REVIEW ARTICLE

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Fundamental Molecules in the Pathways and Regulation of Apoptosis

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Abstract

Apoptosis plays a role in many events such as the remodeling of cells starting from embryo formation, the elimination of faulty or dysfunctional cells, and is important for cellular balance. Bcl-2 family and various mediators, of which caspases are the basis, play a role in maintaining this balance in apoptosis. These mediators are important in the initiation, continuation and arrest of apoptosis pathways, and in the regulation of cellular balance. Damages to these mechanisms can cause undesirable results and diseases. In this review, we tried to give information about the pathways of apoptosis and the basic molecules involved in the regulation of apoptosis.

Keywords: Apoptosis, Bcl-2, caspase, pathways, regulation.

INTRODUCTION

Apoptosis occurs throughout life, starting from the embryonic period. It is a programmed cell death that is genetically regulated in eliminating unnecessary tissues, reorganizing them, eliminating cells that have completed their life, and maintaining intracellular balance (Khan et al., 2010). It consists of the words 'apo' and 'ptosis', which are used to mean separate and fall. It is an energy requiring process (Karalezli, 2016). First, in Kerr et al., (1972) observed condensed chromatin fragments in the nuclei of cells that died physiologically in their microscopic examination and, realizing that the organelles were preserved, they defined this phenomenon as "shrinkage necrosis". While *Caenorhabditis elegans* nematode, which is frequently used in apoptosis studies, passed from the hermaphrodite period to the adult stage, a decrease of 131 cells was determined in the number of cells that apoptosis result was 1090 (Hengartner and Horvitz, 1994). The cell death abnormal-3 (ced-3), cell death abnormal-4 (ced-4), and cell death abnormal-9 (ced-9) genes are involved in the regulation of apoptosis in these nematodes. It was observed that when ced-3 and ced-4 of these genes are inactivated, apoptosis is not observed in cells and these genes are the genes that stimulate apoptosis, while the cell death abnormal-9 (ced-9) gene is the gene that suppresses apoptosis. These genes have been named as caspases (Cysteine aspartate-specific proteases), Apaf-1 (Apoptotic protease activating factor) and Bcl-2 (B-cell lymphoma protein-2) accordingly in humans (Hengartner, 1996).

MORPHOLOGICAL CHANGES OBSERVED IN APOPTOSIS

The main structural changes observed in apoptosis are cell shrinkage, chromatin condensation and subsequent fragmentation. This fragmentation, which causes the DNA to show blebs in fluorescent staining, occurs through the enzyme endonuclease, and DNA is fragmented from its internucleosomal domains. When immune electrophoresis is performed, a ladder-like structure called 'ladder pattern' is observe (Wyllie, 1980). Budding forms on the cell membrane of apoptotic cells and the cell fragments into apoptotic bodies (Figure 1) (Cotter et al., 1992). These cells are then phagocytosed by macrophages and neighboring cells without the occurrence of inflammation (Kerr et al., 1972).

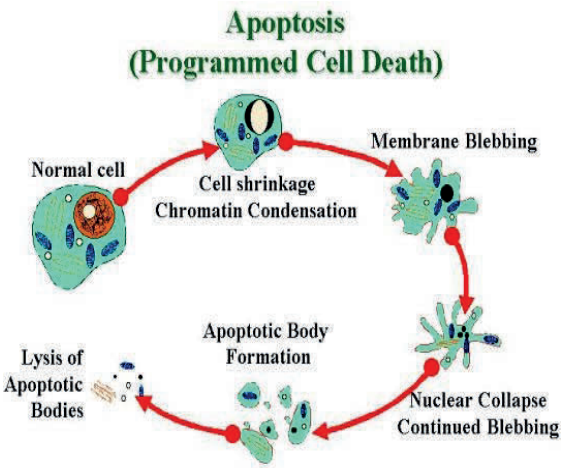

Figure 1. A schematic of a cell in apoptosis (https://www.quora.com/Why-do-cells-undergo-apoptosis)

MECHANISMS OF APOPTOSIS

Extrinsic and intrinsic pathways constitute the basis of apoptosis. Proteases called caspases are involved in these pathways, and both pathways enter a common pathway with the conversion of procaspase-3 to its active form. In addition to these pathways, there is also the

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perforin/granzyme pathway in which cytotoxic T cells and perforin/granzymes are involved (Elmore, 2007).

