Review Article

Apoptosis: Molecular mechanism

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ABSTRACT

Cell death is one of the essential processes. Balance between cell division and cell death is of utmost importance for the development and maintenance of multi-cellular organism. Disorders of either process have pathologic consequences and can lead to disturbed embryogenesis, neurodegerative diseases, or the development of cancers. This article reviews the apoptotic as well as anti-apoptotic molecules along with molecular pathways, which may alter in many diseases.

Key words: Apoptosis, caspase, p53, Bcl2 family

INTRODUCTION

Apoptosis, commonly referred to as "cell suicide," is a highly conserved process in most eukaryotes, and is just as essential for normal cell development as mitosis. Within the human body alone, over 10 billion cells are made each day to balance those dying of apoptosis. The term apoptosis comes from Greek origin: *apo* meaning "from" and *ptosis* meaning "falling" or together, "dropping off."[1]

Apoptosis is characterized by specific biochemical and morphological features that culminate in shrinkage of the cell to apoptotic bodies that are engulfed by neighboring macrophages.[2] Apoptosis occurs during normal cell turnover, tissue homeostasis, embryogenesis, induction and maintenance of immune tolerance, development of the nervous system, and endocrine-dependent tissue atrophy.[3] There are various forms of cell death. Amongst these, two well-described pathways are necrosis and apoptosis. Other less described cell death pathways are mitotic catastrophe, autophagy, slow cell death, and paraptosis.[4]

MECHANISMS OF APOPTOSIS

The molecular mechanisms of apoptosis were highly conserved through evolution so that higher organisms, including humans and other mammals, possess an apoptotic program that, albeit more complex, which exhibits significant similarities to the one described in *C. elegans* (nematode worm *Caenorhabatitis elegans*). A number of physiological and pathological stimuli including lack of nutrients, activation of cell surface death receptors, chemicals, ionizing radiation, and direct physical injury can activate the apoptotic program. These stimuli activate different pathways leading to apoptosis[5] [Figure 1].

Major inducers of apoptosis

1. Irreparable DNA damage
2. Cell cycle perturbation
3. Aberrations in cells metabolism
4. Loss of extracellular molecules that inhibit cell death or promote cell survival
5. Death ligands, e.g., Fas ligand
6. Growth factor withdrawal
7. Calcium influx
8. Glucocorticoids
9. Free radicals
10. Heat shock
11. Viral infections and bacterial toxins
12. Radiation therapy
13. Chemotherapy drugs

Upon receiving specific signals instructing the cells to undergo apoptosis, a number of distinctive changes occur in the cell. A family of proteins, known as caspases, is typically activated in the early stages of apoptosis. These proteins breakdown or cleave key cellular components that

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are required for normal cellular function including structural proteins in the cytoskeleton and nuclear proteins such as DNA repair enzymes. The caspases can also activate other degrading enzymes such as DNases, which begin to cleave the DNA in the nucleus. Apoptotic cells display distinctive morphology during the apoptotic process. Typically, the cell begins to shrink following the cleavage of laminin and actin filaments in the cytoskeleton. The breakdown of chromatin in the nucleus often leads to nuclear condensation and in many cases, the nuclei of apoptotic cells take on a "horse-shoe"- like appearance. Cells continue to shrink, packaging themselves into a form that allows for their removal by macrophages. These phagocytic cells are responsible for clearing the apoptotic cells from tissues in a clean and tidy fashion that avoids many of the problems associated with necrotic cell death. In order to promote their phagocytosis by macrophages, apoptotic cells often undergo plasma membrane changes that trigger the macrophage response. One such change is the translocation of phosphatidyl serine from the inside of the cell to the outer surface. The end stages of apoptosis are often characterized by the appearance of membrane blebs or blisters process. Small vesicles called apoptotic bodies are also sometimes observed.

