Review

Apoptosis: to Die or to Live? A Holistic Review

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Review

Apoptosis: to Die or to Live? A Holistic Review

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Abstract

Apoptosis is the process of programmed cell death. It is a physiological process needed and necessary for the maintenance of proper cellular homeostasis. Its involvement in the physiological process of morphogenesis is well known and documented. Also well documented are the pathologies (e.g. Alzheimer disease) that may arise as a result of any aberration of this process. This review gives an outlook of the essential biochemistry of apoptosis.

Keywords: apoptosis; necrosis; PCD; cell death; caspases; Bcl2

Introduction

Apoptosis, or programmed cell death, is a crucial biological process essential for maintaining cellular homeostasis and organismal development. The delicate balance between cellular survival and death governs tissue homeostasis, immune system function, and the prevention of diseases such as cancer. Understanding apoptosis involves delving into a myriad of cellular mechanisms, including intrinsic and extrinsic pathways, the role of various proteins, and the impact of genetic and environmental factors.

The review article titled "Apoptosis: To Die or Live? A Holistic Review" provides a basic overview of the currently known apoptosis transduction pathways and their various interplays at the molecular and cellular levels and how these are regulated. This review is designed as notes for students, researchers and clinicians, who are looking to understand at first-hand the multifaceted nature of apoptosis in receptor and transduction.

Morphological Hallmarks of Apoptosis

A classical paper (Kerr *et al*., 1972) described a house keeping process of cell deletion whose morphology differs distinctly from the already well-known process of necrosis. The term apoptosis was coined to describe this process. The characteristic morphological hallmarks differentiating apoptosis from necrosis include intracellular shrinkage, extensive blebbing resulting in the formation of apoptotic body and final subsequent destruction of dying cells by phagocytosis (Saraste and Pulkki, 2000). A principal denominator differentiating apoptosis from necrosis is that the whole process does not involve inflammation and through it a dying cell remains physiologically functional to its last moment. This would not be the case in necrosis which involves swelling up of a dying cell followed by membrane rupturing and subsequent release of intracellular content of the cell into the extracellular milieu thus eliciting inflammatory stimuli.

Molecular Pathways of Apoptosis

Three models of apoptosis have been characterized. A pathway that follows transduction from intracellular signaling originated within the mitochondria is called the intrinsic pathway. Another pathway that is elicited extracellularly by the action of ligand binding to specific cell receptors

 doi:10.20944/preprints202510.0405.v1

localized on cell surfaces is called the extrinsic pathway. There is also the granzyme-perforin pathway that is orchestrated via the activities of certain death molecules.

Extrinsic Pathway

Two molecular mechanisms of receptor/ligand caspase dependent initiation of apoptosis have been characterized. In these mechanisms apoptosis is initiated by the binding of a ligand to its specific cell surface receptor. Apoptotic receptors are protein macromolecules consisting of characteristic DD (death domains) which serves as recognition and attachment site for specific ligand (Ashkenazi and Dixit, 1998).

FasL AND TRAIL Signaling

FasL (Fatty acid synthetase ligand) and TRAIL (TNF-related apoptosis inducing ligand) ligates their respective receptors FasR (Fas receptor) and DR5 (death receptor 5) respectively by first recruiting an adaptor protein called FADD (Fas-associated death domain) to the cell's surface (Boldin *et al.,* 1995; Chinnaiyan *et al.,* 1995; Elmore, 2007, Ricci and El-Deiry, 2007). FADD carries a DD similar to that found on the receptor. Also present on FADD is another domain called the DED (death effector domain).

FADD facilitate death signaling by binding both the receptor and CP8 (caspase 8) which at this stage is present in its inactive form. CP8 belongs to a family of cysteinyl aspartate-specific proteinases which mediates apoptosis (Alnemri *et al,* 1996). These protein family of proteolytic enzymes are distinct by having a common cysteine motif and function by cleaving their substrates after specific aspartic residues. CP8 (synthesized as the inactive zymogen proCP8), also has a death effector domain, DED (Hsu *et al.,* 1996), which is similar to the one present on FADD.

