

## Apoptosis-Biochemistry: A Mini Review

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### Abstract

Apoptosis or programmed cell death is a normal component of the development and health of multicellular organisms. Homeostasis is maintained through a balance between cell proliferation and cell death. It is a process of controlled cellular death whereby the activation of specific death-signaling pathways leads to deletion of cells from tissue. The distinct morphological features of apoptosis are Cell shrinkage, Chromatin condensation, Membrane blebbing and formation of apoptotic bodies, which are phagocytosed by neighbouring macrophages without any inflammatory response. Derailment of apoptosis in regulation can lead to several diseases or ailments with either too much or too little apoptosis. Understanding the molecular mechanism of apoptosis including death genes, death signals, surface receptors and signal pathways will provide new insights in developing strategies to regulate cell survival/death. The current knowledge on the molecular events of apoptotic cell death and their significance in health and disease is reviewed.

**Keywords:** Apoptosis; Necrosis; Hemostasis; Caspases

### Introduction

The existence of advanced forms of life became possible only through evolving definite mechanisms to regulate proliferation, differentiation, aging and death of the constituent cells by which the eukaryotic organisms maintain homeostasis. Necrosis and apoptosis are the two cell death processes in living organisms [1]. Apoptosis, also called Programmed Cell Death (PCD), has attracted great attention in recent years. Apoptosis is the physiological way for nucleated cells to die and also takes care of unwanted, injured, or virus-infected cells [2].

Human body is composed of nearly 104 cells. Every day billions of cells are produced by mitosis and a similar number of cells die by apoptosis to maintain tissue homeostasis [3]. Physiological cell death occurs when a cell within an organism dies by a mechanism orchestrated by proteins encoded by the host's genome. Apoptosis refers to both the initiation and execution of the events whereby a cell commits suicide and it is distinct from other forms of cell death [4]. It is a process of controlled cellular death whereby the activation of specific death-signaling pathways leads to deletion of cells from tissue [3]. It provides an efficient mechanism of eliminating unwanted cells during normal embryonic and adult development. Physiologically, all multicellular organisms use apoptosis during development, homeostasis, defense, metamorphosis, terminal differentiation, immune response, cellular response to growth factors and hormones [3].

Apoptosis has been extensively studied in immune system where it appears to play an important role in deletion of self-reacting lymphocytes. Cytotoxic T lymphocytes, Killer cells & Non Killer cells have been described to induce apoptosis in their targets. Apoptosis plays an important role in both carcinogenesis and cancer treatment. The loss of balance between cell division and cell death leads to cancer. It affects scattered individual cells, which have the following distinct morphological features such as Cell shrinkage, Chromatin

condensation, Membrane blebbing, Formation of apoptotic bodies and Shows no inflammatory response [3]. Eventually the dying cell degrades into apoptotic bodies that are sub cellular membrane bound vesicles that are ultimately removed by phagocytosis [4]. The transportation of Phosphatidyl serine from the inner membrane to the outer membrane is essential for the phagocytosis. Genetic analysis revealed the involvement of many death and survival genes in apoptosis which are regulated by extracellular factors.

The regulation of apoptosis is elusive, but defective regulation leads to etiology of various ailments [1]. Derailment of apoptosis in regulation of immune system leads to several diseases with either too much or too little apoptosis. Apoptosis prevents malignant transformation whereas abnormal apoptosis can predispose to cancer. Understanding the molecular mechanism of apoptosis including death genes, death signals, surface receptors and signal pathways will provide new insights in developing strategies to regulate cell survival/death. The current knowledge on the molecular events of apoptotic cell death and their significance in health and disease is reviewed.

### Apoptotic Pathways

The apoptotic cascade is initiated by two main pathways, involving either the activation of cell death receptors to respond to death ligands, or the release of cytochrome c from mitochondria. Both pathways will trigger a specific family of cysteine proteases, the caspases, to execute the self-killing process. The third less well known pathway also exists called endoplasmic reticulum stress-induced pathway.