Extrinsic Pathway

Transmembrane receptors, which are included in the TNF (tumor necrosis factor) receptor family, are involved in this pathway. These receptors have a death domain containing almost 80 amino acids in their structure responsible for signal transmission (Park et al., 2014). Events occurring in the extrinsic pathway are schematized by FasL/FasR (Fas ligand/Fas Receptor) and TNF-α/TNFR1. The interaction of the Fas receptor with its ligand provides binding to the death domain FADD (Fas associated death domain) which the Fas receptor is associated. The interaction of the TNF receptor with its related ligand allows the TNF receptor to bind to TRADD (TNFR-1-related death domain) via FADD and RIP (receptor interacting protein). Subsequently, FADD dimerizes and can thus bind to procaspase-8. This binding causes the formation of the DISC complex (death inducing signaling complex) and the conversion of procaspase-8 to the caspase-8 form by autocatalytic activation (Falschlehner et al., 2007). If active caspase-8 is at high levels, it is degraded to terminator caspases such as caspase-3 as a result of autoproteolytic cleavage, or activated caspase-8 is triggered by caspase-8 signaling to break Bcl-2 family members Bid and release cytochrome-c from mitochondria to stimulate apoptosis. Depending on this, it also mediates the realization of events that result in caspase-3 activation (Mayer and Oberbauer, 2003). This pathway can be suppressed by a protein called c-FLIP (cellular FLICE-like inhibitory protein) (Golks et al., 2005).

Intrinsic Pathway

Instead of receptors, many stimuli such as growth factor deficiency, oxygen deficiency, high temperature, viral factors play a role in the initiation of this pathway. These stimuli cause Bcl-2 family member and apoptosis-stimulating proteins to migrate to mitochondria and attach to the mitochondrial outer membrane, resulting in the formation of the mitochondrial transition pore (Giorgi et al., 2012). This pore is involved in the release of cytochrome-c from the mitochondria to the cytosol (Giorgi et al., 2012).

Cytochrome-c then forms a complex called apoptosome with Apaf-1, ATP and procaspase-9. The apoptosome complex is involved in the transition of procaspase-9 to its active caspase-9 form (Elumalai et al., 2012). The conversion of procaspase-9 to its active form can be inhibited by a protein called Aven (Chau et al., 2000). Activated caspase-9 causes caspase-3 activation as at the end of the extrinsic pathway. Proteins such as AIF (apoptosis-inducing factor), endonuclease G and Smac/DIABLO and Omi/HtrA2, which translocate to the nucleus and cause DNA segmentation and chromatin condensation, are also released from the mitochondria and neutralize the effect of IAPs (apoptosis-inhibiting protein) on apoptosis (Savitskaya and Onishchenko, 2015).

Perforin/Granzyme Pathway

In this pathway, apoptosis is induced via GranzymeA or GranzymeB. Caspase-3 activation is observed in all three of the extrinsic, intrinsic and GranzymeB pathways. Cytotoxic T cells and natural killer (NK) cells contain perforin and granzyme proteins in their secretory granules. Secreted perforins cause pore formation in the cell after the interaction of cytotoxic T cells with the target cell (Turner et al., 2019). While Granzyme B enables the conversion of caspase-3 to the active form, either directly or by the activation of procaspase-10; granzyme A stimulates the cell to enter the apoptosis pathway by single-stranded DNA damage. (Martinvalet et al., 2005).

Path of Execution

It begins with the activation of terminator caspases to the active form in the final stage of the extrinsic and intrinsic pathways. Terminator caspases are involved in the activation of endonucleases, which cause nuclear material fragmentation, and proteases responsible for cytoskeletal proteins and nuclei fragmentation. Caspase-3, has an important role in apoptosis. Caspase-3, leads to the activation of the endonuclease CAD (caspase-activated DNase) and cleaves the CAD inhibitor, ICAD, leaving CAD free for DNA fragmentation and chromatin condensation and apoptosis is terminated by phagocytosis of apoptotic cells (Larsen and Sørensen, 2017).

REGULATION OF APOPTOSIS

Regulation of the apoptotic process is provided by many molecules. Bcl-2 family proteins, caspases, mitochondria, cytochrome-c, calcium are among the molecules responsible for regulation (Akşit et al.,2008)

Calcium

During apoptosis, the cell is exposed to a constant flow of calcium. As a result of the increase in intracellular Ca^{+2} concentration, endonucleases are activated and this causes the initiation of the cell death cascade. The increase in calcium concentration occurs with the increase of Ca^{+2} influx from Ca^{+2} channels and Ca^{+2} release from organelles such as mitochondria and ER (Sukumaran et al., 2021).

Bcl-2 Family

There are antiapoptotic and proapoptotic members of this protein family that suppress and stimulate apoptosis. Although the Bcl-2 family does not differ much in structure, they have similar protein structures with α helix tangles. These are called BH domains and are numbered up to 4. In addition, they are examined in 3 main groups (Gross et al., 1999).

Group I: Proteins in this group are located in the mitochondrial outer membrane, nuclear membrane, and endoplasmic reticulum and are involved in the suppression of apoptosis by inhibiting the release of procaspase, AIF and cytochrome-c. Bcl-2, Bcl-xL, Mcl-1, Bcl-w, Bfl-1/A1, boo proteins constitute the members of this group (Gross et al., 1999).

