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Apoptosis- a sequence of events culminating in cell demise

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ABSTRACT

Type I programmed cell death, also called as apoptosis is a general phenomenon and a basic component of development in multicellular organisms. It is a self-destructive process and serves to maintain the cellular homeostasis by balancing the process of cellular proliferation. Apoptosis is not the only processes of cell death in multicellular organisms. Other forms of cell death also occur in multicellular organisms and these include autophagy (Type II programmed cell death) and necrosis (Type III programmed cell death). Apoptosis is a well-regulated process in which the activation of certain death specific signals lead to the removal of unwanted cells. Apoptotic cells show various morphological features including chromatin condensation, membrane blebbing, cell shrinkage, apoptotic body formation and DNA fragmentation, which enable the neighbouring cells to recognize and phagocytize them. Apoptosis is an essential contestant in diverse processes encompassing embryonic development, normal adult development of tissues, immune system maintenance and normal cell turnover. Derailment of apoptotic regulation can lead to various diseases including cancer, neurodegeneration and autoimmune diseases. This review focusses on various processes of apoptosis and the involvement of various proteins like caspases, BCL2 proteins and IAP's in these processes. It also throws light on the involvement of apoptosis in various diseases like neurodegeneration and cancer.

Keywords: *blebbing, karyorrhexis, neurodegeneration, phagocytosis, pyknosis.*

I. INTRODUCTION

The existence of different mechanisms to regulate cell death, differentiation, aging and proliferation by cells to maintain homeostasis led to the evolution of advanced forms of life. Apoptosis, a form of programmed cell death was first discovered by Carl Vogt in 1842. The term apoptosis was first used in 1972 by Kerr, Currie and Wyllie. Apoptosis is a genetically controlled, normal physiological and irreversible process which allows a cell to commit suicide [1-4]. It is an efficient process which allows an organism to eliminate unwanted, unhealthy, harmful, abnormal, damaged and virus infected cells [5]. Apoptosis is a process used by all multicellular organisms during development, immune response and differentiation. It also occurs during the response of cells to hormones and growth factors [6]. The phenomenon of apoptosis involves the initiation and execution of events which culminate in the demise of the cell. These events are choreographed in a cell by proteins encoded by the genome [7]. The process of apoptosis has been immensely studied in immune system where it leads to the elimination of self-reactive lymphocytes. The process of apoptosis plays a vital role in the development of cancer and cancer treatment. Malignant transformation is inhibited by apoptosis whereas anomalous apoptosis can induce cancer in cells. The total number of cells in multicellular organisms is a balance between the process of cell division and cell death and the loss of this equilibrium results in the development of cancer [8,9]. During the treatment of cancer drugs used for chemotherapy or irradiation induce DNA damage. The DNA damage results in the activation of p53 and hence induction of apoptosis by a p53 dependent pathway [10]. The process

of apoptosis is prompted by a variety of pathological and physiological stimuli but all cells do not necessarily require the same stimulus to die. Some stimuli lead to apoptotic death in some cells whereas the other cells remain unaffected e.g, corticosteroids which induce apoptotic death in thymocytes whereas the other cells remain unaffected or even get stimulated [10]. The type and the degree of the stimuli determines whether a cell dies by apoptosis or necrosis, the two cell death processes that occur sequentially, independently as well as simultaneously [11,12]. A variety of stimuli such as radiation, heat, cytotoxic anticancer drugs and hypoxia can induce apoptosis at low doses but the same stimuli result in induction of necrosis at higher doses [10].