### Caspase-dependent Cell Death

#### Extrinsic pathway

Also called Cell death receptor pathway, the death receptor-FADD-caspase-8 pathway begins when death ligands bind to death receptors. Death ligands bind to death receptors which sequentially signal the

self-destruction process or instruct the other cell to kill itself which is called instructive apoptosis. Best known death receptors are TNF Receptor (TNFR1) and Fas (CD95) and their ligands are called TNF and Fas ligand (FasL) respectively and the others include TRAIL receptors DR4 (TNF-related apoptosis-inducing ligand receptor 1, TRAIL-R1) and DR5 (TRAIL-R2) [5]. Death receptors have an intracellular death domain that recruits adapter proteins such as TNF Receptor-Associated Death Domain (TRADD) and Fas-Associated Death Domain (FADD). When Fas is activated by FasL, caspase-8 will be recruited via an adapter molecule FADD to make a ligand-receptor-adapter protein complex known as Death-Inducing Signalling Complex (DISC) [6]. DISC then initiates/activates caspase 8 which is an initiation caspase which consecutively activates caspase cascade leading to apoptosis. The cells which require DISC mediated signals to complete the cascade are classified as type I cells, while the cells which require the contribution of mitochondrial pathway to complete the apoptotic process are classified as type II cells. This is achieved by a Bcl-2 family member BID cleaved by caspase 8 producing a truncated form (tBID) which is translocated to mitochondria and activates the intrinsic apoptotic pathway through the conformational change of Bax and Bak.

However, caspase-9 has also been shown to be activated directly by a specific type of receptor, called "dependence receptors". Dependence receptors include a family of unconventional receptors which play opposing roles depending on availability of ligands. To date, 15 receptors have been shown to display these two opposite activities: p75NTR, DCC, Neogenin, UNC5H receptors family, Androgen Receptor, Patched, RET, MET, TrkC, ALK, EphB4. In its presence they mediate a positive signal of survival, differentiation, and migration, but without it they trigger a negative signal which leads to cell death. Ligand free dependence receptors precipitate cell death by molecular mechanisms like localization in lipid rafts, monomerization, recruitment of a caspase activated complex and cleavage by caspases [7].

### Intrinsic pathway / mitochondrial pathway

Intrinsic pathway means which is initiated within the cell. The mitochondrial pathway can be induced by extra- or intracellular stress (hypoxia, DNA-damage, insufficient amount of growth factors, high concentration of cytosolic calcium, or due to oxidative stress). This pathway causes increased permeability of the outer mitochondrial membrane leading to the release of pro-apoptotic molecules such as cytochrome c into the cytoplasm. The membrane permeability is controlled by members of the BCL-2 family which are further classified as pro-apoptotic and anti-apoptotic. Cytoplasmic release of cytochrome c activates caspase 3 and form a complex apoptosome with ATP, APAF-1 (apoptotic protease activating factor 1) and caspase-9. Family members of IAP (inhibitors of apoptosis) can bind directly to caspases and inhibit their activity. IAPs are negatively regulated by proteins from the mitochondrial intermembrane such as second mitochondria-derived activator of caspase (Smac), Direct IAP Binding Protein with Low pI (DIABLO) and Omi/high temperature requirement protein A (HtrA2) [8,9]. In addition to caspase activator protein, some other molecules such as AIF (Apoptosis Inducing Factor) and endonuclease G has also been found to be released that causes apoptosis by chromatin condensation and high molecular weight DNA fragmentation.

### The endoplasmic reticulum pathway

Third pathway and is less well known and is believed to be caspase 12-dependent and mitochondria-independent. A variety of ER stresses result in unfolded protein accumulation, which triggers the unfolded protein response which activates unique pathways that lead to cell death through apoptosis in response to injury by cell stress Bax/bak get activated which are localized in the endoplasmic reticulum, leading to calcium depletion and murine caspase 12 (C-12) activation. Recent evidence has shown that in addition to PUMA, p53 and NOXA are novel components of the ER stress-induced apoptotic pathway, and both contribute to ER stress-induced apoptosis via mitochondrial apoptotic pathway mediated by Apaf-1 [10].

### Caspase Independent Apoptosis

In recent years, it has become evident that although caspases are key players in the apoptotic process, the caspase activation is not the only determinant of decisions in programmed cell death. These alternative caspase-independent models include autophagy, paraptosis, mitotic catastrophe, and apoptosis-like or necrosis-like PCD, as well as senescence [11]. Caspase-independent pathways are considered safeguard mechanisms to protect the organism against unwanted and potential harmful cells when caspase-mediated routes fail but can also be triggered in response to cytotoxic agents or other death stimuli such as cathepsins, calpains, and other proteases. These type of cell death differ morphologically and biochemically and each type depends on the type of cell system, kind and intensity of stimuli [12].