FADD and the cell receptor associate through their DD domains. A similar association is formed between FADD and proCP8 through their DED domains. Therefore, a stable constitution of a DD-DD and DED-DED interaction is formed on the cell surface (Salvesen and Riedl., 2009; Scott *et al*., 2009). This assembly of ligand, receptor, FADD and proCP8 forms a structural organization called DISC (death inducing signaling complex). Subsequent aggregation of multiple procaspases through DISC formation leads to proximal auto-proteolysis of proCP8 and its activation. Activated CP8 cleaves and activate other downstream executioner caspases 3, 6 and 7 triggering the death signaling cascade (Muzio *et al*., 1996; Zhuang *et al.,* 1999; Volpe *et al*., 2016)

TNF Signaling

TNF (tumour necrotic factor) is a pro-inflammatory cytokine which is also involved in the maintenance of cell survival and cell death (Kaltschmidt *et al.,* 2002; Wang *et al*., 2005; Zhao *et al*., 2020; Webster and Vucic, 2020). There are two TNF-inducing apoptotic signaling complexes.

A well know mechanism of TNF induced apoptotic signaling involves the formation of a DISC-like complex consisting alpha chain of the TNF ligand TNF-α, its receptor TNFR1 and TRADD, (TNF receptor associated death domain). TRADD function as the primary adaptor protein by recruiting FADD as the secondary adaptor protein through a DD-DD interaction. CP8 is also recruited and activated in a similar manner leading to the activation of executional caspases 3, 6, and 7 (Micheau and Tschopp, 2003; Eum *et al*., 2011; Pobezinskaya and Liu, 2012; Dostert *et al*., 2019).

A new mechanism of TNF induced apoptosis which does not lead to activation of CP8 but rather leading to the activation of transcription factor NF-KB (nuclear factor light chain kappa B) had also been described. This pathway proceeds through the proteolytic breakdown (ubiquitination) of IKB (inhibitor of KB), the protein responsible for the sequestration of KB within the cell. TRAF2 (TNFR-associated factor-2), as well as RIP (receptor interacting protein) binds directly to TNFR1 or indirectly through TRADD. This complex activates NIK (NF-KB inducing kinase) which in turn activates IKK (inhibitor of KB kinase). IKK releases NF-KB by phosphorylating IKB. NF-KB once freed translocate

 doi:10.20944/preprints202510.0405.v1

into the nucleus of the cell where it transduces apoptosis (Rothe *et al*., 1995; Baichwal and Baeuerle, 1997; Baeuerle, 1998; Kuwano and Hara, 2000; Israël, 2010).

Intrinsic Pathway

Stimuli which may include but not limited to irradiation, hypoxia, toxins, depletion of growth factors, starvation, hyperthermia, viral infections and free radical generation may impact immense mechanical injury on a cell severe enough to compromise the mitochondrial membrane potential. The depolarization of mitochondria membrane potential results in the stimulation of the so-called mitochondria permeability transition, MPT (Lamaster *et al*., 1998; Zorova *et al*., 2018; Webster, 2002). MPT are channels-like structures that cuts through the mitochondrial membrane and facilitates the inward and outward movement of molecules. It has been suggested that the MPT is constructed from two proteins i.e. VDAC (voltage dependent anion channel) and ANT (adenine nucleotide translocator), both molecules are membrane bound proteins of the mitochondria (Baines *et al*., 2007; Baines, 2009; Tsujimoto *et al*., 2006) though ANT is suggested as the major protein of the inner mitochondrial membrane, VDAC on the other hand is the major protein of the outer membrane.

A plausible viewpoint for the formation of MPT is obtained from studies on the activities of the pro-apoptotic members of the Bcl2 (B-cell lymphoma 2) family i.e. Bax (Bcl2-associated X protein) and Bak (Bcl2-antagonist killer). These proteins have been reported to stimulate MPT formation either directly or in association with other proapoptotic proteins (Nutt *et al*., 2005; Jurečeková *et al*., 2011; Wolf *et al*., 2022). Most prominent of these proteins include Cyt C (cytochrome c) as well as other pro-apoptotic proteins such as SMAC (second mitochondria derived activator of caspases), DIABLO (direct inhibitor of apoptosis binding protein with low pI), HtrA2/Omi (high temperature requirement A2) are released by this event (Wang and Youle, 2009; Rehm *et al*., 2003; Maas *et al*., 2010). Cyt C binds with cytosolic pro-apoptotic adaptor protein Apaf-1 (apoptosis activating factor-1) to form a macromolecular structure referred to as the apoptosome which recruits and activate procaspase 9 which then recruits executioner caspases 3, 7 (Shi, 2002; Bratton and Salvesen, 2010; Shakeri *et al*., 2010).

