: Intrinsic pathway (Mitochondrial pathway)



2. Conclusion:

Apoptosis has been found to be a ubiquitous way of cell death in both physiological and pathological conditions. It has also been proposed that diseases can arise from dysregulation of cellular suicide mechanism such as apoptosis. Both intrinsic and extrinsic pathways of apoptosis leads to the cellular death. This process is intricately and precisely regulated inside the cell by various factors. Once the understanding of the apoptotic pathway comes to the edge of our knowledge, it becomes the next step to identify the methods to localise the apoptotic cells in the tissues and this identification can be based on the events occurring during apoptosis pathway like the cleavage of cellular proteins, activation of certain caspases and antibodies directed against the degraded proteins. If we achieve the goal of localizing the apoptotic cells in a tissue, it can lead us to correlate the pathophysiology of various proposed diseases with altered apoptosis and then various antiapoptotic manipulations can be applied to bring about protective and preventive measures against these diseases.

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