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APOPTOSIS: PATHWAYS OF PROGRAMMED CELL DEATH A REVIEW

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ABSTRACT

Apoptosis, programmed cell death, is an energy-dependent biochemical process which alters the cellular morphology that leads to death of cell. Apoptosis plays a major role in various cellular processes like proper functioning and development of the immune system, normal cellular turnover, hormone-induced atrophy, embryonic stage of development and chemical-triggered cell death. Disproportionate apoptosis either more or less is a major factor for illness in many human beings including autoimmune diseases, neurodegenerative disorders, ischemic damage, and different types of cancer. The potential to stabilize the life or death of a cell is an index for its intense therapeutic property. Therefore, various signaling pathways and cell cycle machinery are the research area to focus on the control of cell cycle arrest and apoptosis. The research in the field of apoptosis moving forward at very rapid rate. Presently many key proteins are identified which are responsible for apoptosis but the mechanisms of action of these proteins are still to be elucidated. The aim of this review is to provide current knowledge in the field of apoptosis which includes the role of apoptosis in relation to health and various disease states, detection methods, and a potential alternative forms of apoptosis.

Keywords Apoptosis, Caspases, Tumor necrosis factor, signal transduction

INTRODUCTION

The term apoptosis (an a-po-toe-sis) was coined by Kerr, Wyllie, and Currie in the year 1972 to describe a distinct form of the cell death morphologically **[1]**. The mechanism of apoptosis involved in mammalian cells came from programmed cell death investigation which occurs during the development of the

nematode Caenorhabditi selegans For animal development, Cell death is essential and crucial **[2, 3]**. From the studies of a motoneuron in chick embryo development, it is first came to know that the cell death is regulated during normal animal growth. Nerve growth factor (NGF) The first regulator of cell survival was discovered from these studies **[4]**. Amphibian and insect Metamorphosis reveales that developmental cell death is a common place and predictable **[5]**. The term programmed cell death (PCD) was coined to acknowledge the reproducibility and renewal.

Apoptosis plays major roles from tissue sculpting to cell numbers controlling, and then quality control (Box 1). In case of Drosophila Melanogaster PCD appears to be important for viability and for vertebrates defective PCD results cancer, neurodegeneration, and developmental abnormalities **[6, 7]**. 1090 somatic cells are generated in this organism to form the adult worm, out of which 131 cells undergo apoptosis or "programmed cell death." At particular points these 131 cells die during the development process, which is essentially same between the worms, which denote the probable accuracy and control of the system. Since then apoptosis has been regarded and accepted as an important and distinct form of "programmed" cell death, which involves elimination of genetically determined cells. However, it is also important to conclude that other forms of programmed cell death may yet to be discovered **[8]**.

During development and aging apoptosis occur as a homeostatic mechanism to maintain cell populations in tissues. When cells are damaged by diseases or in immune reactions' apoptosis also occurs as a defense mechanism. There are a wide variety of physiological and pathological conditions, that can trigger apoptosis, not necessarily all cells die in response to the similar stimuli. Aniticancer drugs for chemotherapy results in the damage to DNA in some cells, which leads to apoptotic death. Some hormones, like corticosteroids, lead to apoptotic death in some cells (e.g., thymocytes) however other cells are unaffected. Fas or TNF receptors also lead to apoptosis through protein cross-linking and ligand binding. Certain cells have defect death pathway that is blocked by a survival factor such as a growth factor or hormone.

Apoptosis and necrosis are the two processes that can occur sequentially, independently, and simultaneously **[9]**. In some cases it is the degree of stimuli or type of stimuli that determines if cells die by necrosis or apoptosis. Various types of injurious stimuli such as heat, hypoxia,

radiation, and cytotoxic anticancer drugs can induce apoptosis at low doses, but these same stimuli can result in necrosis at higher doses. Thus, cells cause their own demise for development, by activating a selected self-culling cascade. Now D. melanogaster and C. elegans are used as model systems for most of developmental cell death studies. Regarding this, we will describe the mechanisms of developmental cell death and their control Finally, apoptosis is a coordinated and energy-dependent process that stimulate a number of cysteine proteases known as "caspases" and a cascade of events that initiates the stimuli for final demise of the cell.