Caspase family

The caspases are a conserved family of enzymes that are essential for initiation and execution of apoptosis. The term caspases comes from cysteine-dependent aspartate-specific protease: Their proteolytic activity cleaves their substrate after Asp residues. Their activity is irreversible, and once activated, commits the cell to death. An initiator caspase invariably contains an extended N-terminal prodomain (>90 amino acids) important for its function, whereas an effector caspase frequently contains 20-30 residues in its prodomain sequence. All caspases are synthesized in cells as catalytically inactive zymogens and must undergo an activation process. The activation of an effector caspase, such as caspase-3 or -7, is performed by an initiator caspase, such as caspase-9. An initiator caspase, however, is autoactivated under apoptotic conditions, a process usually requiring and facilitated by multi-component complexes. For example, the apoptosome is responsible for the activation of caspase-9. The effector caspases, most importantly caspase-3, are responsible for the morphologic and biologic changes seen in apoptotic cells. Their targets include structural component (actin and nuclear laminin), regulatory protein, and caspase-activated deoxyribonuclease (CAD) activation. CAD is responsible for the degradation of the chromosome, chromatin condensation, and formation of distinctive DNA fragments. Completion of the execution pathway leads to phagocytic uptake, in the final step of apoptosis. Externalization of phosphatidylserin on the surface of apoptotic cells causes non-inflammatory phagocytic recognition, uptake, and disposal of the cell [Table 1].[1]

Apoptosis can be classified as caspase-dependent and caspase-independent apoptosis [Figure 2].

Caspase-dependent apoptosis

Caspases are the cysteine protease enzymes. These are key mediators of apoptosis. They are essential for the initiation and execution of apoptosis. They are inactive in normal conditions but get activated by the various apoptotic stimuli. These proteins breakdown key cellular components and also activate other enzymes.

Table 1: Types of caspases

Type of caspases	Mammalian caspases
Initiator caspases	2, 8, 9, 10 (Ced-3 in C. elegans)
Effector caspases	3, 6, 7
Inflammatory caspases	1, 4, 5
Other cellular caspases	11, 12, 13, 14

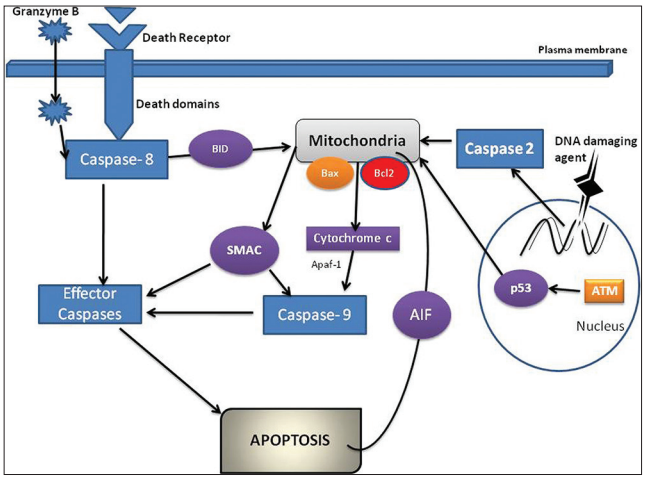

Figure 1: Synopsis of the basic molecular mechanisms of apoptosis

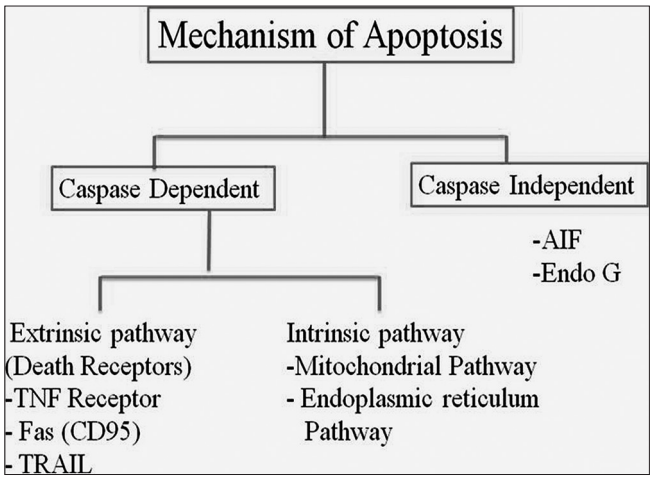

Figure 2: Schematic representation of mechanism of apoptosis

The caspase-dependent apoptosis is divided into intrinsic and extrinsic apoptotic pathways.[5]

Extrinsic pathway

Extrinsic pathway is mediated by death receptors. They play an important role in apoptosis and can activate a caspase cascade within seconds of ligand binding. Therefore, induction of apoptosis via this mechanism is very rapid [Figure 3].