Pro-apoptotic proteins SMAC/DIABLO and HtrA2/Omi function as antagonists and inhibitors of anti-apoptotic proteins including inhibitor of apoptosis protein, IAP and X-chromosome-linked inhibitor of apoptosis protein, XIAP (Martins *et al*., 2002; Hedge *et al*., 2002; Martinez-Ruiz *et al*., 2008) Other pro-apoptotic proteins are also released during terminal stages of a cell that has already committed to die through apoptosis. This will be looked into in more detail in later section in this review.

Perforin/Granzyme Pathway

Granzymes are proteolytic granule enzymes produced by certain death cells i.e. CTL (cytotoxic T lymphocytes) and NK (the natural killer) cells. There are two types of granzymes i.e. granzymes A and B. Scientific investigations had revealed how CTL and NK cells transduces apoptosis in a caspase dependent and independent manner via the production of granzymes and perforin respectively (Goping *et al*., 2003; Froelich *et al*., 2004; Rousalova and Krepela, 2010; Martinvalet, 2019). CTL and NK cells are members of CD8 (cluster of differentiation 8) family of immunologic killer cells sensitive to cells expressing MHC class I (major histocompatibility complex) peptides sequence on their surface membrane (Sutton *et al*., 2000; Stadnisky *et al*, 201; Rosenberg and Huang, 2018; Nutt and Huntington, 2019). CTL and NK cells scan for changes in this signature protein on T-cell receptors (TCR) present on target cells.

Mechanistically, the formation of MTOC (microtubule organizing center), similar to the spindle structure formed in mitosis has been described in facilitating CTL and NK cells degranulation and prevent bystander killing (Topham and Hewitt, 2009; Mentlik *et al*., 2010; 2012; Hsu *et al*., 2016). Lytic granules enclosed in vesicles converged and arranged themselves like tiny beads on MTOC inside mature killer cells. Cells flagged for apoptosis are approximated by killer cells followed by release of

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lytic granules initially into the IS (immunological synapse) i.e. space between killer cell and target cell (Krzewski, and Coligan, 2012; Ham *et al*., 2022). Internalization of lytic granules of lytic granules into a target cell is by passage through porins or intramembrane channels that are formed across the plasma membrane (Voskoboinik *et al*., 2015).

A different model for perforin action was also described which suggests its involvement in the movement Ca^{2+} from its external stores into the cytoplasm of a target cell. Increase in the intracellular concentration of this ubiquitous second messenger leads to the depolarization of the plasma membrane of the cell and subsequent influx of lytic granules possibly facilitated by passive diffusion (Takayama *et al*., 1987; Lancki *et al*., 1987; Maul-Pavicic *et al*., 2011).

Granzymes are serine proteases that cleave their substrates at specific aspartate residues in the same manner as the caspases and as such elicit similar apoptotic signaling cascade as the caspases. Once delivered, granzyme translocate into the nucleus of the target cell where it initiates apoptosis through pathways that involve both caspase activation and cleavage of BH3 (Bcl2 homology 3) only protein Bid (BH3 interacting domain death agonist) (Goping *et al*., 2003; Froelich *et al*., 2004; Rousalova and Krepela, 2010).

Execution Phase of Apoptosis

The execution pathway in apoptosis describes the series of biochemical and morphological events occurring during the terminal stages of a dying cell. All known caspase dependent pathways of apoptosis including extrinsic, intrinsic and perforin/granzyme converge at the execution phase. The key players in the execution phase are the executioner caspases 3 and 7 (Elmore, 2007). Once activated caspase 3 launched the execution phase of apoptosis by the proteolysis of a number of other pro-apoptotic proteins. For example, ICAD (inhibitor of caspase activated DNase) and DFF (DNA fragmentation factor) are key caspase 3 substrates involved in the nuclear chromatin condensation and disintegration (Wilson, 1998; Tang and Kidd, 1998; McIlroy *et al*., 1999). ICAD is a constitutively expressed inhibitor of CAD (caspase activated DNase). It inactivates CAD and prevents its translocation into the nucleus of a target cell. Cleavage of ICAD by caspase 3 activates CAD which then translocate into the nucleus. Hematopoietic cell divalent cation dependent neutral endonuclease (NUC70) and other members of the NUC family of endonucleases have also been reported to be caspase 3 substrates (Urbano *et al*., 1998; Robertson *et al*., 2000).