APOPTOSIS BY THE EXTRINSIC/DEATH RECEPTOR PATHWAY

Apoptosis is triggered by two main pathways: the intrinsic pathway or mitochondrial pathway and the extrinsic pathway or death receptor pathway. The extrinsic apoptosis pathway is regulated by tumor necrosis factor (TNF), gene superfamily death receptors (DR) at the surface of cell membrane provide an efficient and rapid form of apoptosis. Death receptors are transmembrane proteins and through intracytoplasmic death domain (DD) component signal transduction occurs. Receptor interaction with death ligand require an adapter protein known as Fas-associated death domain protein (FADD) to form death inducing signalling complex (DISC), then binds with procaspase- 8 molecules, and activation of caspase-8 occurs **[10]**. Activation of, caspase-8 leads to activation of downstream caspases like caspase 3 and caspase 7 that initiates apoptosis.

Another type of death receptor which activate caspase-8 and directly bind with FADD through TRAIL-R1 and TRAIL-R2, but in case of TNF–TNFR1 complex which utilises an extra intermediate like binding of TNF to TNFR1, then trimerisation occurs. An adaptor molecule TRADD (TNFR-associated death domain) is essential to help the binding of FADD to the receptor complex with the recruitment of procaspase-8. TRADD also recruit secondary adaptors like RIP (a serine–threonine kinase receptor-interacting protein) and TRAF2 (TNF receptor-associated factor 2) to mediate signalling. By this way it activates the JNK/AP-1 and NF-κB survival pathways, and stop the apoptotic signal thus ensure survival of the cell. Fas and TRAIL systems are able to activate both the intrinsic and extrinsic pathways of apoptosis. The Bid molecule (a pro-death member of the Bcl-2 gene family) is cleaved by procaspase-8. The active part of the cleaved Bid translocates into mitochondria, binds Bax or Bak (pro-apoptotic members of the Bcl-2 family)

with resultant mitochondrial fragmentation, cytochrome c release and apoptosome formation as before.

Bid is the intermediate between the extrinsic and intrinsic pathways. In some cells (known as type 1) caspase-8 activation is sufficient to initiate apoptosis through its downstream effector caspases-3 and -6 activation. But in other cells (termed type II) which are not able to form the DISC thus the extrinsic pathway requires amplification through the mitochondrial pathway. It is hereby cleared that, uncontrolled death receptor activation would be hermfull for an organism. To restrict this, receptors signaling are regulated in different ways, ike anti-apoptotic proteins inside the cells, and the of transcription factors activation.