Induction of apoptosis by TNF receptor

TNF receptor are specific cell membrane receptors, which belong to tumor necrosis factor (TNF) family, are collectively known as death receptors, including TNF-receptor 1, Fas, DR3, DR4, DR5, and DR6. Upon binding of specific ligands, such as TNF-α, lymphotoxin, Fas ligand, and TRAIL, these receptors undergo conformational changes that allow them to interact with specialized intracellular adaptor proteins.[5,6] Following ligand binding, a conformational change in the intracellular domains of the receptors reveals the presence of a "death domain" such as TRADD (TNFR-associated death domain), which allows the recruitment of various apoptotic proteins to the receptor. This protein complex is often called the DISC or death inducing signaling complex. The final step in this process is the recruitment of one of the caspases, typically caspase 8, to the DISC. This results in activation of caspase 8 and the initiation of apoptosis. TRADD can also associate with FADD (FAS-associated death domain), which leads to the induction of apoptosis via the recruitment and cleavage of pro-caspase 8.[6]

Intrinsic pathway

The other mechanism of caspase-dependent apoptosis is the intrinsic pathway, also called mitochondrial-mediated apoptosis. This pathway is induced by intracellular signals, such as hypoxia, radiation, viral infections, and notably, DNA damage. The Bcl2 families of proteins are the main mediators of this process. After release of specific pro-apoptotic proteins, such as cytochrome c, smac/DIABLO, AIF and Endo G, the execution pathway begins with activation of caspase-3. The intrinsic pathway is primarily dominated by the Bcl2 family of proteins. This family has up to 4 conserved domains, known as the Bcl2 homology (BH) domains. The Bcl2 family contains both pro-apoptotic members and anti-apoptotic members. The pro-apoptotic members can be further subdivided in to the multi-domain proteins (also known as the Bax family), that consist of Bax, Bok and Bak, and BH-3 only proteins, such as Bid, Bad, and Bim. These proteins, despite their homology, are localized in different regions of the cell. In the normal cell, Bax is found in the cytosol. Both of these proteins undergo conformational changes to render them active upon apoptotic stimulation. The anti-apoptotic members of the Bcl2 family contain all 4 conserved domains, such as Bcl2 and Bcl_{xl}. Bcl2 are found exclusively within intracellular membranes and within the cytosol. These proteins function by preventing apoptosis through heterodimerization with pro-apoptotic proteins and self over-expression. The main purpose of the Bcl2 family is to release apoptotic-signaling factors from the mitochondria through mitochondrial outer membrane permeabilization (MOMP) via opening of the membrane permeability transition pore. This achieved through oligomerization of pro-apoptotic Bcl2 members on the outer mitochondrial membrane, interaction with membrane pore channels and/or loss of mitochondrial membrane potential. This releases two groups of pro-apoptotic proteins from the inter-membrane space into cytosol. The first group of proteins released are cytochrome c, Smac/DIABLO, and HtrA2/Omi, and these are caspase-dependent, meaning they work downstream of the caspase cascade, leading to apoptosis. Its main purpose is to bind and activate Apaf-1 and procaspse-9, leading to formation of the apoptosome. This leads to the activation of caspase-9 and subsequently, effector caspase-3 and -7, which finalizes the apoptotic pathway.