Apart from the DNA, the cytoskeletal structure of the cell consisting of an array of fine protein filaments responsible for maintaining native architecture of the cell is also disrupted. These intracellular and nuclear proteins including PARP (poly-ADP-ribose polymerase), α-fodrin, and NUMA (nuclear mitotic apparatus) are all cleaved by caspase 3 to provoke the morphology observed in apoptotic cells during the execution phase. Equally important is gelsolin which after its activation by caspase 3, cleaves actin; the principal cellular cytoskeletal protein (Kothakota *et al*., 1997; Sakahira *et al*., 1998).

Granzymes once delivered into the cell initiate cell execution in a caspase dependent or independent manner. In caspase dependent, the activation of CP3 as well as other proapoptotic proteins by granzyme leads to cell dissolution resulting in the activation of execution phase already described. In caspase independent, granzyme B transduces apoptosis through the cleavage of Bid to tBid (truncated form of Bid). tBid associates with and activates important pro-apoptotic members of Bcl2 family, Bax and Bak. The subsequently translocation of these two proapoptotic proteins into the nucleus initiates the execution phase (Barry and Bleackley, 2002; Russell and Ley, 2002).

Evidence from various studies have provided insights on the crucial role of mitochondrial generated reactive oxygen **s**pecies, (ROS) in connection to granzyme induced execution phase of apoptosis in a caspase independent manner (Fulda *et al*., 2010; Schenk *et al*., 2015; Martinvalet., 2019). ROS are generated by the mitochondria during electron transport chain by the incomplete reduction of molecular oxygen (Sisein, 2014; Ifeanyi, 2018; Alkadi, 2020). ROS consists of free radicals and non-radicals having unpaired electrons. Radical members of ROS include hydroxyl radical ($\cdot OH$), nitric oxide, and peroxynitrite, ($ONOO^{-}$). Non-radical members are singlet oxygen, ($^{\cdot}O$) and super oxide

 doi:10.20944/preprints202510.0405.v1

anion, ($\cdot O_2$). Other ROS members having no unpaired electron are hydrogen peroxide, (H_2O_2), and hypochlorous acid (HOCl).

Granzyme B induces apoptosis in a ROS dependent manner by which it targets the enzyme of complex I electron transport chain, NADH ubiquinone oxidoreductase and also various other proteins of complex I such as NDUFS1-3 (NADH dehydrogenase [ubiquinone] iron-sulfur protein 1, 2 and 3). Cleavage of these enzymes results in the generation and accumulation of ROS within the cell leading to undesirable effects such as lipid peroxidation and protein oxidation (Martinvalet, *et al*., 2015; Jacquemin *et al*; 2015; Cigalotto and Martinvalet, 2024).

Final House Keeping

Overall, the interplay of CP3 activities and other executioner caspases in the breakdown and rearrangement of the cell cytoskeleton coupled to the actions of ROS resulting in the fragmentation of nuclear chromatin and degradation of the nucleus culminate concomitantly into the manifestations of apoptotic morphology and the formation of apoptotic bodies called apoptosomes (Porcuna *et al*., 2016; Doncel *et al*., 2017; Povea-Cabello *et al*., 2017). These apoptotic bodies are rapidly taken up (phagocytosed) and destroyed by neighbouring house keeper cells i.e. phagocytes and macrophages. This engulfment of apoptotic fragments is facilitated by the externalization of phosphatidylserine on membrane surface of targets cells and serves as a "eat me" beacon to nearby house keeper cells (Fadok *et al*., 1998, 2001; Balasubramanian and Schroit, 2003; Nagata *et al*., 2016)