### Apoptosis and Carcinogenesis

Cancer involves various genetic changes/mutations, in which normal cell gets transformed into malignant one, leading to excessive proliferation and decreased apoptosis. As early as the 1970's, Kerr et al. had linked apoptosis to the elimination of potentially malignant cells, hyperplasia and tumor progression [13]. Dysregulation of apoptosis or its resistance causes progressive accumulation of genetic alterations within the molecules and plays an important role in carcinogenesis. Cancer cells have the capacity to engulf apoptotic bodies which reutilizes mutated DNA, by transfer of genetic information between somatic cells, which constitutes a novel mechanism for propagation of genetic instability and/or diversity in tumors. A number of human diseases have been related to pathologic acceleration or retardation of the physiologic apoptotic rate. Apoptosis deregulation contributes to nearly half of all human diseases as a result of alterations in one or more elements of the apoptotic machinery.

### Apoptosis in Neurodegenerative Diseases

In neurodegenerative diseases activation of neuronal cell membrane receptors may bring about caspase activation, enhanced calcium levels and the generation of reactive oxygen species consequently inciting a course of events that eventually brings about cell death. The chief molecules responsible for apoptosis are caspases wear as high calcium levels and ROS contribute to the mitochondrial pathway.

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by accumulation of Amyloid- $\beta$  (A $\beta$ ) in the extracellular plaque and intracellular neurofibrillary tangles (NFTs) composed of microtubule-binding tau proteins, hyperphosphorylation of which results in loss of neurons and progressive dementia. Caspase 3 sequentially cleaves Amyloid Precursor Protein (APP) and tau

protein at its C terminus bringing about the generation of A $\beta$  and accumulation of NFTs [14,15]. In addition caspase 6 dependent axonal degeneration is activated by the actuation of death receptor 6 (DR6 also known as TNFRSF21) for which N-terminal APP fragment acts as a ligand [16].

Parkinson's Disease (PD) has been linked to mutations in several genes such as parkin, DJ-1, and a gene codifying for a mitochondrial kinase, (PTEN)-Induced Kinase 1 (PINK1) [17]. Down-regulation of PINK1 and other anti-apoptotic proteins such as Bcl-2 has been confirmed by Gene-expression profiling. PINK1 function is related to the inhibition of mitochondria-dependent apoptosis however extrinsic pathway contribution can likewise happen. Furthermore loss of PINK1 results in elevated levels of caspase activation (caspases 3 and 9). Additionally, death receptors such as FAS, TNFRSF10B and TNFRSF21 were up-regulated in PD-affected neurons [18].

Huntington's Disease (HD) is caused by a mutation in the gene encoding the huntingtin protein (htt). Different proteases including caspases cleave mutant htt causing the accumulation of caspase cleaved fragments which is an early pathological finding in brains of HD patients. Caspases 6 cleave mutant htt and is responsible for the development of the characteristic behavioral and neuro-pathological symptoms. Furthermore Hippo-Hip complex formation activates caspase 8. Mutated htt increases the free cellular concentration of HIP-1 which in turn favor the pro-apoptotic Hippo-Hip complex formation [19,20].

## Apoptosis in Autoimmune diseases

Physiologic regulation of cell death is essential for the removal of potentially autoreactive lymphocytes at the time of development and also for the removal of excess cells after the completion of immune response. Failure in removing these autoimmune cells which are produced during development or as a result of somatic mutation results in autoimmune disease [21]. Thus autoimmune diseases can arise both from defective clearance of autoreactive cells or by delayed elimination of autoantigens. The autoimmune prone *lpr* (lymphoproliferation) and *gld* (generalized lymphoproliferative disease) mouse strains display a defective apoptosis due to mutations in Fas (CD95) and the Fas-ligand, respectively, supports this concept [22].

Patients with Systemic Lupus Erythematosus (SLE) have elevated levels of soluble Fas, which inhibit Fas ligand-Fas interactions. The decrease in Fas mediated apoptosis contributes to accumulation of autoimmune cells in SLE. Furthermore it has been suggested that a defect in the clearance machinery and a subsequent overload of apoptotic cells is a potential mechanism for the breakdown of self-tolerance in SLE [23]. Autoimmune Lymphoproliferative Syndrome (ALPS) is a rare human disorder, in which apoptosis plays a crucial role in the maintenance of lymphocyte homeostasis. Defective cell death has been observed in *IL-2<sup>-/-</sup>*, *IL-2R $\alpha$ <sup>-/-</sup>* and *Bim<sup>-/-</sup>* mice, leading to the development of ALPS features such as splenomegaly, lymphadenopathy and the production of autoantibody. Whereas dysregulated Activation Induced Cell Death (AICD) leads to impaired apoptosis of activated autoreactive T cells in Multiple Sclerosis (MS). Additionally, Bcl-2 proteins are differentially expressed in lymphocytes from MS patients in a way that promotes resistance to apoptosis [23]. Rheumatoid Arthritis (RA) is another autoimmune disease in which defects in apoptosis appear to play an important role in disease pathogenesis. Hyperproliferation of peripheral CD4(+) T

cells in murine model of RA, with increased expression of c-FLIP and impaired AICD, may lead to accumulation of autoreactive T cells in the periphery [23].