II. HALLMARKS OF APOPTOSIS

Apoptotic cells are characterized by various morphological changes which distinguish them from the healthy neighbouring cells [13]. Pyknosis, a consequence of chromatin condensation is a visible characteristic of early apoptosis. Apoptotic cells also undergo shrinkage which is characterised by dense cytoplasm, smaller size and more tight packing of cellular organelles [10]. Pyknosis and cell shrinkage are visible during early apoptosis by light microscopy [14]. During histological examination with hematoxylin and eosin stain, the apoptotic cells emerge as a round mass with crowded purple chromatin fragments and dark cytoplasm [10]. Apoptotic cell also undergo a process called budding during which substantial blebbing of plasma membrane occurs. The process of blebbing is followed by karyorrhexis which results in the formation of apoptotic bodies. Apoptotic bodies are small cell fragments consisting of a small cytoplasm with densely packed intact cell organelles all enclosed within an intact plasma membrane. Apoptotic bodies may or may not contain a chromatin fragment from nucleus. After the formation of apoptotic bodies, they are phagocytosed by macrophages and degraded within phagolysosomes [2]. The process of apoptosis and the elimination of apoptotic bodies is not associated with any inflammatory response because the apoptotic cells do not spill their contents into the surrounding tissues. The apoptotic cells are also readily phagocytosed by the macrophages and the macrophages which englufs the apoptotic cells do not generate the anti-inflammatory cytokines [15,16]. One of the first changes that occur in the plasma membrane of apoptotic cells is the flipping of the phospholipid phosphatidylserine from the inner leaflet to the outer leaflet. This loss of membrane asymmetry is detected by the binding of annexin V to the negatively charged phospholipid, phosphatidylserine [18]. Apoptotic signals increase the intracellular levels of calcium and this increase in calcium level inhibits the activity of the enzyme translocase which is responsible for the translocation of phosphatidylserine and phosphatidylethanolamine to the inner leaflet of the plasma membrane [21,22]. The increase in the intracellular calcium levels also increases the activity of the enzyme scramblase, an enzyme responsible for indiscriminate migration of all glycerophospholipids across the lipid bilayer, leading to loss of membrane asymmetry [21,23]. The biochemical changes in the plasma membrane of apoptotic cells are mandatory for the recognition and removal of these cells by phagocytes. During apoptosis the organization of the cytoskeletal elements is also lost. After chromatin condensation and the exposure of phosphatidylserine to the outer surface of the cell there is a simultaneous aggregation of vimentin and cytokeratin in the cell. These intermediate filaments are aggregated and cleaved proteolytically during the early phases of apoptosis. However, the microtubules and microfilaments are not proteolytically cleaved during apoptosis but are present in the apoptotic bodies as aggregated filaments [17]. During apoptosis the membranes

of some cells also become vulnerable to secretory phospholipase A2 hydrolysis [19,20]. Apoptotic cells also undergo degradation of chromosomal DNA by endogenous DNases. These DNases cut the double-stranded DNA in the internucleosomal region resulting in the formation of DNA fragments which are 180-200 base pair in size [24]. These DNA fragments contain single base overhangs at the 3' end and are also characterised by the presence of blunt ends [25,26]. The DNA isolated from the apoptotic cells runs in a ladder pattern during electrophoresis. The fragmentation of DNA in the internucleosomal region is used in the detection of apoptosis [24].

III. CONTESTANTS IN APOPTOSIS

3.1 Caspases

The process of apoptosis is a highly complex sequence of events mediated by a family of proteins called as caspases. Caspases are a family of endoproteases indispensable for maintaining cellular homeostasis by synchronizing the processes of inflammation and cell death [27]. Caspases are the main players in the process of apoptosis. These are initially synthesized as inactive zymogens called as procaspases and all nucleated cells contain these inactive precursors to mediate their own death after receiving proper death signal. Hence the caspases are tightly regulated to ensure that the apoptotic program is initiated only when needed. The process of caspase activation requires the dimerization of procaspases which is followed by cleavage of the procaspase to active caspase. Procaspase dimerization is mediated by adapter proteins which bind to procaspase prodomain. Caspases are characterized by the presence of different protein-protein interaction domains within their prodomains, allowing different caspases to interact with different adapter proteins. Caspases 1,2,4, 5 and 9 are characterized by the presence of caspase activation and recruitment domain (CARD), however, the CARD domain is absent in caspase 8 and 10 which possess the death effector domain instead [28]. Procaspases consist of a large and a small subunit in addition to the N-terminal prodomain. The crystal structure of the active forms of caspase 1 and 3 show that each enzyme exists as a heterotetramer, consisting of two large and two small subunits [29]. After receiving an appropriate death signal, inactive procaspases in the cell are converted to active caspases which then cleave critical substrates within the cell leading to the pronounced morphological changes in the apoptotic cell [29]. The proteolytic activity of caspases depends upon the presence of catalytic cysteine residues in the active site of the enzyme. Caspases are able to cleave the target proteins only after specific aspartic acid residues [27]. After activation caspases can activate other procaspases leading to a proteolytic cascade in which one caspase activates other thus amplifying the apoptotic signal. After the initial caspase activation the cell programmes towards irreversible cell death (10). Caspases are not only involved in apoptosis but also play a very important role in regulating the inflammatory responses. The caspases may therefore be either inflammatory caspases like caspase 1, 4, 5 and 12 in humans and caspase 1,11 and 12 in mice or apoptotic caspases like caspase 3, 6, 7, 8 and 9. The caspases involved in the process of apoptosis may further be classified as initiator caspases and executioner caspases depending upon their mode of action [27]. Initiator caspases are crucial in activating the effector caspases in response to death stimuli and include caspase 2, 8, 9 and 10 [30]. The initiator caspases are activated by induced proximity dimerization in which the dimerization of procaspases leads to conformational changes in the procaspases to mediate their activation. These caspases do