Group II: Mostly containing 3 sometimes 2 BH domains, these group members are Bax, Bak, Bcl-Xs. They are present in the cytoplasm and, unlike group I, they stimulate apoptosis by positively affecting the release of AIF and cytochrome-c (Gross et al., 1999).

Group III: Among the group members are BH3 interacting-domain death agonist (Bid), Bcl-2-interacting killer (Bik), Bcl-2-interacting mediator of cell death (Bim), Bcl-2-associated death promoter (Bad), p53 upregulated modulator of apoptosis (Puma), NADPH oxidase activator (Noxa), which contain only the BH3 domain and are associated with the mitochondrial outer membrane. Members of this group connect both with each other and with mitochondrial membrane proteins. They

play a role in the release of cytochrome-c into the cytosol by forming a pore in the mitochondrial membrane. Proteins in this group do not exist in the active form and after activation, they allow the activation of group II proteins (Gross et al., 1999).

Caspases

Cysteine aspartate-specific proteases are released in the form of procaspase and are activated by cleavage of the protein from the aspartate amino acid after stimulating apoptosis. All caspases contain catalytic small and large subunits in their structure. It is associated with the length and shortness of the N-terminal front region found in the classification structures of caspases classified as initiator and terminator. A long N-terminal anterior region is observed in initiator caspases, while a short N-terminal anterior region is observed in terminator caspases (Stennicke and Salvesen, 2000).

Caspase 3-6-7-14 terminator, caspase 1-2-4-5-8-9-10-11-12-13 are initiator caspases and initiator caspases contain a CARD (caspase-recruitment domain) or DED (death effector domain) domain in the long N terminal region. Of these caspases, caspase-8 and caspase-10 have a DED domain, while other initiator caspases have a CARD domain. Terminator caspases do not have CARD and DED domains (Ramirez and Salvesen, 2018). Caspase-8, 9, 10 causes caspase-3 activation and initiation of the execution phase (Ramirez and Salvesen, 2018).

Inhibitors such as XIAP (X-linked apoptosis protein inhibitor) and IAPs can prevent caspase-3 activation (Nachmias et al., 2004). In addition, caspase-1, 4, 5, 11, 12, 14 plays different roles such as inflammation and keratinocyte maturation. Additionally, caspase-2 specifically reverts to its active form with the PIDDosome complex. This complex consists of PIDD, RAIDD and caspase-2 and is stimulated by p53 as a result of DNA damage (Parrish et al., 2013).

p53

The p53 gene, which is responsible for the regulation of apoptosis and tumor suppression as a result of oxygen deficiency and the formation of free radicals, provides the necessary time for DNA repair by preventing the cell from transitioning to S phase when DNA damage occurs. If there is a damage that cannot be repaired, it stimulates apoptosis by increasing the production of Fas, Bax and Apaf-1 and inhibiting Bcl-2, Bcl-xL. The p53 gene is suppressed by the transcription regulator factor Mdm-2 (murine double minute2) (Vousden and Lu, 2002).

Fas/Apo-1/CD95

Fas receptor, which is a member of TNF receptor family and located on the surface of natural killer and cytotoxic T cells, is activated as a result of its association with Fas protein, and when it binds to the FADD molecule, procaspases turn into active form and apoptosis is stimulated (Volpe et al., 2016).

Apaf-1

It is a protein that plays a role in the initiation of apoptosis and has CARD and ATPase regions in its structure. After cytochrome-c is released into the cytoplasm in the intrinsic pathway, its structure changes as a result of the interaction of cytochrome-c with the CARD domain. And through the CARD domain, Apaf-1 and caspase-9 interact to form apoptosome complexes. This complex is involved in the transition of effector caspases to the active form (Droin and Green, 2004).

Cytochrome-c

It is an important protein in the intermembrane space of mitochondria and is involved in the production of ATP. Observation of cytochrome-c in the cytoplasm indicates that the cell is in an irreversible process of apoptosis. Although the transition of cytochrome-c to the mitochondrial outer membrane remains unclear, some members of the Bcl-2 family are involved in the regulation of this process (Gross et al., 1999).

RESULT

Many molecules are involved in apoptosis, which play a role in the elimination of unwanted, expired cells, mediating the ability of cells to perform their vital activities, and many pathways are observed for the continuation of the apoptosis process. In this review, we have mentioned the main molecules involved in the pathways and regulation of apoptosis. We think that as a result of the studies, more molecules will be discovered and the mechanisms will be understood more clearly, so that it will be a guide in terms of the occurrence and treatment of many diseases.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authorship contributions

Concept: S.T., Design: S.T., B.O., Data Collection or Processing: S.T., B.O., Analysis or Interpretation: S.T., B.O., Literature Search: B.O., Writing: S.T., B.O.

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