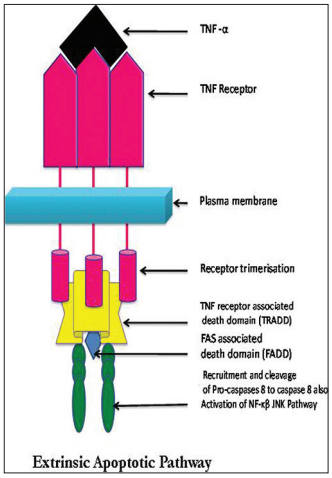

Figure 3: Extrinsic apoptosis pathway

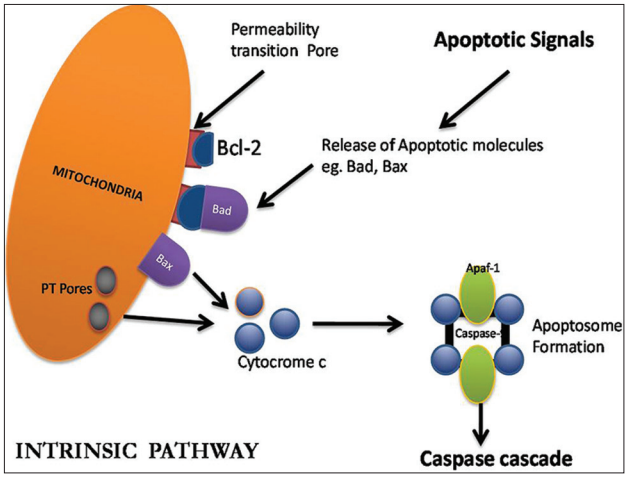

Figure 4: Intrinsic apoptosis pathway

It is the apoptosome formation and effector caspase activation that causes the token apoptotic events, such as chromatin condensation, plasma membrane asymmetry, and cellular blebbing[3,5,6] [Figure 4].

Caspase-independent apoptosis

The proteins involved in the caspase-independent apoptosis induce the decision of apoptosis upstream of caspase activation. In caspase-independent apoptotic mechanism, various factors are involved, like apoptosis-inducing factor (AIF), endonuclease G, Omi/HtrA2, and endoplasmic reticulum.[4]

Apoptosis-inducing factor

Apoptosis-inducing factor is a mitochondrial protein that plays a pivotal role in PCD. AIF becomes an active cell killer when it is released to the cytosol; it then translocates to the nucleus and triggers, possibly together with endonuclease G, peripheral chromatin condensation, and high molecular weight (50 kb) DNA loss. The lysosomal protease cathepsin D trigger AIF release independent of the caspase cascade. The lethal effects of AIF are controlled by the anti-apoptotic protein heat shock protein 70 that interacts with AIF and protects against its apoptogenic effects.[4]

Endonuclease G

Endo G like AIF translocates to the nucleus and along with AIF triggers the apoptosis.[4]

Endoplasmic reticulum

The ER is an important sensor of cellular stress that can withhold protein synthesis and metabolism to restore cellular homeostasis. If the damage to the ER is too extensive, this can initiate PCD via the unfolded protein response or via release of calcium into the cytoplasm. This leads to activation of caspase 12, possibly via translocation of the Bcl-2 family member Bim to the ER. Caspase 12 in its inactive state is localized at the cytosolic face of the ER, but it triggers downstream caspases and apoptosis when it becomes activated. In addition and independent of caspase 12 activation, ER stress can induce permeabilization of the mitochondrial membrane and thus activates the classic apoptotic pathway as well as other mitochondrial death pathways. Bcl-2 family proteins as well as cytoplasmic calcium shifts orchestrate the cross-talk between the mitochondria and the ER.[4]

Inhibitor of apoptosis proteins

The IAPs are a family of proteins characterized by one or more 70-80 amino-acid baculoviral IAP repeat (BIR) domains. So far, eight human IAP homologues have been identified, among others NAIP, c-IAP1, c-IAP2, XIAP, and Survivin. The IAP proteins function as endogenous caspase inhibitors, as well as participate in cell cycle regulation and in the modulation of receptor-mediated signal transduction.[7,8]

Survivin

Survivin is a 16.5 kDa structurally unique IAP protein, organized as a stable dimer, and containing a single BIR and a –COOH terminus coiled-coil, but no other identifiable domain. Survivin is a mitotic gene, whose expression at cell division is tightly transcriptionally-controlled. Survivin cytoprotection may be more selective than that of other IAPs.

Functions of survivin

1. Survivin prevent caspase-9 activation within a functional apoptosome and its modulation by binding to mitochondrially-released Smac/DIABLO.
2. Control of mitosis- Survivin can enhance microtubule stability in metaphase spindle formation.[9]

p53: Guardian of the genome

The tumor suppressor protein p53 was discovered independently by David Lane and Arnold Levine in 1979 as a cellular protein in complex with the large T antigen (LT) of SV40.[10] The p53 gene is a DNA-binding protein localized to the nucleus; and is located on chromosome 17p13.1, and it function primarily by controlling the transcription of several other genes. p53 is the most common target for genetic alteration in human tumors; suggesting that the p53 protein functions as a critical gatekeeper against the formation of cancer.[11]