not require cleavage activation [31-33]. However in certain cases, the intrinsic proteolytic activity of initiator procaspases leads to the cleavage and activation of other initiator procaspase in the dimer and hence activating each other [34]. The activation of the initiator caspases lead to the cleavage and activation of executioner caspases which are the main effectors of apoptosis. The executioner procaspases include caspase 3, 6 and 7. Executioner caspases are synthesised as inactive procaspase dimers and these dimers are activated by cleavage mediated by initiator caspases. The cleavage between the large and small subunits of executioner caspases leads to a conformational change that brings the active sites of the two executioner caspases in the dimer together to produce a functional caspase [35]. After activation the executioner caspase activates other executioner caspases producing a cascade of caspase activation which culminates in the demise of the cell.

3.2 Bcl-2 Proteins

The Bcl2 proteins are the very important regulators of apoptosis. More than 25 members of the BCL-2 family have been discovered [36,37]. BCL-2 family members are dived into two groups: the proteins that promote apoptosis and hence the term proapoptotic and the proteins which inhibit apoptosis and hence the term antiapoptotic. The balance between the proapoptotic and antiapoptotic proteins is essential for the maintenance of cellular homeostasis. Various proapototic stimuli like DNA damage increase the expression of many members of proapoptotic protein family, shattering the tie between proapoptotic and antiapoptotic proteins. This disruption of the delicate balance results in the induction of apoptosis [38].

Bcl-2 proteins are characterised by the presence of one or more Bcl2 homology (BH) domains. Four Bcl-2 homology domains have been identified till date and each Bcl-2 family member contains at least one. The BH1, BH2 and BH3 domains in the Bcl-2 family members are required for the homo or heterodimerization of these proteins. On the basis of presence of several BH domains, the proapoptotic class of Bcl-2 family has been further divided into two sub-categories [37]. The members of the Bax sub-class of proapoptotic Bcl-2 family possess the BH1, BH2 and BH3 domains, however the members of the BH3 sub class have sequence homology only to the BH3 domain [39]. The antiapoptotic Bcl-2 proteins like Bcl-2, Bcl-x_L and Bcl-w are characterised by the presence of all the four Bcl2 homology domains [37]. In addition to Bcl2 homology domains, some of the members of the Bcl-2 family also contain a hydrophobic domain at their carboxyl end. This domain is important for the membrane localization of these proteins [37,40]. The membrane localization pattern of the proapoptotic and antiapoptotic proteins varies greatly. The proapoptotic proteins like Bax and Bid are localized in the cytosol prior to activation. Upon activation these proteins are localized to the mitochondrial membrane to inhibit the activity of antiapoptotic proteins like Bcl2 which are already localized on the outer mitochondrial membrane. The localization of the antiapopototic proteins like Bcl2 on the outer mitochondrial membrane is important in the maintenance of membrane integrity [41,42]. The disruption of the membrane integrity of mitochondria results in the release of cytochrome *c* from the intermembrane of mitochondria. This release of cytochrome *c* from the mitochondria is a critical step in the initiation of apoptosis [40]. The structure of Bcl-2 family members has revealed how these proteins are regulated at molecular level. These studies have also thrown light on the mechanism of action of these proteins. Although there is a remarkable difference in the sequence and function of Bcl-2 family members, they all are characterised by the presence of a remarkably identical fold consisting of

two hydrophobic α-helices at the centre. These two α-helices are surrounded by six or seven α-helices, which are amphipathic in nature [38].

