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FUNDAMENTALS OF APOPTOSIS

OSNOVE APOPTOZE

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Summary

Apoptosis is evolutionary conserved, programmed pattern of cell death with an essential role in various physiological processes, such as normal cell turnover and embryonic development, hormone – regulated cell demise, aging, immune system functioning and development and removal of defective and harmful cells. There are two general pathways for activation of apoptosis: the intrinsic and extrinsic pathways. While the intrinsic apoptotic pathway can be triggered by a cytotoxic accumulation of intracellular Ca^{2+}, followed permeabilization of mitochondrial membrane and release of pro-apoptotic proteins into the cytosol from mitochondria, the extrinsic mechanisms of apoptosis include the participation of death receptors of tumor necrosis factor-α (TNF-α), receptor superfamily such as TNFR-1, Fas, and TNF-related apoptosis – inducing ligand receptors (TRAIL-R) located on the plasma membrane. There is also the perforin – granzyme pathway that involves T-cell mediated cytotoxicity. All three pathways converge on the same execution pathway, resulting in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross – linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells. In this review we summarize data from recent studies focusing on apoptotic proteins that have been identified and molecular mechanisms of apoptosis. Understanding apoptotic mechanism might provide useful information and a new approach to prevention and development of new therapies for variety of diseases.

Key words: *apoptosis, programmed cell death, intrinsic/extrinsic pathway, perforin-granzyme pathway, caspase.*

Sažetak

Apoptoza je evolutivno očuvan mehanizam programirane ćelijske smrti, koji ima važnu ulogu u fiziološkim procesima, kao što su embrionalno razviće, hromonima regulisana ćelijska smrt, funkcionisanje imunog sistema i uklanjanje oštećenih ćelija. Dva osnovna puta indukcije apoptoze su unutrašnji i spoljašnji. Dok unutrašnji put apoptoze može pokrenuti unutarćelijska akumulacija Ca^{2+} jona, koja je praćena permeabilizacijom membrane mitohondrija i oslobađanjem pro-apoptotskih proteina iz mitohondrija u citoplazmu, spoljašnji put uključuje aktivaciju membransih receptora za TNF-α (engl. Tumor Necrosis Factor-α), kao što su TNFR-1 (engl. Tumor Necrosis Factor receptor 1), Fas, i TRAIL-R (engl. TNF-related apoptosis-inducing ligand receptors). Pored toga, postoji i perforin-granzim put koji uključuje aktivaciju citotoksičnih T-limfocita. Sva tri puta rezultiraju fragmentacijom DNK, degradacijom citoskeleta i nuklearnih proteina, unakrsnim povezivanjem proteina, formiranjem apoptotskih tela, ekspresijom liganada za receptore na fagocitima i na kraju fagocitozom. U ovoj reviji su objedinjeni podaci iz nedavno objavljenih studija koje su fokusirane na proteine uključene u proces apoptoze i molekularne mehanizme apoptoze. Razumevanje mehanizama apoptoze može da pruži korisne informacije i nove pristupe u prevenciji i razvoju novih terapija za različite bolesti.

Ključne reči: *apoptoza, programirana ćelijska smrt, unutrašnji/spoljašnji put, perforin-granzim put, kaspaze*

INTRODUCTION

The term apoptosis was first used by Kerr, Wyllie, and Currie in 1972 [1; 2; 3; 4]. Apoptosis is an evolutionary conserved, regulated and programmed pattern of energy – dependent cell death without any following inflammatory reaction [5]. An essential role of apoptosis is well known in various physiological processes, such as normal cell turnover and embryonic development, hormone – regulated cell demise, aging, immune system functioning and development and removal of defective and harmful cells [6]. Apoptotic process is considered to be a homeostatic mechanism and is a defense mechanism when cells are damaged by any disease or harmful agents [6; 7]. Apoptosis includes processes like shrinkage of the cell cytoplasm, cleavage of DNA and condensation of chromatin in the nucleus, consequently forming apoptotic bodies, which all eventually lead to cell death [8].