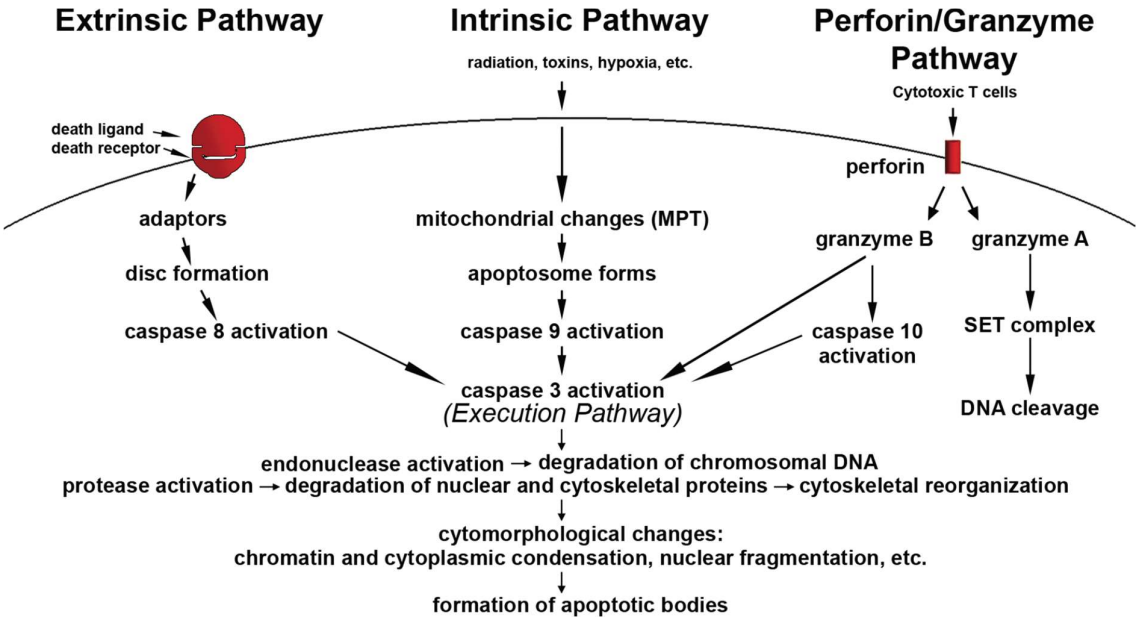

Figure 1: Schematic representation of apoptotic events

APOPTOSIS BY THE INTRINSIC/MITOCHONDRIAL PATHWAY

The Bcl-2 family proteins by regulating the motichondrial outer membrane permeabilization (MOMP) controls the intrinsic apoptosis pathway centers. Within at least one to four Bcl-2 homology (BH) domains, Bcl-2 family members share a homology which are required for the homo- and heterotypic interactions that determine the decision to undergo MOMP. Bax and Bak are the pro-apoptotic members contain BH1–3 and are essential for the execution of apoptosis through the intrinsic pathway **[11, 12, 13]**. The existence of Bax molecules

as cytosolic monomers in which the NH2-terminal alpha-helix and the COOH-terminal are embedded within the protein structure. Exposure of the COOH-terminal mediates targeting of Bax to the outer mitochondrial membrane. Following mitochondrial translocation, Bax projects its NH2 terminus and forms dimers and then homo-oligomers that result in MOMP and cytochrome c release **[14, 15, 16, 17]**.

It is not fully understood about the induction of MOMP and cytochrome c release by Bax oligomers. However, many recent studies have provided ideal mechanistic pathways. Bax central helices are inserted with the mitochondrial outer membrane, inducing curvature potentially and MOMP **[18, 19, 20]**. After apoptosis induction ring structures of various size and shape are formed by Bax to represent pores, and these are not present in other mitochondrial proteins. **[21]** Bax rings formation on the mitochondria is not adequate for maximal release of cytochrome c, and other proteins such as Drp1 involved in mitochondrial structural changes. Activation of the enzyme mitochondrial protease OMA1 results downstream of Bax oligomerization that leads to cristae remodelling and cytochrome c release by activation and cleavage of OPA1 **[22]**.

The regulation of Bax oligomerization is important for the determination of death commitment point. With the help of anti-apoptotic Bcl-2 family members, Bax is held in check through inhibitory processes and Bax is also inhibited by other non-Bcl-2 family proteins such as parkin, humanin, and clusterin **[23, 24, 25, 26]**. Pro-apoptotic group of Bcl-2 family proteins such as BH3 proteins that induces apoptosis by deinhibition of Bax. Along with de-inhibition of Bax, certain BH3 proteins have the capacity to directly bind and activate Bax. This “direct activation” is helpfull for the induction of MOMP **[27]**. Cells deficient for all eight BH3 proteins (Bim, Bid, Puma,

Bad, Bik, Bmf, Hrk, Noxa) by the help of CRISPR technology it is demonstrated that Bax/Bak-dependent apoptosis could be possible in the absence of BH3 proteins by immediate knockdown of Mcl-1 and Bcl-xL. Thus direct activation is not required for the induction of intrinsic apoptosis, whereas de-inhibition of Bax/Bak does **[28]**.