3.3 Inhibitors Of Apoptosis (Iap's)

Inhibitors of apoptosis are a family of highly conserved proteins that are primarily involved in the downregulation of caspase activity and hence apoptosis. These proteins also play a vital role in regulation of tumor cell migration and shape [43]. These antiapoptotic proteins inhibit the activity of caspase 3,7 and 9. However they don't influence the activity of caspase 8. Inhibitors of apoptosis are also known to regulate the processes like cell cycle progression, cell division [44]. The two prime pathways of caspase activation i.e the death receptor pathway and the mitochondrial pathway are inhibited by the IAP's. Another minor pathway of apoptosis, the granzyme B pathway which involves caspase 3 is also inhibited by IAP's [45,46]. Since IAP's are primarily involved in the regulation of caspases and cell death, they also modulate a variety of cellular processes and hence are pleiotropic [47]. IAP's mediate the activation of NF-κB and MAPK pathways in a ubiquitin dependent manner, thereby enhancing the expression of genes driving cell survival, migration, immunity and inflammation. IAP's actually functions as E3 ubiquitin ligases, transferring ubiquitin from E2 to various target molecules, and hence regulating the stability of these substrates [48].

IAP's are also known as BIR domain containing proteins (BIRC's), as these proteins are characterised by the presence of Baculovirus IAP Repeat (BIR) domain. BIR domain is a zinc finger domain and is important for mediating protein protein interactions [49]. Mammalian cells are characterised by the presence eight IAP's BIRC1 also called as neuronal IAP/NAIP, BIRC2 also called as cellular IAP1/cIAP1/HIAP2, BIRC3 also called as cellular IAP2/cIAP2/HIAP1, BIRC4 also called as X-linked IAP/XIAP/hILP, BIRC5 also called as surviving, BIRC6 also called as BIR containing ubiquitin conjugating enzyme/BRUCE/Apollon, BIRC7 also called as melanoma IAP/ML-IAP/Livin and BIRC8 also called as IAP like protein 2/ hILP2/Ts-IAP(43). A large number of cellular processes regulated by IAP's are deranged in cancer which directly or indirectly contribute to cancer development. Small pharmacological inhibitors of IAP's called as Smac-mimetics (SM) are undergoing clinical trials for cancer treatment [50].

IV. PATHWAYS OF APOPTOSIS

Apoptosis involves an extensively complex and sophisticated cascade of energy dependent molecular events that are categorised into two main apoptotic pathways- The intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway. However, research based evidence suggests the presence of an additional pathway i.e. the perforin/granzyme pathway which involves T-cell mediated cytotoxicity and perforin-granzyme-dependent killing of the cell [51]. All the three pathways converge on the same terminal pathway which eventually leads to DNA fragmentation, degradation of nuclear and cytoskeleton proteins, cross-linking of proteins, expression of ligands for phagocytic cell receptors, formation of apoptotic bodies, and finally uptake by phagocytic cells.

4.1 Intrinsic Pathway

The intrinsic pathway is a series of mitochondrial-initiated events activated by intracellular signals that act directly on targets within the cell. The events culminate in opening of the mitochondrial permeability transition