Apoptosis of Type I And Type II Cells: A Mechanistic Crossover

Cells have been categorized as type I or II in relation to their response to the apoptotic stimuli. Type I are cells that adhere to the typical ligand-receptor induced signaling cascade leading to characterized structural and biochemical events of apoptosis. In type II cells, death signals are weak; failure of DISC formation occurs and consequently termination of the apoptotic process. This debacle is circumvented in type II cells by amplification of death signals through the mitochondria pathway (Özören and El-Deiry, 2002; Sharon and Finkelshtein, 2009; Hao and Mak, 2010). This is a mechanistic crossover between the extrinsic and the intrinsic pathways. It involves cleavage of C-terminal of the BH3-only member of Bcl2 family Bid from its inactive form (Bid) to its active form (tBid). tBID translocation into the mitochondria and its subsequent interaction with important pro-death Bcl2 members Bax and Bak facilitate the release of Cyt C and other pro-death factors including AIF and CP9 (caspase 9). This crossover mechanism ensures that any initiated apoptotic process is driven through in type II cells.

Caspase Independent (AIF) Pathway

Results obtained from different studies on apoptosis have consistently speculated an alternative pathway of apoptosis which was presumed to be independent of caspases. This was evident from the observation of apoptosis in both green plants and fungi despite the apparent lack of caspases in these species. Also, the appearance of apoptotic phenotype in the nematode *Caenorhabditis elegans* (C. elegans) genetically knocked out for a variety of genes expressing Apaf-1, CP9 and Bcl-xl, proves a redundancy in caspase activity with regards to apoptosis. Furthermore, the Fas/TRAIC induced apoptosis pathway has also been found not to be perpetually truncated but rather partially retarded by blocking caspase activation in some cells (Susin *et al*., 1999, 2000). These revelations are substantiating another pathway of apoptosis distinctive and independent of caspase activity in some cells. The involvement of a the flavoprotein (flavin adenine dinucleotide, FAD) in the activation of this pathway has been demonstrated (Lorenzo *et al*., 1999; Candé *et al*., 2002). The protein called AIF (apoptosis inducing factor) resides within the intermembrane space of the mitochondria where it is held by the mitochondrial localization sequence (MLS), a peptide expressed in the mitochondria. MLS is responsible for keeping AIF locally inactive until when released into the cytosol (Sevrioukova, 2011).

 doi:10.20944/preprints202510.0405.v1

Delocalization of AIF is said to be triggered by the lysosomal protease, cathepsin D (cath D). Once released, AIF translocate into the nucleus where in synergy with endonuclease G, (EndoG) - also released by the mitochondria - causes nuclear chromatin condensation and DNA fragmentation. In this way AIF serves an oxidoreductase and apoptotic trigger (Daugas *et al*., 2000; Cande, C., *et al*., 2004).

Apoptosis Control and Regulations: Modulations by BCL2 Family

Regulation of apoptosis by members of the Bcl2 family of proteins is perhaps the most important mechanism of regulation in apoptotic cell death. Bcl-2 was the first member to be discovered as part of a family of anti and pro apoptotic proteins sharing unique homology in their peptide sequences. Earlier pioneering studies of Tsujimoto and co-workers conducted on human follicular cancer of B cell origin originally discovered Bcl-2 as an oncogene involved in the regulation apoptosis (Bakhshi *et al*. 1985; Cleary and Sklar 1985; Tsujimoto *et al.* 1985; Tsujimoto and Croce 1986). Also, studies carried out on the nematode C. elegans (*Caenorhabditis elegans*) have indicated that the worm's pro-survival gene Ced-9 is a functional homologue of the pro-survival Bcl-2 gene found in mammals (Hengartner, 1992; Conradt and Horvitz, 1998). Indeed, other studies have demonstrated that Bcl-2 is in fact an inner mitochondrial membrane protein that blocks programmed cell death (Hockenbery *et al*., 1990; Hengartner and Horvitz, 1994; Chao and Korsmeyer, 1998).

Members of the Bcl2 proteins have been categorized into three groups bearing four different conserved Bcl2 homology (BH) domains i.e. BH1, BH2, BH3 and BH4 (Adams and Cory, 1998; Burlacu, 2003).

Group 1 members – Bcl-2, Bcl-X_L, Bcl-W, Mcl1, A1 and DIVA are collectively regarded as the anti-apoptotic members of the Bcl2 family. These group 1 members have synonymous anti-apoptotic action and also bear the four homology domains BH1-BH4.

Group 2 members including Bax, Bak and Bok lack BH4 homology domain but bear the three domains, BH1-BH3. They are generally referred to as pro-apoptotic or pro-death members of Bcl2 family.