Two general pathways of apoptosis activation are known: the intrinsic and extrinsic pathways [9]. Extrinsic pathway is regulated through death receptors as opposed to intrinsic pathway, which is regulated by Bcl-2 protein family members. Both pathways are linked and lead to the same result of activation of cascade of proteolytic enzymes and caspase family members [9]. There is also the perforin/granzyme pathway that involves T-cell mediated cytotoxicity [6; 10]. The perforin/granzyme pathway induces apoptosis *via* either granzyme B or granzyme A [6; 10]. The intrinsic, extrinsic and perforin/granzyme pathways converge to the same execution pathway that is initiated by the cleavage of caspase-3 and -7 [6; 10].

Intrinsic Mechanism of Apoptosis

The intrinsic apoptotic pathway can be triggered by a cytotoxic accumulation of intracellular Ca^{2+}, followed by changes in the inner mitochondrial membrane, loss of transmembrane potential and release of pro – apoptotic proteins into the cytosol from mitochondria, which triggers an apoptotic cascade [11; 12].

The Bcl-2 family of proteins is critical in the regulation of the intrinsic apoptotic pathway. These proteins include anti – apoptotic (Bcl-2, Bcl-xL, and Bcl-w) and pro – apoptotic (BAX, Bcl-2 homologous antagonist killer (BAK), Bcl-xS and BAD) groups [8]. The anti – apoptotic members of the Bcl-2 family are localized in the outer mitochondrial membrane and play an important role in maintaining cell survival [8]. Conversely, the pro – apoptotic members have a crucial role in cell death induced by trophic factor withdrawal and injury [8].

Extrinsic Mechanism of Apoptosis

Extrinsic mechanisms of apoptosis, also known as the "death receptor pathway", include the participation of death receptors of tumor necrosis factor-α (TNF-α) receptor superfamily such as TNFR-1, Fas, and TNF – related apoptosis – inducing ligand receptors (TRAIL-R) located on the plasma membrane [4; 11; 13]. These cell surface death receptors bind death ligands such as TNF-α, Fas ligand (FasL), and TRAIL which further result in the activation of initiator caspase-8 and subsequent activation of effector caspase-3 [11]. Namely, a transcription factor forkhead 1 stimulates expression of FasL, resulting in activation of caspase-3 [11]. Furthermore, FasL binds to its receptor and initiates extrinsic mechanisms of apoptosis resulting in recruitment of the cytoplasmic adaptor protein Fas-associated death domain protein (FADD), which binds to procaspase-8 [11]. After just a few seconds of Fas receptor engagement, FasL–Fas–FADD–procaspase-8 complex, also known as death – inducing signaling complex (DISC), is assembled [11]. DISC mediates activation of caspase-8, which is then released into the cytoplasm and initiates activation of caspase-3 by direct or mitochondrial – dependent mechanisms [11], and activated caspase-3 can further cleave procaspase-8 leading to increase of extrinsic cell death [11].

An extrinsic mechanism of apoptosis can also involve the mitochondrial – dependent pathway using an intermediate BH3 interacting-domain death agonist (BID) [8].

Caspase – Dependent Apoptosis

Caspases are the central enzymes of apoptosis involved in processes of initiation and execution [4; 14].

The caspase family is divided into two categories: the caspase – activated DNase (CED) subfamily consisting of caspases-2, 3, 6, 7, 8, 10, which are activated during apoptosis; and the interleukin-1-beta converting enzyme (ICE, also known as caspases-1) subfamily consisting of caspases-1, 4, 5, 11, 12, which undergo activation during inflammatory responses [4]. Caspases can be further divided into initiators (caspases-2, 8, 9, 10, 12) and effectors (caspase-3 and 7) [11]. Initiator caspases cleave inactive pro – forms of other caspases activating them, while effector caspases cleave various cellular proteins to begin apoptosis [8].