The mitochondrial localization plays a vital role in the activation of Bax by, Bim, Puma and tBid **[29]**. In vitro studies have concluded that the BH3 proteins Puma and Bim for apoptosis by a series of toxic stimuli, that includes reactive oxygen species, DNA damage, proteosomal inhibition, endoplasmic

reticulum stress, amyloid and excitotoxic stress. Depending on current evidence other BH3-only proteins like Noxa and Hrk do not take part such a vital role in apoptosis **[30]**. The very recent antibody identification which can prevent mitochondrial translocation of cytosolic Bax but activate mitochondrial Bax increases hope towards the development of more Bax-inhibiting compounds may be possible. The recent discovery of a substance that inhibits apoptosis, cytochrome c release and promotes long-term Bax activation holds good for the development of apoptosis inhibitors **[31]**.

PERFORIN/GRANZYME PATHWAY

Cytotoxicity mediated by T-cell is a variant of type IV hypersensitivity reaction in which CD8+cells kill antigen-bearing cells. These cytotoxic T-lymphocytes (CTLs) via extrinsic pathway are able to kill target cells and the interaction of FasL/FasR is the predominant way of CTL-induced apoptosis **[32]**. However, a novel pathway that involves secretion of the transmembrane pore-forming molecule perforin able to exert their cytotoxic effects on virus-infected cells and tumor cells with a subsequent release of granules from cytoplasm through the pore into the target cell **[33]**.

Granzyme A and granzyme B serine proteases are the most important component within the granules. At aspartate residues Granzyme B cleaves proteins and therefore activates pro-caspase-10 then cleave ICAD (Inhibitor of Caspase Activated DNAse) factors. Granzyme B utilizes the mitochondrial pathway by induction of cytochrome c release and specific cleavage of Bid for amplification of the death signal **[34]**. Granzyme B can also activate caspase-3 directly. In this way, there is direct induction of apoptosis and the upstream signaling pathways are bypassed. It is suggested that both direct activation of caspase-3 and the mitochondrial pathway are critical for granzyme B induced apoptosis.

Recent findings proof that neither caspases nor death receptors are responsible for the T cell receptor-induced apoptosis because their isno effect on apoptosis by blocking their ligands. On the other hand, granzyme B has no effect on Fas-Fas ligand interaction, adapter proteins with death domains and caspases which are involved in the apoptosis and regulation of cytotoxic Type 1 helper cells.

Granzyme A activates caspase independent pathways and also important in cytotoxic T cell induced apoptosis. Granzyme A initiates DNA nicking through a tumor suppressor gene product DNAse NM23-H1 **[35]**. This DNAse has a vital role through

the induction of tumor cell apoptosis in immune surveillance to prevent cancer. The nucleosome associated protein SET which inhibits the NM23-H1 gene. Granzyme A helps for the breakdown of SET complex for inhibition of NM23-H1, resulting in degradation of apoptotic DNA. As well as inhibiting NM23-H1, the SET complex has vital functions in DNA repair and chromatin structure. The different complexes like Ape1, SET, pp32, and HMG2 work together to provide protection for chromatin and DNA structure. **[36]** Therefore, granzyme A inactivates this complex and contributes to apoptosis by blocking the chromatin structure integrity and maintenance of DNA structure.

CONCLUSIONS

Apoptosis is regarded as an energy dependent regulated process, characterized by specific biochemical and morphological features in which activation of caspases plays a vital role. In the apoptotic pathways many of the key proteins are activated or inactivated. The activation of these proteins and molecular mechanisms of action are not fully understood and must be the focus of next research. It is vital to understand the importance of mechanistic machinery of apoptosis because programmed cell death is a part of both disease and health , which is initiated by various pathologic and physiologic stimuli. In the pathophysiology of various diseases the excess involvement of apoptosis leads to therapeutic intervention at different checkpoints. At the molecular level to understand the mechanism of apoptosis, and other types of programmed cell death, that provides deeper idea into different disease processes and it may influence new therapeutic strategy.

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