Group 3 members are also pro-apoptotic members of the Bcl2 family but are generally designated as BH3-only members differentiating them from their group 2 counterparts. This group is also so-named because they bear only the BH3 homology domain. Members in this group of regulatory proteins include Bid, Bim, Bik, Bad, Noxa and PUMA (p53 upregulated modulator of apoptosis).

Pro-survival members of Bcl2 family, Bcl2 and Bcl-X_L interact antagonistically with cytosolic pro-death members Bax and Bak repressing their activity. This restriction on the activity of the proapoptotic proteins is relieved by the binding of BH3-only members resulting in the release of Bax and Bak, both of which oligomerizes and inserts into the mitochondrial membrane. Subsequent translocation of these proteins into the mitochondria culminates in the eventual release of Cyt C (Gross *et al*., 1999; Emily *et al*., 2001). BH3-only members are believed to be activated by a variety of stimuli, for example DNA damage induced by p53 in the case of Noxa and PUMA (Aubrey *et al*., 2018; Roufayel *et al*., 2022), and growth factor deprivation in the case of Hrk and Bim (Sanz *et al*., 2000; Biswas and Greene, 2002).

Bid, another pro-death of Bcl2 family member is activated via CP8 cleavage into its active form, tBid which associate in a similar manner with other members of the family causing the release of Cyt C and activation of CP9 (Brunelle and Letai, 2009; Burlacu, 2003; Hardwick and Soane 2013; Czabotar *et al*., 2014)

 doi:10.20944/preprints202510.0405.v1

Regulation of Apoptosis by IAP

IAP (Inhibitors of apoptotic proteins) are a group of anti-apoptotic molecules which function by blocking the activation of effector caspase 9 and executioner caspases 3 and 7. This group of proteins are characterized as consisting of a unique baculovirus inhibitor repeat (BIR) domain at their N-termini, caspase recruitment domain (CARD) and a RING-finger-like domain at their C-termini (Deveraux and Reed, 1999). Members of this inhibitory proteins includes X- chromosome linked IAP (XIAP), cellular IAP (CIAP1 and CIAP2) and melanoma associated IAP (ML-IAP). All the IAP bears at least one out of three known BIR domains. For instance, XIAP, CIAP1 and CIAP2 have the BIR1, BIR2 and BIR3 domains while ML-IAP have only one BIR domain.

IAP inhibit caspase activation by binding them through the BIR domains. XIAP for example, inhibit CP9 activation by associating with it through its BIR3 domain. Similarly, CP3 and CP7 are inhibited by XIAP through its BIR2 domain. In addition to inactivation of caspases, IAPs also possesses a ubiquitin ligase activity also mediated through the BIR domains. By self-ubiquitination, via ubiquitin ligase, IAP target themselves and other proteins including caspases for proteolytic degradation.

IAP restriction on caspase activation and repression of apoptosis is however regulated by other regulatory proteins such as Smac/DIABLO and HtmrA2/Omi. These regulatory proteins bind to and effectively reverse the restriction imposed by IAP on caspases thereby releasing for subsequent activation (Martins *et al*., 2002; Jin *et al*., 2003; Martinez-Ruiz *et al*., 2008). It has also been demonstrated that certain IAP are capable of blocking the activity of other IAP. For instance, CIAP was observed to exert an inhibitory effect on XIAP by binding the latter through its RING-finger domain. Binding of both IAP in this way, target them for proteolytic degradation leading to a significant reduction in IAP accumulated activity (Silke *et al*., 2005; Cheung *et al*., 2008).

Regulation by Death and Decoy Receptors

As already mentioned in earlier sessions of this review, tumor necrotic factor, TNF forms a family of ligand-binding death receptors found on cells surface membrane. The unique structural property of this family of membrane receptor is the possession of a death domain, DD, an intracellular region consisting of amino acid sequences which bears varying degree of homology. There are six members of the TNF receptor family including TNF-R1, FasR, DR3, DR4, DR5, and DR6. The ligands that bind these receptor molecules also form a family of related cytokines which include TNFα, FasL, Apol3L, LTα and TRAIL. The binding of a ligand to its death receptor transduces apoptotic signal which is executed downstream caspase cascade leading to cell demise. However, a subgroup of TNF-ligand binding receptors also exist which function as inhibitor rather than transducers of apoptosis. DCR1 and DCR2 are membrane bound protein receptors that actively bind TRAIL1 and TRAIL2 respectively. The interaction of TRAIL1/DCR1 and TRAIL2/DCR2 are unable to transduce apoptosis signal as DCR1 lacks the death domain DD and DCR2 only bears a truncated one. In this way these decoy receptors function as inhibitors of apoptosis by competing for ligand binding with death receptors. In a similar manner DCR3, a soluble protein receptor block FasR mediated apoptosis by competing for FasL (Ashkenazi and Dixit, 1999; Sheikh and Fornace, 2000).