Mitochondria play a key role in activation and regulation of apoptosis in mammalian cells [4]. Mitochondria act as a reservoir for multiple apoptogenic proteins such as cytochrome *c* (cyt *c*), small mitochondria – derived activator of caspases/direct IAP-binding protein with low PI (SMAC/DIABLO), apoptosis – inducing factor (AIF), proteases-activating factor-1 (APAF-1), and endonucleases and procaspases-2,3,8,9 [4]. It is believed that Bcl-2 family members regulate the movement of proteins across mitochondrial membranes that once in the cytosol, activate caspase proteases [15]. Cyt *c*, SMAC/DIABLO and the serine protease HtrA2/Omi are the first of pro-apoptotic proteins released from the mitochondrial inner membrane space into the cytosol, followed by procaspase-9, and APAF-1, thus participating in activation of the caspase – dependent mitochondrial pathway [8; 11]. Cyt *c* is able to bind to APAF-1 as well as procaspase-9 to form an "apoptosome" in the presence of deoxy ATP (dATP) or ATP [12], and apoptosome activates procaspase-9, which further activates executor caspase-3 [12]. Caspase-3 can cleave many substrate proteins, like endonucleases, lamin, spectrin, huntingtin, gelsolin, and poly (ADP-ribose) polymerase (PARP), etc. [4; 11; 16]. Inactivation of PARP leads to DNA injury and subsequently to apoptosis, while excessive activation of PARP causes energy depletion and ultimately leads to necrosis [11].

Members of the anti – apoptotic Bcl-2 family as well as anti – apoptotic proteins kinase B (Akt) and extracellular signal – regulated kinase (ERK) are involved in the protection of the mitochondrial integrity by inhibiting pro – apoptotic Bcl-2 family members [11]. On the other hand, these apoptosis inhibitor proteins can be deactivated by the mitochondrial proteins SMAC/DIABLO and OMI/HtrA2, which bind to them and promote apoptosis [12]. Hence, OMI/HtrA2 participates in caspase – dependent cell death, but it can also act as an effector protein in necrosis, using its protease activity [17; 18; 19].

The endonuclease G is also one of mitochondrial proteins that can potentially contribute to both caspase – independent and caspase – dependent apoptosis. Endonuclease G is able to induce DNA fragmentation independent of caspase action in an isolated nucleus [19; 20].

Caspase – Independent Apoptosis

Joza et al. [21] first provided genetic evidence of a mitochondria – regulated cell death pathway independent of APAF-1 and caspase-9 [21]. The same authors reported that the first wave of apoptosis in the early mouse embryo requires the AIF, but not caspases [21]. AIF, first described by Susin et al. [22], is localized in the mitochondria inner membrane space acting as oxidoreductase [19; 22; 23]. Yu et al. [24] also showed that AIF can be released from mitochondria in a caspase – independent manner by excessive Ca^{2+} influx resulting in over-activation of PARP-1 and subsequent cell damage [24]. After AIF is translocated to the nucleus, possibly together with endonuclease G, AIF induces peripheral chromatin condensation and DNA fragmentation [19; 24; 25; 26; 27]. Conversely, AIF may interact with heat shock protein 70 and play an important role in protection against apoptogenic effects of AIF [19; 28]. Certain evidence indicates that AIF can perform as an additional response mechanism to facilitate the completion of caspase – dependent apoptosis, whereas it is capable of executing caspase -independent apoptosis in other cell types [19; 29].

Beside the vital role of mitochondria in caspase – independent apoptosis, lysosomes and endoplasmic reticulum (ER) play an essential role in the release and activation of death factors such as cathepsins, calpains, and other proteases [19]. Additionally, the pro-apoptotic proteins BAX and BID are represented as key participants in caspase – independent apoptosis [30].

Conclusion

Apoptosis plays an important role in the maintenance of tissue homeostasis, and it is critically involved in the pathophysiology of variety of diseases such as cancer, neurodegeneration, diabetes mellitus, stroke etc. In this review we focused on apoptotic proteins that have been identified, but the molecular mechanisms of action of these proteins are not fully understood. Understanding apoptotic mechanisms involving these proteins is essential for better understanding of occurrence and development of various diseases. Understanding apoptotic mechanism might provide useful information and new approaches for prevention and development of new therapies for variety of diseases.

Acknowledgements

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