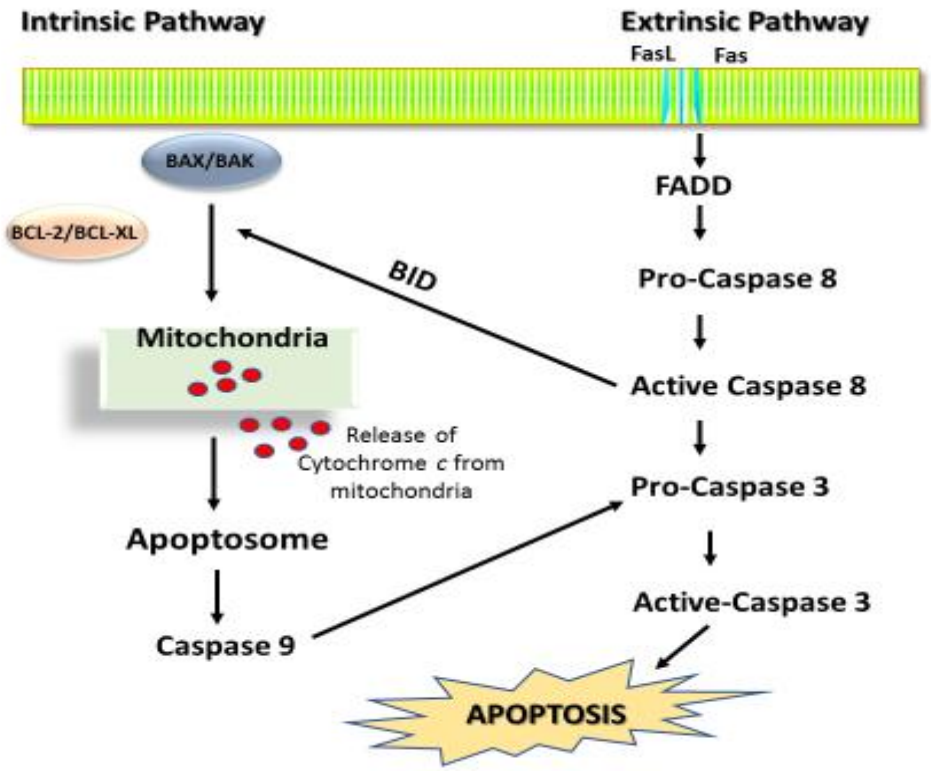
(MPT) pore, drop of the mitochondrial transmembrane potential and release of two important groups of otherwise sequestered pro-apoptotic proteins from the intermembrane space into the cytosol [52]. The first group comprises of cytochrome c, Smac/DIABLO, and the serine protease HtrA2/Omi [53-56] all of which activate the caspase-dependent mitochondrial pathway. Cytochrome c binds and activates Apaf-1 as well as procaspase-9, forming an "apoptosome" [57] which further leads to activation of caspase-9.

The second group of pro-apoptotic proteins include AIF, endonuclease G and CAD which are released as a late event from the mitochondria after the cell has committed to die. AIF translocates to the nucleus and leads to DNA fragmentation into ~50–300 kb pieces and peripheral nuclear chromatin condensation [58]. Endonuclease G translocates to the nucleus and causes cleavage of nuclear chromatin [59]. CAD after cleavage by caspase-3, leads to oligonucleosomal DNA fragmentation and much more pronounced chromatin condensation [60]. The regulation of these mitochondrial events of apoptosis occurs through members of the Bcl-2 family of proteins [61].

4.2 Extrinsic Pathway

The extrinsic pathway involves transmembrane receptor-mediated interactions. These include death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily [62] which bear similar cyteine-rich extracellular domains with a cytoplasmic domain of about 80 amino acids known as the "death domain" [63]. This death domain is indispensable in transmitting the apoptotic signal from the surface of the cell to the intracellular signaling pathways. The best-characterized ligands and corresponding death receptors include FasL/FasR, TNF-α/TNFR1, Apo2L/DR4, Apo2L/DR5 and Apo3L/DR3 [63-67].

The sequence of events starts with the clustering of receptors and binding with the homologous trimeric ligand. Upon binding with ligands, there is recruitment of cytplasmic adapter proteins that exhibit corresponding death domains to bind with the receptors. The binding of Fas ligand to Fas receptor leads to the binding of the adapter protein FADD. Also, the binding of TNF ligand to TNF receptor leads to the binding of the adapter protein TRADD with recruitment of FADD and RIP [68,69]. FADD associates with procaspase-8 through dimerization of the death effector domain. After this, a death-inducing signaling complex (DISC) is formed which results in the auto-catalytic activation of procaspase-8 [70].