Other Control Mechanisms of Apoptosis

SODD (silencer of death domains) are inhibitory proteins that attenuate apoptosis by preventing DD aggregation of both TNFR1 and DR3 (Tschopp *et al*., 1999; Takada *et al*., 2003).

c-FLIP (cellular FLICE [FADD-like IL-1β-converting enzyme] inhibitory protein) prevent the activation of CP8 and CP10 or by binding to FADD or TRAIL receptor 5 (DR5) prevents the cellular formation of DISC and so inhibits the extrinsic pathway of apoptosis (Day *et al*., 2008).

p53 has been demonstrated to transcriptionally regulate the expression of a variety of apoptotic genes including Fas, FasL, and DR5 (Maecker *et al*., 2000; Wu *et al*., 2002).

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Calcium in Homeostasis and Apoptosis

Principal regulators of apoptosis, the Bcl2 proteins have long been found to be localized within organelles (mitochondria and endoplasmic reticulum ER) responsible for calcium ion (Ca^{2+}) homeostasis. Therefore, an intricate connection between the concentration of Ca^{2+} in the ER and the mitochondrial has been speculated. The existence of this connection became apparently substantive when it was proven that cytosolic Ca^{2+} increases during early and late stages of apoptosis (Pinton *et al*., 2008). This Ca^{2+} mediated apoptosis transduction is suggested to proceed either by the activation of a number of Ca^{2+} dependent enzymes such as Calpains, Calmodulins, Nitric oxide synthase and DAP kinase. These enzymes in turn advance the progression of apoptosis by activating other downstream proteins (Timmins *et al*., 2009). Another Ca^{2+} transduction pathway that was proposed involves the deployment of Ca^{2+} from the ER store and the uptake of the same by the mitochondria resulting in mitochondrial polarization and permeabilization and the subsequent release of Cyt C (Rasola and Bernardi, 2011; Giorgi *et al*., 2012).

The actual definitive mechanism through which Ca^{2+} is released from the ER is still largely controversial and elusive. However, several works have reported the involvement of the pro-apoptotic proteins Bax and Bak in this connection. It is still not clear though if these proteins directly mediate MPT pore opening by stimulation of VDAC and ANT as it has been proposed or by impinging on ER therefore facilitating Ca^{2+} release. Research is still needed in this regard to de-mystify this puzzle as there are many controversial roles of Ca^{2+} homeostasis in apoptosis transduction that has been reported in literatures.

Conclusions

Apoptosis is a well-defined cellular phenomenon of programmed cell death (PCD) involved in the physiological remodeling of tissues and organs of the body by elimination of cells in the body. The derangement of apoptosis may lead to pathological conditions including AIDS, Alzheimer's disease and cancer. Over the past decades, a lot of works have been done to completely elucidate and broaden our knowledge about this highly important process and have been learnt in this regard, this may not be exhausted. However, future works should be focused on lime lighting the crucial role of apoptosis in pathological states. Hence more works are still needed on this topic especially as apoptosis present a robust and promising future as a target for cancer therapy.

Statement of Compliance to Helsinki Declaration: This review article, titled "Apoptosis: To Live or Die - A Holistic Review", complies with the ethical standards set forth in the Declaration of Helsinki, as revised in 2013. As this is a review of existing literature and does not involve new human or animal subjects research, no direct human participant data was collected or analyzed. However, the authors affirm that all studies reviewed, which involved human participants, were conducted in accordance with the principles of the Helsinki Declaration. Ethical approvals for the original studies were obtained where applicable, and participant confidentiality was maintained. The authors have ensured that all research cited adheres to ethical guidelines and that the review promotes transparency, integrity, and respect for the rights and dignity of all individuals involved in the original research.

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