## Major Players in Apoptosis

### Caspase

The “c” of “caspase” refers to a cysteine protease, while the “aspase” refers to the enzyme's unique property to cleave after aspartic acid residues. Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. Activated caspases cleave many vital cellular proteins and break up the nuclear scaffold and cytoskeleton. The caspases which play a central role in apoptosis are -2,-3,-6,-7,-8,-9,-10,-11 which can be further classified as initiation caspases(-2,-8,-9,-10,-11) and effector caspases(-3,-6,-7). Initiation caspases initiate the apoptotic pathway whereas effector caspases carry out coordinated program of proteolysis, resulting in the destruction of cytoplasm and cytoskeleton [24]. Furthermore caspase activated DNAase helps in further degradation of the nuclear DNA. Family members of IAP (inhibitors of apoptosis) (c-IAP1, c-IAP2, X-IAP, survivin) can bind directly to caspases and inhibit their activity [25]. As they play a key role in the extrinsic and intrinsic pathway it is believed that low level or dysfunction of these can lead to dysregulation of apoptosis and helps in carcinogenesis.

### Bcl2

All family members contain at least one of the BCL-2-homologous domains (BH1-BH4). BH3 is responsible for the anti or proapoptotic behaviour, and certain pro-apoptotic members contain only BH3 domain. There are two main groups of the Bcl-2 proteins, namely the pro-apoptotic proteins (e.g. Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim and Hrk) made up of the BH-3 only proteins, so named because at the time of cellular stress they get activated and initiate apoptosis and the anti-apoptotic proteins (e.g. Bcl-2, Bcl-X L, Bcl-W, Bfl-1 and Mcl-1) contain all four BH domains and they protect the cell from apoptotic stimuli by blocking the mitochondrial release of cytochrome-c [26]. Disruption of the balance between pro and anti-apoptotic members can lead to dysregulation of apoptosis. In many human tumors, decreased production of pro-apoptotic molecules like Bax and over expression of the anti-apoptotic proteins causes the survival of the tumor cells [27].

### Inhibitor of apoptosis proteins IAPs

IAPs are a family of proteins characterized by a zinc-binding region rich in cysteine and histidine residues termed baculoviral IAP repeat (BIR). All IAPs share a specific BIR region of 70 amino acids required to provide the antiapoptotic effect [28]. To date eight IAPs have been identified, namely, NAIP (BIRC1), c-IAP1 (BIRC2), c-IAP2 (BIRC3), X-linked IAP (XIAP, BIRC4), Survivin (BIRC5), Apollon (BRUCE, BIRC6), Livin/MLIAP (BIRC7) and IAP-like protein 2 (BIRC8). They regulate apoptosis, cytokinesis and signal transduction. They can bind directly to caspases and inhibit their activity or by keeping them away from their substrates. IAP activity is tightly regulated at transcriptional and post transcriptional levels. Dysregulated IAP expression has been reported in many cancers [29].

**p53**

The p53 protein, also called tumor protein 53, is one of the best known tumor suppressor proteins. It plays a multitude of important cellular responses that may vary from protecting the integrity of the genome, inducing apoptosis, regulating glycolysis and autophagy, to even promoting cell differentiation [30]. Cells committed to die via p53-dependent apoptosis typically follow the mitochondrial pathway, although p53 can also modulate cell death through death receptors. Furthermore, most evidence suggests that the key contribution of p53 to apoptosis is primarily dependent on transcriptional activity. p53 has the ability to activate transcription of various proapoptotic genes, including those encoding members of the Bcl-2 family, such as the BH-3 only proteins Bax, Noxa, and Puma [31]. In addition, it has been found that when the p53 mutant was silenced, it resulted in reduced cellular colony growth in human cancer cells, which was found to be due to the induction of apoptosis. Recent studies have shown that finely tuned, complex control of p53 by Mdm-2 (mouse double minute-2, an oncoprotein) is a key step in Ursodeoxycholic Acid (UDCA) modulation of p53-triggered apoptosis [32].

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