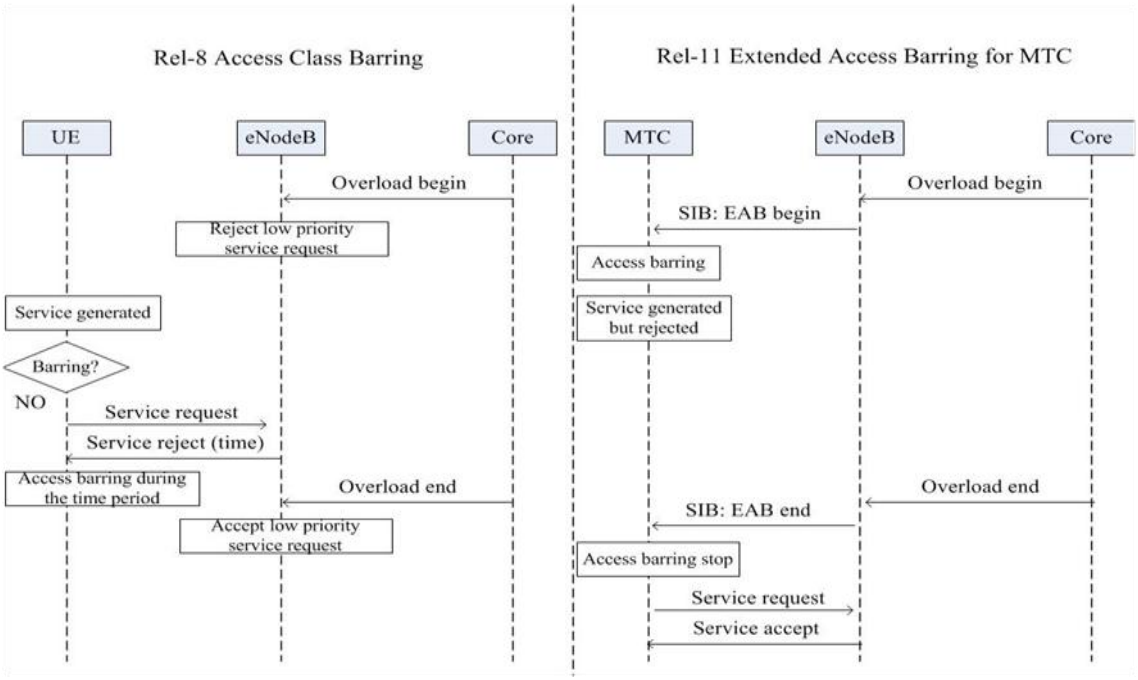
4.3 Perforin/granzyme Pathway

The granzyme A and granzyme B, serine proteases, are the most important components within the granules [71]. Granzyme A activates caspase independent pathways and leads to DNA nicking via DNAse NM23-H1, a tumor suppressor gene product [72]. Granzyme B cleaves proteins at aspartate residues. It activates procaspase-10 and cleaves factors like ICAD (Inhibitor of Caspase Activated DNAse) [73]. It has also been shown that granzyme B utilizes the mitochondrial pathway for amplification of the death signal by specific cleavage of Bid and enhancement of cytochrome c release [74,75]. However, granzyme B also directly activates caspase-3 thus bypassing the upstream signaling pathways. It has been suggested that both the mitochondrial pathway and direct activation of caspase-3 are vital for granzyme B-induced killing [76]. Granzyme B cytotoxicity is an important control mechanism for T cell expansion of type 2 helper T (Th2) cells [77].

4.4 Terminal Pathway

The extrinsic and intrinsic pathways both terminate at the point of the execution phase, regarded as the final pathway of apoptosis. This phase is initiated by the activation of the execution caspases Caspase-3, caspase-6, and caspase-7. They activate cytoplasmic endonucleases, which degrade the nuclear material, and proteases which degrade the nuclear and cytoskeletal proteins, cleaving various substrates including cytokeratins, gelsolin, PARP, the plasma membrane cytoskeletal protein alpha fodrin, the nuclear protein NuMA and others, that eventually cause the morphological and biochemical changes seen in apoptotic cells [78]. The last component of apoptotic cell is the phagocytic uptake. The appearance of phosphotidylserine on the outer leaflet of apoptotic cells promotes noninflammatory phagocytic recognition, enabling their early uptake and disposal [79]. This

process of early and efficient uptake with no release of cellular constituents, results in essentially no inflammatory response.



V. CONCLUSION

Apoptosis, a type I programmed cell death, is a sequence of events characterised by various morphological changes which enable macrophages to recognise these cells and engulf them. Cells dying by apoptosis die neatly in contrast to cells dying by necrosis which spill their contents and cause inflammatory responses. However there is no inflammatory response associated with apoptosis. The process of apoptosis involves various proteins like caspases, Bcl-2 family proteins and IAP's. These proteins are very important in regulating the process of apoptosis. Apoptosis may occur by intrinsic, extrinsic, perforin or terminal pathway. All these processes terminate in cell suicide.

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