The response of cells to DNA-damaging agents is complex, involving recognition and repair of the lesions in DNA to minimize the risk of genetic instability. A central player in protecting the integrity of the genome is p53 (human p53 gene or protein), which is present at low levels under unperturbed conditions but becomes rapidly stabilized and activated in response to a variety of stimuli including DNA damage.[12]

Ataxia telangiectasia mutated (ATM) activated by mechanism that sense double-stranded DNA breaks. Transmit signals to arrest cell cycle after DNA damage. It acts through p53 in the G1-S checkpoint. Primary regulation of p53 is conducted by Mdm2, which is a product of a proto-oncogene, amplified or otherwise over-expressed in a significant number of human tumors. Its binds tightly to p53 and renders it inactive. P53 has been referred to as the 'guardian of the genome' due to its ability to decide the fate of the cell. The cell has a choice to either arrest in G1/S phase and repair the DNA damage or choose apoptosis if the damage is too extensive. If the cell is chosen for apoptosis, p53 mediates this decision through activation and regulation of pro-apoptotic Bcl-2 family members.[1]

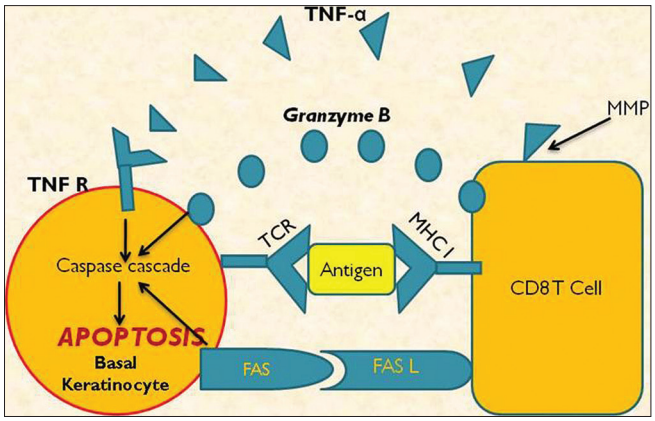

Figure 5: Pathogenesis of lichen planus; TCR- T cell receptor, MHC- Major histocompatibility receptor, TNF- Tumor necrosis factor, MMP- Matrix metalloproteinases

Physiological functions of p53:[1]

a. DNA repair via expression of GAAD45.
b. Inhibition of angiogenesis through Thrombospondin-1 (TSP-1) and VEGF expression.
c. Inhibition of metastasis through increased expression of maspin.
d. Most importantly, cell cycle arrest and apoptosis

Diseases associated with apoptotic deficiency

Oral cancer and, in particular, squamous cell carcinoma have been repeatedly linked to apoptotic dysregulations. Mounting evidence suggests that oral carcinogenesis is correlated with a progressive accumulation of genetic alterations in molecules that play crucial roles during apoptosis. The presence of genetic changes in pre-cancerous lesions of oral mucosa underscores the significance of apoptotic deviations during the early steps of malignant transformation.[5,13,14] Reduced apoptotic index and altered p53, MDM2, bcl-2, bcl-xL, and bax have been detected in salivary gland tumors, lymphomas, and sarcomas of the oral and maxillofacial region.[5] Links between common oral diseases of possible autoimmune etiology and apoptosis have been suggested. For example, phagocytosis of apoptotic cells by intraepithelial mononuclear leukocytes has been observed in recurrent aphthous stomatitis.[5,15] In addition, lichen planus, a mucocutaneous disorder with frequent oral involvement, is characterized by common detection of apoptotic bodies in the basal layer of epithelium corresponding to a significant increase in apoptosis within epithelium compared to normal oral mucosa. These changes have been linked to alterations in the protein expression of various apoptotic molecules in lichen planus, including Fas, FasL, p53, and members of the bcl-2 family[5,16] [Figure 5].

Diseases associated with excess apoptosis

Acquired immune deficiency syndrome (AIDS), hematologic diseases like aplastic anemia and cyclic neutropenia, myocardial infarction, various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, spinal muscular atrophy, and amyotrophic lateral sclerosis are associated with increased apoptosis.[5]

CONCLUSION

Apoptosis has emerged over the last three decades as an important biological process involved in normal physiology and in the pathogenesis of a variety of diseases. The elucidations of the molecular mechanisms of apoptosis and their correlation with a vast array of human diseases have generated optimism for implementation of this knowledge for treatment purposes.

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